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Autor

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Listado de abreviaturas

A	Antheraxanthin
A_c	Conductive area
Acm	Acuminate apex
Acu	Acute apex
AI	Aridity Index
A_N	Photosynthesis net assimilation
$A_{N, leaf}$	Photosynthesis net assimilation per leaf
A_p	Phloem area
A_{pet}	Petiole cross-section area
ARID	Arid macroclimate
A_x	Xylem area
BIO1 or MAT	Annual mean temperature
BIO2	Mean diurnal range
BIO3	Isothermality
BIO4	Temperature seasonality
BIO5	Max temperature of warmest month
BIO6	Min temperature of coldest month
BIO7	Temperature annual range
BIO8	Mean temperature of wettest quarter
BIO9	Mean temperature of warmest quarter
BIO10	Mean temperature of warmest quarter
BIO11 or T_{min}	Mean temperature of coldest quarter
BIO12 or MAP	Annual precipitation
BIO13	Precipitation of wettest month
BIO14	Precipitation of driest month
BIO15	Precipitation seasonality
BIO16	Precipitation of wettest quarter
BIO17	Precipitation of driest quarter
BIO18	Precipitation of warmest quarter
BIO19	Precipitation of coldest quarter
bio20	Precipitation heterogeneity
C_a	CO_2 concentration surrounding the leaf
Cir	Circular shape
COLD_T	Cold Temperate macroclimate
Cre	Crenate margin
CT	Condensed tannin concentration
DEC	Deciduous

Den	Dentate margin
$\mathbf{d_h}$	Hydraulic diameter
$\mathbf{d_{hp}}$	Hydraulic diameter of phloem
dhx	Hydraulic diameter of xylem
$\mathbf{d_i}$	Diameter
Dim1	First principal component
Dim2	Second principal component
Dpu	Densely pubescent
ECf	Final electrical conductivity
ECi	Initial electrical conductivity
ECr	Relative electrical conductivity
Ell	Elliptic shape
Ent	Entire margin
EVE	Evergreen
$\mathbf{F_0}$	Minimum chlorophyll fluorescence in the dark-adapted state
FAMD	Factor analysis of mixed data
$\mathbf{F_V / F_M}$	The dark-adapted, maximum quantum efficiency of PSII
Gla	Glabrous
GR	Glutathione reductase
$\mathbf{g_s}$	Stomatal conductance
$\mathbf{g_{s,\ leaf}}$	Stomatal conductance per leaf
Hv	Huber value
ILB	Index of lobation
$\mathbf{K_h}$	Hydraulic conductivity
$\mathbf{K_{hp}}$	Hydraulic conductivity of phloem
$\mathbf{K_{hx}}$	Hydraulic conductivity of xylem
$\mathbf{K_s}$	Specific conductivity
$\mathbf{K_{sx}}$	Specific conductivity of xylem
LA	Leaf area
Lan	Lanceolate shape
LAR	Leaf area ratio
LES	Leaf economic spectrum
LMA	Leaf dry mass per unit area
Lob	Lobated margin
LSC	Leaf-specific conductivity
LW	Length-width ratio
M	Dry mass
MDAR	Monodehydroascorbate reductase
MED	Mediterranean macroclimate
NAR	Net assimilation rate

NPQ	Non-photochemical quenching
Obl	Oblong shape
Obo	Obovate shape
OLS	Ordinary least squares
Ova	Ovate shape
P_{50}	Xylem pressure inducing 50% loss of hydraulic conductivity
P_{88}	Xylem pressure inducing 88% loss of hydraulic conductivity
PC1-Clim	First principal component of the morphological PCA
PC1-Morph	First principal component of the morphological PCA
PC2-Clim	Second principal component of the morphological PCA
PC2-Morph	Second principal component of the morphological PCA
PCA	Principal component analysis
PET	Potential evapotranspiration
PGLS	Phylogenetic generalized least squares
PLA	Phloem to leaf area ratio
PRI	Physiological reflectance index
PSII	Photosystem II
Pu	Pubescent
RGR	Relative growth rate
ROS	Reactive oxygen species
Rou	Rounded apex
Ser	Serrate margin
SLA	Specific leaf area
SOD	Superoxide dismutase
Spi	Spinose margin
Str	Straight apex
T	Temperature
T_c	Critical temperature
TLP	Turgor loss point
Und	Undulate margin
V	Violaxanthin
VOC	Volatile organic compound
VPD	Vapor pressure 6éficit
WARM_T	Warm Temperate macroclimate
WdT-TROP	Winter dry temperate to Tropical macroclimate
WF	Work of fracture
XLA	Xylem to leaf area ratio
Z	Zeaxanthin
η	Viscosity
λ	phylogenetic signal
ρ	Density

Φ_{exc}	Intrinsic PSII efficiency
ΦPSII	Photosystem II efficiency
Ψ	Leaf water potential
Ψ_{MD}	Midday leaf water potential
Ψ_s	Soil water potential

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Resumen

El género *Quercus* cuenta con más de 400 especies distribuidas por todo el hemisferio norte. Esta biodiversidad, sumada a las dispares historias evolutivas de los diferentes clados subgénericos, se traduce en una gran diversidad de morfologías foliares, estrategias fisiológicas y ecológicas que les permiten colonizar, e incluso dominar, numerosos hábitats en climas muy diferentes.

El clima mediterráneo, por ejemplo, es uno de los más restrictivos por la presencia de una sequía estival y unas temperaturas invernales bajas. En la cuenca mediterránea, una especie paradigmática de *Quercus* asociada a este clima es la encina. Esta especie cuenta con un comportamiento bimodal asociado a dos morfotipos que presentan diferencias en sus estrategias para enfrentarse a dichos factores de estrés. Así, el morfotipo Ballota, que ocupa condiciones generalmente más continentales y frías, presenta mayor resistencia a la cavitación, vasos más pequeños en su xilema y mayor tolerancia al frío que el morfotipo Ilex, que habitualmente está presente en zonas más suaves por su proximidad al mar.

La encina define un síndrome foliar característico del clima Mediterráneo, que comparte con las especies que ocupan climas áridos y algunas otras especies mediterráneas. Este síndrome se caracteriza por presentar hojas pequeñas, esclerófilas, perennifolias, generalmente redondeadas, pubescentes y de bordes enteros, a veces con espinas o mucrones. Otras especies mediterráneas, junto con las templadas presentan hojas grandes, malacófilas, caducifolias, obovadas y lobuladas. Las especies tropicales tenderían a desarrollar hojas más largas, ligeramente esclerófilas, perennifolias, de bordes serrados, con ápices acuminados y glabras.

El hábito foliar también define dos estrategias anatómicas diferentes en *Quercus* a nivel de pecíolo. Por un lado, las especies caducifolias tienden a aumentar el tamaño de sus vasos en el xilema y las células del floema para satisfacer las mayores demandas hidráulicas y fotosintéticas que implica desarrollar una mayor lámina foliar. Por el contrario, las especies perennifolias tienden a seguir una estrategia más conservadora, aumentando su capacidad hidráulica especialmente a través de la inversión en superficie conductora.

Por último, queda demostrado cómo las especies de *Quercus* tienden a maximizar una única estrategia para defenderse de los herbívoros, ya sea de naturaleza física, química o de tolerancia a la presión biótica. Las especies más esclerófilas optimizarían una estrategia defensiva de tipo físico, mientras que el resto apostarían o bien por incrementar sus concentraciones de taninos condensados o su ratio relativo de crecimiento.

Abstract

Genus *Quercus* comprises more than 400 species distributed across the Northern Hemisphere. This biodiversity, combined with the varying evolutionary histories of different subgeneric clades, results in a wide diversity of leaf morphologies, physiological, and ecological strategies that enable them to colonize and even dominate numerous habitats in very different climates.

For instance, the Mediterranean climate is one of the most restrictive, characterized by summer droughts and low winter temperatures. In the Mediterranean Basin, a quintessential *Quercus* species associated with this climate is the holm oak. This species exhibits a bimodal behavior linked to two morphotypes that show differences in their strategies to cope with these stressors. The Ballota morphotype, which occupies generally more continental and colder conditions, exhibits higher cavitation resistance, smaller xylem vessels, and greater cold tolerance compared to the Ilex morphotype, which is typically found in milder coastal areas.

The holm oak defines a characteristic leaf syndrome of the Mediterranean climate, shared with species occupying arid climates and some other Mediterranean species. This syndrome is characterized by small, sclerophyllous, evergreen leaves that are usually rounded, pubescent, with entire margins and sometimes with bristle-tips or spines. Other Mediterranean and temperate species have large, malacophyllous, deciduous, obovate, and lobed leaves. Tropical species tend to develop longer, slightly sclerophyllous, evergreen leaves with serrated margins, acuminate tips, and the absence of trichomes.

Leaf habit also determines two different anatomical strategies in oaks at the petiole level. Deciduous species, on one hand, tend to increase the size of their xylem vessels and phloem cells to meet the higher hydraulic and photosynthetic demands of larger leaf laminae. Evergreen species, on the other hand, generally adopt a more conservative strategy, increasing their leaf-specific conductivity primarily through investment in conductive surface area.

Finally, it is demonstrated that *Quercus* species tend to maximize a single strategy to defend against herbivores, whether it be physical, chemical, or tolerance-based. The most sclerophyllous species optimize a physical defense strategy, while the rest either increase their concentration of condensed tannins or their relative growth rate.

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Capítulo I

Introducción general

1. Introducción

1.1. El género *Quercus*: diversidad, clasificación y distribución

Quercus L. es el género más biodiverso de su familia (Fagaceae), contando con más de 400 especies de árboles y arbustos distribuidas por todo el hemisferio norte. La gran diversidad de especies se corresponde con una amplia variedad de estrategias morfológicas, ecológicas y fisiológicas que les sirven para ocupar hábitats muy diferentes en toda su área de distribución. Esto convierte al género en el principal contribuyente arbóreo de todo el hemisferio norte (Nixon, 2006). De hecho, conforman el género de especies forestales con mayor diversidad y biomasa en México y Estados Unidos de América, región en la cual se encuentra su *hotspot* de biodiversidad (Valencia, 2004; Cavender-Bares, 2016; Hipp *et al.*, 2018). Su importancia radica en la gran cantidad de funciones que desempeñan en los ecosistemas: a través de la producción de bellotas, hojas y madera, proveen hábitat y alimento a todos los niveles de la cadena trófica (Cavender-Bares, 2019); son un componente imprescindible de la rizosfera, influyendo en la microbiota y el ciclo de nutrientes del suelo (Suz *et al.*, 2014; García-Guzmán *et al.*, 2017); son reguladores del clima, la hidrología y la calidad del aire (Kroeger *et al.*, 2014); varias especies son resistentes al fuego, que sumado a una gran capacidad de rebrote, las convierten en un pilar fundamental de la sucesión ecológica (Madrigal-González *et al.*, 2017). Muchas especies son resistentes a la sequía y a las heladas, estando las especies de *Quercus* (quercíneas) entre las últimas angiospermas arbóreas en los límites de vegetación forestal hacia extremos áridos y fríos (Tardif *et al.*, 2006; Poulos, 2009; Asouti y Kabukcu, 2014; Yang *et al.*, 2020).

El género *Quercus* fue inicialmente descrito por Linneo en 1753 en su obra magna *Species Plantarum*, con 14 especies. Desde entonces y hasta la actualidad, numerosos botánicos (Loudon, Trelease, Camus o Menitsky, entre los más destacados) han contribuido a incrementar el número de especies descritas por todo el globo. Conforme el número de especies crecía, también lo hacían los esfuerzos por intentar clasificar dichas especies en grupos que compartieran rasgos comunes, inicialmente en base a caracteres botánicos y, más recientemente, complementados con caracteres genéticos. Actualmente, tras la última gran revisión infragenérica (Denk *et al.*, 2017), el género se divide en dos subgéneros (subgen. *Cerris* y subgen. *Quercus*) y ocho secciones (*Cyclobalanopsis*, *Ilex*, *Cerris*, *Lobatae*, *Protobalanus*, *Ponticae*, *Virentes* y *Quercus*), con historias evolutivas muy diferentes (Fig. 1). El origen del género data de principios del Eoceno (ca. 56 Ma; Hofmann *et al.*, 2011; Hipp *et al.*, 2020) y la radiación en los dos subgéneros y las diversas secciones se dio en etapas muy tempranas de su evolución.

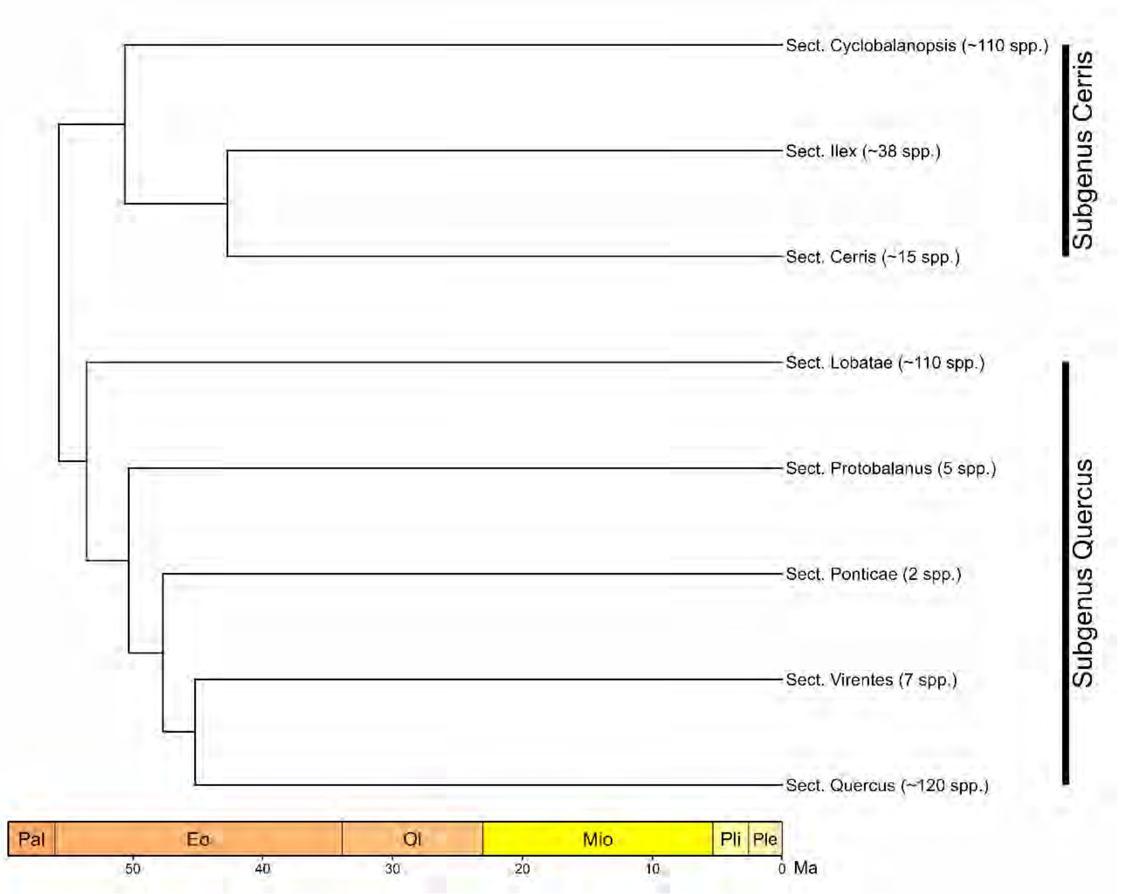

Figura 1. Árbol filogenético del género *Quercus* hasta el nivel de sección. Entre paréntesis se indica el número de especies aceptadas para cada sección. En la parte inferior se añade una escala de tiempo geológico desde el origen del género hasta el presente.

El subgénero *Cerris*, "robles del Viejo Mundo", o "robles de latitudes medias", tiene su origen en zonas paleotropicales del este de Asia durante el Eoceno (36-38 Ma) (Hipp *et al.*, 2020; Jiang *et al.*, 2019). Se compone de tres secciones: *Cyclonalanopsis*, *Ilex* y *Cerris* (Fig. 2). La sección *Cyclobalanopsis*, conocidos como robles con cúpula en anillo, costa de unas 110 especies, todas ellas perennifolias, distribuidas únicamente en el continente asiático en regiones que van desde lo subtropical del sur de Corea y Japón hasta lo ecuatorial de Indonesia. La sección *Ilex* presenta unas 38 especies perennifolias y esclerófilas cuya mayor biodiversidad se halla en la mitad sur de China y las cadenas montañosas adyacentes. Miembros de esta sección migraron hacia el oeste favorecidas por el ascenso del sistema de los Himalayas y las condiciones subtropicales del óptimo climático del Mioceno hasta llegar a Oriente Medio y, finalmente, al Mediterráneo occidental (Jiang *et al.*, 2019). La sección *Cerris*, también conocidos como robles turcos, consta de unas 15 especies mayoritariamente caducifolias. Solo un clado basal, de tres especies, permanece en el lugar de origen en regiones subtropicales y templadas del este de Asia. El resto de las especies acompañaron en su migración a los miembros de la sección *Ilex*, resultando en una distribución actual circummediterranea y por regiones montañosas de Oriente Medio (Denk *et al*., 2023).

El subgénero *Quercus*, “robles del Nuevo Mundo”, o “robles de latitudes altas”, tiene su origen en zonas neárticas del norte de Norteamérica durante el Eoceno (45-48 Ma) (Cavender-Bares, 2019), desde donde fue colonizando el continente hacia el sur hasta llegar a Colombia recientemente. Este subgénero se compone de las cinco secciones restantes (Fig. 2). La sección *Protobalanus* consta de tan solo 5 especies, todas ellas perennifolias y esclerófilas, cuya distribución se reparte por zonas mediterráneas y áridas del extremo occidental de Norteamérica. La sección *Virentes* está compuesta por 7 especies perennifolias o brevideciduas que habitan en torno al Golfo de México y el extremo sur de la península de California. La sección *Ponticae* presenta únicamente dos especies disyuntas entre las montañas de Siskiyou (EE. UU.; *Q. sadleriana*) y los Alpes Pónticos (Turquía y Georgia; *Q. pontica*). Las dos secciones restantes: los robles rojos (Sect. *Lobatae*) y los robles blancos (Sect. *Quercus*) son un claro ejemplo de evolución paralela. Constan de un número similar de especies (ca. 110 sp. para *Lobatae* y ca. 120 para *Quercus*) de habito foliar muy variable que ocupan en muchos casos los mismos nichos en simpatría. En ambas secciones, (i) los clados basales están representados por unas pocas especies californianas, (ii) un número significativo de especies caducifolias son formadoras de bosques templados y, (iii) en torno al 60% de las especies restantes forman los grupos mexicanos de los robles rojos (*Erythromexicana*) y blancos (*Leucomexicana*), de origen más reciente (Hipp *et al*., 2018). La mayor diferencia biogeográfica entre ambas secciones se presenta en un clado de unas 20 especies de robles blancos (*Roburoides*) que llegó, primero, a Europa a través de los llamados “puentes del Atlántico norte” durante el Mioceno (Denk *et al*., 2010) y, posteriormente, al Lejano Oriente, resultando en una distribución holártica de la sección *Quercus*. Por el contrario, la sección *Lobatae* permanece en la actualidad exclusivamente en el continente americano, aunque hay indicios palinológicos de que pudo haber estado presente en Europa (Barron *et al*., 2017)

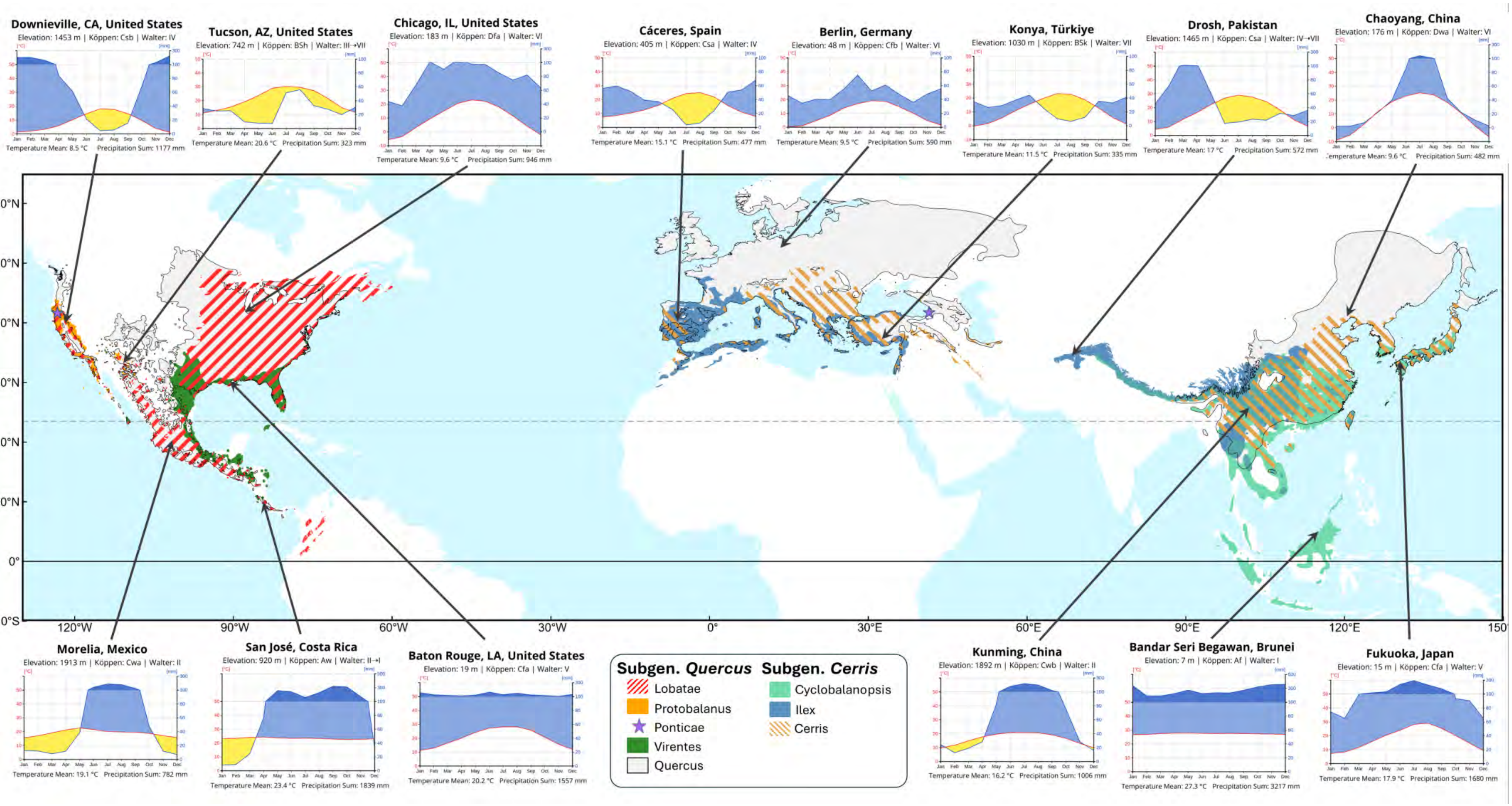

Fig. 2. Mapa de la distribución global del género *Quercus*. Cada una de las ocho secciones en la que se divide el género están representadas con una infografía diferente (ver leyenda). Además, se muestran con diagramas ombrotérmicos de Walter-Lieth ejemplos representativos de los principales climas en los que habitan las diferentes especies, indicando el clima según la clasificación climática de Köppen-Geiger y según los zonobiomas de Walter (Walter y Breckle, 2013). El mapa ha sido elaborado extrayendo los puntos de presencia de cada especie individualmente de la base de datos de *Global Biodiversity Information Facility* (GBIF; https://www.gbif.org/es) y agrupándolas por secciones tras su limpieza. Los ombrotermogramas han sido obtenidos de climatecharts.net (Zepner *et al*., 2020; https://climatecharts.net).

1.2. Clima y formaciones vegetales

Wladimir Köppen y Andreas F.W. Schimper fueron los dos primeros científicos que hicieron una gran clasificación bioclimática con base fitogeográfica del mundo en sus respectivas obras (Köppen, 1900; Schimper, 1898). Desde entonces, numerosas modificaciones y nuevas clasificaciones se han ido sucediendo a lo largo del siglo XX, siendo probablemente la más usada en la actualidad la de Köppen-Geiger (Köppen y Geiger, 1936). Dichas clasificaciones básicamente toman como referencia la temperatura, la precipitación y la estacionalidad de ambas para definir un bioclima. Dada la amplia distribución de las especies de *Quercus* por el mundo, éstas son capaces de habitar en numerosos —aunque no todos— de los bioclimas propuestos por cualquier clasificación.

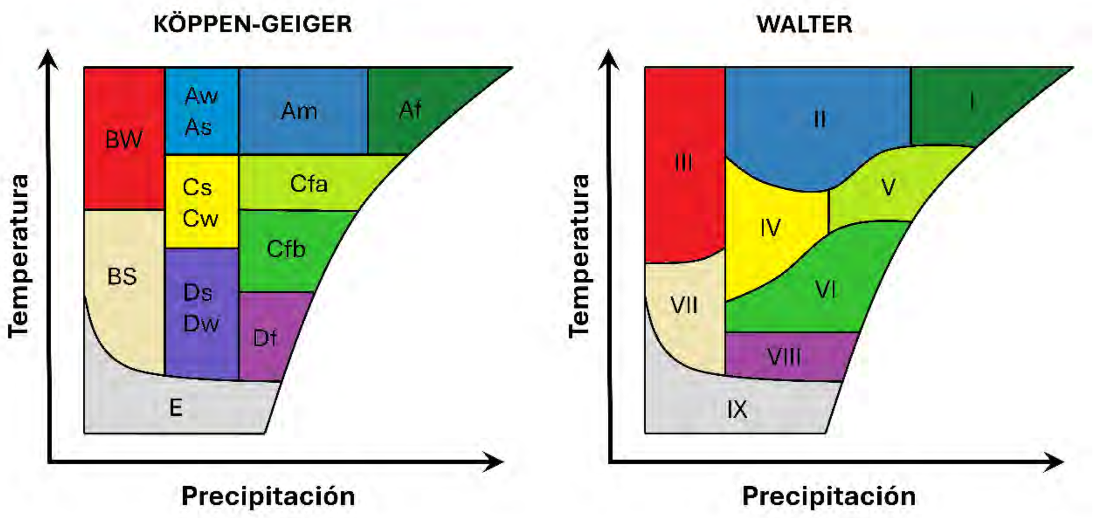

Figura 3. Síntesis comparativa entre los principales climas de la clasificaciones de Köppen-Geiger (Köppen y Geiger, 1936; izquierda) y los zonobiomas de Walter (Walter y Breckle, 2013; derecha) de acuerdo con gradientes de precipitación y temperatura. Se ha omitido la estacionalidad de las precipitaciones como tercer factor para definir los climas con el fin de facilitar su visualización.

Tomando como referencia la clasificación de Köppen-Geiger, que será usada posteriormente en el capítulo III, los climas se definen por una letra mayúscula que viene dada por la temperatura, desde los más cálidos (A) hasta los más fríos (E), con la excepción de la letra B que se reserva para los climas áridos (BS para estepas y BK para desiertos); una segunda letra minúscula que define el régimen de precipitaciones (s, xericidad estival; w, xericidad invernal; f, ausencia de xericidad) y una tercera letra que define la temperatura del verano (desde más cálido (a) a más fresco (d)). Esta variedad de climas de Köppen se puede sintetizar o resumir en una serie de macroclimas que, a su vez, se superponen ampliamente con los nueve zonobiomas de Walter (Walter y Breckle, 2013). El motivo de hacer referencia a varias clasificaciones climáticas es porque si bien todos describen a grandes rasgos las condiciones climáticas que ocupan las especies del género, ninguna clasificación las sintetiza a la perfección. Como se verá a continuación, en la mayoría de los casos los climas de ambas clasificaciones se superponen perfectamente; pero, en otras ocasiones, una clasificación se ve favorecida sobre la otra. Las diferencias entre ambas están resumidas a grandes rasgos en la figura 3.

Así, las especies de *Quercus* son un componente fundamental de los bosques templados caducifolios tanto del Viejo como del Nuevo mundo (Zonobioma VI de Walter; climas D, Cfb y Cfc de Köppen). Este clima se caracteriza por temperaturas frescas, con fuertes y/o recurrentes heladas en invierno, así como por la ausencia generalizada de un periodo seco a lo largo de todo el año. En el extremo opuesto se encontrarían las quercíneas capaces de habitar los semidesiertos en el suroeste de Estados Unidos y Norte de México (Zonobiomas III y VII de Walter; climas B de Köppen). Estas zonas áridas o estepas cálidas se caracterizan principalmente por una escasa precipitación anual que se traduce en un prolongado periodo de xericidad, y, en menor medida, una temperatura anual media relativamente alta. Las comunidades vegetales aquí presentes son principalmente arbustos formando vegetación dispersa similar a un chaparral o una sabana. En latitudes medias, las especies de *Quercus* también son un componente fundamental de dos climas templados con una marcada estación seca: por un lado, el clima mediterráneo (Zonobioma IV de Walter; climas Cs de Köppen) y; por otro, los climas templados de estación seca en invierno (climas Cw de Köppen). El clima mediterráneo, si bien no es el que más biodiversidad de especies de *Quercus* presenta (ca. 40), es uno de los que más diversidad induce en rasgos morfológicos y funcionales. La particularidad del clima mediterráneo radica en que el periodo térmicamente favorable coincide con el hidrológicamente desfavorable y viceversa. En el área de distribución de *Quercus*, este clima se da principalmente en California y en la cuenca mediterránea, y las formaciones vegetales mayoritarias son chaparrales, maquis y dehesas, siendo las especies de *Quercus* un componente dominante de las mismas.

Los climas templados de estación seca en invierno se dan sobre todo en zonas montañosas de México (Sierras Madre Oriental, Occidental y Eje Neovolcánico) y Asia (cordillera de los Himalayas y Montañas Hengduan). Este clima se caracteriza por la existencia de una marcada estacionalidad en los regímenes de temperatura y precipitación, pero donde coinciden en la misma época del año —generalmente verano— el máximo de ambas. A su vez, se da un periodo xérico de variable longitud a finales del invierno y/o comienzos de la primera. Las formaciones más frecuentes de este clima son los bosques de pino-encino madrenses, aunque en ciertos lugares pueden llegar a formar masas con dominancia única de *Quercus* (con un número variable de especies). Dada la posición latitudinal de este clima y la dependencia de la altitud para su existencia, los climas Cw de Köppen normalmente transicionan hacia climas tropicales (Climas Am, Aw o As de Köppen) conforme se pierde altitud; por este motivo, la clasificación de Walter en su zonobioma II es más precisa para describir estas comunidades de *Quercus* que están presentes tanto en climas Cw como A de Köppen. Por otro lado, el clima específico de Köppen Cfa, que sería un clima templado sin estación seca y con heladas débiles o ausentes, es un clima con un remarcable número de especies de *Quercus* en zonas del sur de China, sur de Japón y toda la zona que rodea el Golfo de México. A este clima Walter le da un zonobioma propio, el V y se podría identificar como un clima templado-cálido o subtropical, transicional entre un templado genuino y un tropical húmedo. Por último, algunas especies de *Quercus* son capaces de ocupar nichos en climas ecuatoriales (Zonobioma I de Walter; clima Af de Köppen). Se caracterizan por presentar una media elevada y constante tanto de temperatura como de precipitación a lo largo de todos los meses del año. Las quercíneas ecuatoriales estarían representadas por algunas especies de las secciones *Quercus* y *Lobatae* en Mesoamérica y Colombia, así como por miembros de la sección *Cyclobalanopsis* en el Sudeste Asiático, aunque no presenta una gran biodiversidad en comparación a otros climas. En estos climas las especies de *Quercus* suelen estar, de nuevo, ligadas a zonas montañosas.

Por último, cabe destacar como pueden existir especies capaces de habitar un amplio gradiente climático (eurioicas), especies intrínsecamente ligadas a un clima específico —ya sea por su limitado rango geográfico (estenócoras) o por su intolerancia a otro tipo de condiciones ambientales (estenoicas)— y especies cuyo principal nicho es transicional entre dos climas.

1.3. Adaptaciones funcionales a los climas

La gran heterogeneidad entre los bioclimas impone una serie de restricciones ambientales que terminan por condicionar las diferentes adaptaciones que tendrán que desarrollar las especies para poder ocupar los diversos hábitats. Los climas más fríos comprometen la funcionalidad de los individuos a través de las heladas. Las altas temperaturas de los climas cálidos pueden sobrecalentar las hojas, disminuyendo la tasa fotosintética y provocando daños celulares. Los climas con altas temperaturas son, además, más propensos a provocar sequías. En los climas más lluviosos, el exceso de agua puede suponer un problema para el intercambio de gases. La estacionalidad de las precipitaciones y la escasez de las mismas también afecta de manera directa a la disponibilidad de agua para las diferentes especies cuando más la necesitan.

1.3.1. Adaptaciones morfológicas

Las principales adaptaciones morfológicas se ven reflejadas a nivel foliar. Dado que las hojas son los principales órganos fotosintéticos de las plantas, cualquier modificación de la hoja influye directamente en la capacidad de supervivencia de esta a las diferentes condiciones ambientales. Todas las especies de *Quercus* presentan hojas simples, pero con un amplio rango de variación en su tamaño, forma, esclerofilia y longevidad; no solo a nivel de especie, sino también a nivel poblacional e incluso a nivel individual, dada la gran plasticidad de estas especies.

La superficie foliar en *Quercus* puede oscilar desde hojas nanófilas que apenas sobrepasan el centímetro cuadrado (p. ej., *Q. coccifera*) hasta macrófilos de más de 100 cm^2 (p. ej., *Q. pagoda*, *Q. velutina*). Así, se ha comprobado como las especies tienden a reducir la superficie foliar conforme el clima se vuelve más cálido, seco y/o luminoso (Peguero-Pina *et al.*, 2020; Nicotra *et al.*, 2011). Una menor superficie implica una reducción de la capa límite lo que facilita una mejor disipación del calor (Vogel, 1970). También se ha comprobado como la forma de la hoja responde a diferentes estreses ambientales. Por ejemplo, la aparición de hojas profundamente lobuladas surge como respuesta alternativa —y en algunos casos complementaria— a la reducción foliar en climas secos, como los semiáridos o el mediterráneo (p. ej., *Q. pyrenaica*, *Q. lobata*). Se ha demostrado como esta lobulación consigue mejorar la conductividad hidráulica de las hojas y su termorregulación (Sisó *et al.*, 2001; Sack y Tyree, 2005). En climas húmedos, por el contrario, el objetivo es deshacerse del exceso de agua que puede quedar en la lámina foliar, obstaculizando la fotosíntesis, el intercambio de gases o favoreciendo el desarrollo de hongos patógenos. Para ello, las hojas tienden a desarrollar formas elípticas con ápices acuminados (p. ej., *Q. hypargyrea*), cuya biofísica ayuda a evacuar el exceso de agua (Wang *et al.*, 2020).

Otros géneros de fagáceas son homogéneos en su fenología foliar, con todas sus especies perennifolias (p. ej., *Lithocarpus*) o todas sus especies caducifolias (p. ej., *Castanea*). Los pinos (género *Pinus*), que son el otro gran componente fundamental de los bosques del hemisferio norte, también cuentan con una perennifolia constante en todas sus especies. Las especies de *Quercus*, por el contrario, presentan una amplia diversidad en su fenología, que muestra un gradiente continuo desde especies caducifolias con una esperanza de vida de cinco meses hasta perennifolias con una esperanza de vida de más de tres años (Mediavilla y Escudero, 2003; Wright *et al*., 2004; Harayama *et al*., 2016). Las especies caducifolias se corresponden mayoritariamente con miembros de las secciones *Lobatae* y *Quercus* que aparecen formando los bosques templados en América, Europa y Asia. En cambio, las especies perennifolias más longevas están en climas Mediterráneos (representados por las secciones *Protobalanus* e *Ilex*) y en climas tropicales del sudeste asiático (Sect. *Cyclobalanopsis*). Por último, hay especies brevideciduas o perennifolias cuya esperanza de vida es igual o ligeramente superior a un año, que están representadas por algunas especies de los clados mexicanos y californianos de las secciones *Lobatae* y *Quercus*, así como especies de sección *Virentes* en climas subtropicales del golfo de México y algunas especies de la sección *Cerris*. La caducifolia ayudaría a las especies a lidiar con las intensas y constantes heladas invernales a través de una estrategia evasiva del frío. La perennifolia, por el contrario, se suele encontrar en climas menos estresantes desde un punto de vista térmico, con la excepción del clima mediterráneo y los climas tropicales a gran altura, donde pueden darse heladas invernales. El hecho de tener hojas perennes ayudaría a las especies a extender su periodo fotosintéticamente activo hacia la primavera y el otoño, cuando las especies caducifolias no tienen hojas; a cambio, deberán desarrollar estrategias que les ayuden a soportar los periodos estresantes que se puedan encontrar a lo largo de todo el año (García-Plazaola *et al*., 1999; Corcuera *et al*., 2005).

La esclerofilia, tradicionalmente entendida como hojas con apariencia coriácea, dura y firme, se basa en apreciaciones inconmensurables. No obstante, han sido numerosos los estudios que han tratado de cuantificar la esclerofilia mediante la creación de índices (Loveless, 1961; 1962) o correlacionándola con otras variables, siendo la más utilizada comúnmente el peso seco por unidad de superficie, conocido por sus siglas en inglés como LMA (*Leaf Mass per Area*) (Reich *et al*., 1992; Niinemets, 2001; Alonso-Forn *et al*., 2020). El rango de variación que presentan las especies de *Quercus* en términos de LMA va desde especies malacófilas, o de “hojas blandas”, que están representadas principalmente por los caducifolios de bosques templados (p. ej., *Q. robur*) hasta especies esclerófilas, o de “hojas duras”, cuyo extremo está representado por las perennifolias Mediterráneas (p. ej., *Q. rotundifolia*). La producción de hojas con mayor dureza está intrínsicamente relacionada con el espectro económico foliar, conocido por sus siglas en inglés LES (*Leaf Economic Spectrum*). El LES postula que las hojas que invierten una mayor cantidad de recursos y energía en la producción de sus hojas deben vivir durante el tiempo suficiente como para, al menos, recuperar lo invertido (Wright *et al.*, 2004). El LMA se puede incrementar aumentando cualquiera de sus componentes, que son la densidad y el grosor de la hoja (Sancho-Knapik *et al.*, 2021). Aumentar los valores de LMA; es decir, la dureza, ya sea vía grosor o densidad, implica una mayor inversión de recursos por parte de la planta, por lo que esa hoja esclerófila debería ser fotosintéticamente activa durante el tiempo suficiente como para compensar la inversión en la fabricación de esta. El aumento en LMA es en sí mismo una defensa física frente a estreses tanto bióticos como abióticos, que se traduce en una correlación con una esperanza de vida más larga. Por un lado, una mayor dureza se ha relacionado con una mayor resistencia a la herbivoría, al hacer las hojas menos atractivas por su mayor cantidad de fibras (es decir, paredes celulares) (Kitajima *et al.*, 2012). Por otro lado, una inversión en paredes celulares más gruesas (incremento de LMA vía aumento de la densidad) se ha relacionado con una mayor resistencia a la citórrisis causada por el estrés hídrico (Oertli, 1986).

1.3.2. Adaptaciones fisiológicas

Las dos funcionen más comprometidas por los estreses ambientales —principalmente sequía y frío— son la fotosíntesis y el transporte de agua y nutrientes. Tanto la sequía como el frío pueden provocar la pérdida total de la funcionalidad del xilema (cavitación), siendo este fallo hidráulico la principal causa de mortalidad de las plantas por estreses abióticos (Tyree y Sperry, 1989). Como todas las angiospermas, las especies de *Quercus* presentan traqueidas y elementos de los vasos en su xilema para transportar agua desde las raíces hasta las hojas. De acuerdo con la ley de Hagen-Poiseuille, los elementos de los vasos son el principal contribuyente a la conductividad hidráulica dado su mayor diámetro (Tyree y Zimmermann, 2002). Sin embargo, por este mismo motivo, los vasos son, a su vez, los componentes del xilema más susceptibles a sufrir cavitación (Hacke *et al.*, 2000; Tyree, 2003).

El mecanismo que conduce a la cavitación es diferente según el estrés ambiental. La sequía causa una tensión negativa en el xilema que aumenta progresivamente conforme hay menos disponibilidad de agua en el suelo. Para que un conducto funcional cavite depende de que esté en contacto con otro conducto no funcional, esto es, lleno de aire a presión atmosférica (embolizado). La tensión negativa dentro del conducto funcional succiona el aire del conducto embolizado a través de las punteaduras hasta un punto donde se forma una burbuja que termina por llenar el vaso, primero, de vapor de agua, y finalmente, de aire a presión atmosférica (Tyree y Sperry, 1989). La resistencia a la cavitación se suele expresar como la presión a la cual se pierde la mitad de la conductividad hidráulica del xilema (P_{50}), siendo más resistente la especie cuanto más negativo sea dicho valor. El P_{50} dependerá de factores como son el tamaño del conducto, el grosor de sus paredes celulares o el número y tamaño de las punteaduras intervasculares (Hacke y Sperry, 2001). Otro factor que afecta a la vulnerabilidad a la cavitación es la hipótesis de las punteaduras raras (Wheeler *et al.*, 2005). Esta hipótesis defiende que la embolia en el xilema se inicia en las punteaduras que tienen defectos estructurales o irregularidades, lo que las hace puntos vulnerables al colapso hidráulico. El factor clave relacionado con el frío que condiciona la funcionalidad del xilema son los ciclos de hielo-deshielo (Cochard y Tyree, 1990; Sperry y Sullivan, 1992). Estos ciclos son oscilaciones térmicas entre -2 ºC y +2 ºC que provocan que el agua en el interior del xilema se congele y descongele repetidas veces. Cuando el agua se congela, el aire disuelto en ella forma burbujas de aire que quedan atrapadas entre el hielo, que, al descongelarse, pueden no redisolverse en la fase líquida del agua y cavitar el conducto. A mayor repetición de dichos ciclos aumenta la probabilidad de que los conductos terminen cavitando. Así, la cavitación por frío es endógena, a diferencia de la cavitación por sequía.

En las diferentes especies del género se pueden encontrar una amplia gama de valores medios de P_{50}, desde especies excepcionalmente resistentes a la cavitación a especies muy vulnerables (Choat *et al.*, 2012). Las especies con un P_{50} más negativo suelen coincidir con especies perennifolias (o poblaciones) que ocupan los climas más estresantes, como el mediterráneo o los semidesiertos. En cambio, las especies caducifolias, por lo general, presentan valores menos negativos de P_{50} ya que suelen ocupar climas sin xericidad y evitan la posible cavitación del xilema de sus hojas por frío mediante la caída de éstas. Un ejemplo que merece ser destacado es el quejigo (*Q. faginea*, y su híbrido *Q. subpyrenaica*), una especie caducifolia en clima mediterráneo, que vive frecuentemente en simpatría con la encina (*Q. rotundifolia*), una especie perennifolia altamente resistente a la cavitación. El quejigo ha conseguido desarrollar una segmentación de la vulnerabilidad, donde la hoja actúa como un "fusible hidráulico", cayéndose en condiciones especialmente secas para salvaguardar la funcionalidad del tallo (Peguero-Pina *et al.*, 2015; Peguero-Pina *et al.*, 2016; Alonso-Forn *et al.*, 2021). Finalmente, cabe destacar como la vulnerabilidad del xilema es plástica y no debe ser entendida como un valor exacto específico de cada especie o incluso de cada órgano, pudiéndose encontrar un amplio rango de valores de P_{50} en diferentes poblaciones de una misma especie como respuesta a las condiciones climática locales (Cochard *et al.*, 1997).

Aparte de la sequía en el suelo, representada por un bajo potencial hídrico del mismo (Ψ_s), el aire también puede generar condiciones estresantes para una planta cuando la temperatura es alta y la humedad relativa baja, lo cual acaba derivando en un alto déficit de vapor de agua (*vapor pressure deficit*, VPD) entre la hoja y el aire. Ambas condiciones inducen por sí mismas el cierre estomático mediado por el ácido abscísico. Además, cuando las sequías edáfica y atmosférica se dan simultáneamente —que suele ser lo más frecuente— tienen un efecto sinérgico haciendo que la planta cierre sus estomas incluso antes de lo que lo harían para cada uno de los estreses por separado (Sancho-Knapik *et al.*, 2022). El valor a partir del cual se produce el cierre de estomas determinará si una especie es isohídrica o anisohídrica, correspondiendo la primera estrategia con especies que cierran sus estomas cuando el potencial hídrico o el VPD alcanza niveles moderados para evitar la deshidratación, y la segunda con especies que mantienen sus estomas abiertos incluso a potenciales hídricos más bajos o mayores VPD, tolerando más estrés hídrico antes de cerrarlos (Tardieu y Simonneau, 1998). Así, especies como la encina, han demostrado seguir una estrategia conservadora del agua, cerrando sus estomas a aproximadamente 2-3 kPa de VPD y reduciendo consecuentemente tanto la conductancia estomática como la fotosíntesis (Sancho-Knapik *et al.*, 2022). El quejigo, en cambio, mantiene tasas muy altas de conductancia estomática para niveles similares de estrés edáfico y atmosférico, lo cual le permite mantener tasas fotosintéticas mayores además de ayudar a mantener la hoja refrigerada (Alonso-Forn *et al.*, 2021).

En condiciones normales, cuando la planta está realizando la fotosíntesis, la energía de la luz captada por las clorofilas del fotosistema II (PSII) es usada para romper moléculas de agua y liberar electrones que entrarán a cadena de transporte de electrones y generarán NADPH y ATP. Cuando la planta no reúne las condiciones necesarias para mantener una tasa fotosintética normal y ésta es baja o nula —ya sea causada por un cierre estomático o por unas temperaturas excesivamente bajas—, existe un exceso de energía lumínica que no puede ser destinada a procesos fotosintéticos. Para evitar el posible daño que causaría este exceso de radiación al PSII, las plantas han desarrollado mecanismos de fotoprotección como son la fotorespiración, el ciclo de las xantofilas (Ciclo VAZ) o el ciclo de la luteína epóxido (Lx) (García-Plazaola *et al.*, 2017). Estas dos últimas rutas se encargan de modular la reconversión de la clorofila excitada a clorofila fundamental a través de la disipación de calor de ese exceso de energía, evitando así la formación de radicales libres de oxígeno (ROS) que podrían producir daños moleculares. Este proceso se conoce como quenching no fotoquímico (NPQ) y ha sido descrito en numerosas especies de *Quercus* como respuesta a diversos estreses como son: el exceso de luz, la sequía estival o las bajas temperaturas invernales. A su vez, se ha comprobado como las especies perennifolias que tienen que soportar inviernos fríos —como las especies mediterráneas— tienen una mayor capacidad de fotoprotección que las especies caducifolias o las tropicales (García-Plazaola *et al.*, 2017 y referencias en el mismo).

Por último, otra forma de evitar el exceso de radiación es mediante mecanismos que reduzcan la absorción lumínica, que se pueden agrupar en cambios morfoanatómicos y cambios en la reflectancia foliar. Entre los primeros, se han reportado como las hojas de *Q. coccifera*, por ejemplo, más expuestas al sol son capaces de modular su ángulo para recibir menos radiación (Rubio de Casas *et al.*, 2007). También se ha observado como el enrollamiento de las hojas —que reduce el área efectiva de intercepción de la luz— es común entre las especies mediterráneas de *Quercus* (Niinemets, 2007). Los cambios en la reflectancia pueden ser conseguidos mediante la presencia de tricomas (pubescencia) o la presencia de ceras (glaucescencia), dos caracteres foliares muy variables a lo largo de todas las especies del género *Quercus*.

1.3.3. Adaptaciones ecológicas

Dentro de un mismo clima, las especies de *Quercus* pueden ocupar una amplia gama de hábitats, incluyendo llanuras, riberas, zonas costeras y de alta montaña (Williams *et al.*, 1999; Valencia, 2004; Cavender-Bares, 2019). El éxito de la amplia distribución del género radica en una variedad de estrategias ecológicas que siguen las diferentes especies. En primer lugar, un gran número de especies son grandes árboles que dominan el dosel arbóreo, desde masas monoespecíficas a bosques mixtos, ya sea con otras quercíneas o con otros géneros. No obstante, un número similar de especies son arbustos o pequeños árboles que, o bien conforman el sotobosque, o bien son formadoras de comunidades vegetales tipo matorral, maquis, garriga o chaparral (Nixon, 2006). A su vez, se pueden encontrar desde especies generalistas, indiferentes edáficas o eurioicas hasta especialistas que ocupan condiciones edáficas muy concretas, como suelos de serpentina (p. ej., *Q. euboica*), hidromorfos (p. ej., *Q. bicolor*), arenosos (p. ej., *Q. geminata*), con un rango de pH específico (p. ej., *Q. pyrenaica*) o incluso endemismos insulares (p. ej., *Q. alnifolia*) (Rogers, 1990; Papageorgiou, 1997; Nixon, 2006; Cavender-Bares, 2019).

Cabe destacar que en torno a dos tercios de las especies están intrínsecamente relacionadas a hábitats montañosos, especialmente en latitudes tropicales y subtropicales tanto del Viejo Mundo (Montañas Hengduan, Himalayas, etc.) como del Nuevo Mundo (Sierras Madre Oriental y Occidental, Eje Neovolcánico, etc.) (Valencia, 2004; Le Hardÿ y Lamant, 2010). El relieve heterogéneo favorece la aparición de diferentes nichos que resulta en la coexistencia de numerosas especies en una zona geográfica. De hecho, en el caso de los Himalayas, la colonización y especiación de las especies de *Quercus* acompañó temporal y espacialmente a la orogenia de dicha cadena montañosa (Zhou *et al*., 2007; Meng *et al*., 2017). Así, según los requerimientos de cada especie, se pueden encontrar diferentes comunidades de quercíneas en zonas de sotavento (especies xéricas) o barlovento (especies mésicas). Además, las especies se reparten en los diferentes pisos altitudinales, desde tierras bajas a nivel del mar hasta el límite de vegetación arbórea a más de 4000 metros sobre el nivel del mar (en el caso de *Q. semecarpifolia* en los Himalayas) (Yang *et al*., 2020). Por ejemplo, Kappelle *et al*. (1992) observó como en Costa Rica, las especies de *Quercus* eran más diversas y dominantes en la seca vertiente pacífica en comparación con la húmeda vertiente atlántica, con la excepción de las mayores altitudes, donde las comunidades eran similares. Esta diferencia en la zonación parece estar más relacionada con la estacionalidad de las precipitaciones que con la cantidad de precipitación total, ya que conforme uno viaja hacia el norte —en el sur de México— estas diferencias parecen desvanecerse y se pueden encontrar las mismas especies en ambas vertientes a bajas altitudes.

1.4. Adaptaciones para hacer frente a la herbivoría

La herbivoría es un tipo de estrés biótico que termina por afectar a la producción primaria de un ecosistema al consumir parcial o totalmente los tejidos fotosintéticos de un organismo vegetal. Las plantas, por su parte, han desarrollado una batería de estrategias que se basan en reducir los niveles de herbivoría (resistencia) o, en caso de darse, minimizar su impacto sobre la aptitud biológica (*fitness*) de los vegetales (tolerancia). Las estrategias de resistencia se pueden dividir, a su vez, en evasivas o de antibiosis, según si afectan a la preferencia del herbívoro o a la actuación de éste, respectivamente (Tiffin, 2000). Por último, los mecanismos de antibiosis pueden ser de naturaleza física (mecánica) o química. Todas estas interacciones planta-herbívoro se engloban dentro de la categoría de defensas directas; no obstante, cabe destacar la existencia de mecanismos de defensa indirectos. Un tipo de defensa indirecta sería la producción de compuestos orgánicos volátiles (VOCs, por sus siglas en inglés), que atraería a depredadores naturales de los herbívoros (Dicke y Baldwin, 2010; Lehrman *et al.*, 2013).

Un ejemplo de defensa directa relacionada con la tolerancia es la tasa de crecimiento relativo, conocido por sus siglas en inglés RGR (*Relative Growth Rate*), el cual está directamente relacionado con la disponibilidad de recursos (luz, agua y nutrientes) de un hábitat. Así, las especies colonizadoras de claros, de rápido crecimiento, toleran mejor la herbivoría, al compensar las partes perdidas con la rápida regeneración de las mismas. Por el contrario, las especies tolerantes a la sombra, de crecimiento más lento presentarían mayores daños derivados de la herbivoría. Además, para un mismo nivel de herbivoría, el impacto sobre el *fitness* sería mayor en las especies de sombra que en las de sol (Gianoli y Salgado-Luarte, 2017).

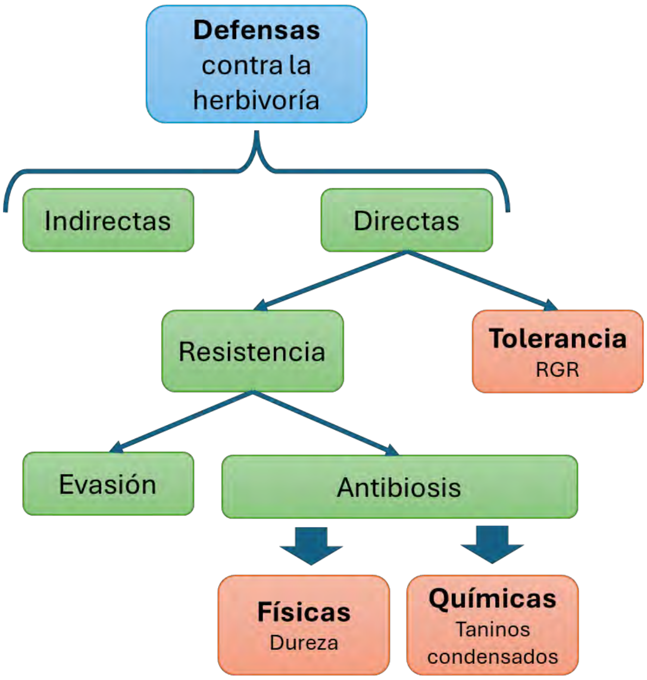

Figura 4. Resumen esquemático de los principales mecanismos defensivos en plantas. En naranja se señalan las categorías que serán estudiadas en el capítulo V.

Las especies del género *Quercus* presentan una gran diversidad de estrategias para defenderse contra los herbívoros, siendo las más estudiadas los mecanismos de resistencia. Así, se ha reportado como individuos de *Q. robur* atacados por el piral del roble (*Tortrix viridana*) —un lepidóptero cuya fase larvaria produce defoliación en varias especies de *Quercus* europeas—, produce compuestos volátiles inducidos por la herbivoría. Estos volátiles hacen de efecto llamada para atraer aún más herbívoros. Ghirardo *et al*. (2012), comprobaron como individuos que producen menos cantidad de estos compuestos presentan menores niveles de herbivoría, representando un claro ejemplo de mecanismos evasivos. Entre los mecanismos de defensa física destacan la producción de tricomas (Karioti *et al*. 2011), el desarrollo de espinescencia (Choong, 1996) o el incremento de la dureza de la hoja (Alonso-Forn *et al*., 2020). Teniendo en cuenta que la dureza está intrínsecamente correlacionada con el LMA y el hábito foliar, encontramos como las especies perennifolias y esclerófilas presentan menores niveles de herbivoría en comparación a las especies caducifolias y/o malacófilas (Mediavilla *et al*., 2018; Alonso-Forn *et al*., 2020). Respecto a las defensas químicas, son numerosos los compuestos que han sido asociados a la disuasión de los herbívoros: fenoles, alcaloides, terpenoides o flavonoides, principalmente (Moreira *et al*., 2018). Por ejemplo, se ha encontrado una relación entre la producción de antocianinas y la protección frente a hojas jóvenes en la coscoja (*Q. coccifera*) (Karageorgou y Manetas, 2006). Por último, Mediavilla y colaboradores (2018) encontraron como hay una compensación entre defensas físicas y químicas, la cual está relacionada con la ontogenia de las hojas. Así, las hojas apostarían por invertir preferentemente en defensas químicas mientras se produce su desarrollo y, una vez maduras, las defensas físicas serían la principal barrera contra los herbívoros.

1.5. Objetivos y estructura de la tesis

1.5.1. Objetivos

El principal objetivo de esta tesis es estudiar y evaluar las diferentes restricciones ambientales que condicionan la funcionalidad en diferentes especies de *Quercus*. Para ello se han establecido una serie de objetivos específicos que se detallan a continuación:

i) Elaborar un sistema de estudio que incluya un clima con diferentes restricciones ambientales (clima mediterráneo) y una especie perennifolia de amplia distribución (*Q. ilex*) que tenga que soportar dichas restricciones. Evaluar y discutir en dicho sistema qué estreses son los que más comprometen la funcionalidad de la especie estudiada.

ii) Estudiar en un diverso set de especies, que intenten representar la mayor cantidad posible de grupos filogenéticos y climas ocupados por *Quercus*, como la morfología foliar es modulada por las diferentes condiciones climáticas de los hábitats; así como discutir la ventajas adaptativas que representan dichas modificaciones.

iii) Analizar como la anatomía de los tejidos conductores del peciolo escalan proporcionalmente a las demandas fisiológicas de la hoja en un variado set de especies caducifolias y perennifolias de *Quercus* que ocupan diferentes climas.

iv) Evaluar las diferentes estrategias posibles para hacer frente a la herbivoría en varias especies de *Quercus* abarcando todo el rango de esclerofilia del género.

1.5.2. Estructura de la tesis

La presente tesis consta de seis capítulos: La introducción general (**Capítulo I**), una revisión bibliográfica que incorpora metaanálisis (**Capítulo II**), tres artículos de investigación (**Capítulos III**, **IV** y **V**), y la discusión general junto con las conclusiones (**Capítulo VI**). Todos los capítulos de investigación han sido publicados en revistas internacionales indexadas en *Journal Citation Reports* (JCR) bajo las categorías *Forestry* y *Plant Science*.

El **capítulo II**, titulado "*Summer y winter can equally stress holm oak (Quercus ilex L.) in Mediterranean areas: A physiological view*" ha sido publicado en la revista *Flora* (Q2 en *Plant Science*) en 2022. Este trabajo aborda el primer objetivo específico haciendo una revisión bibliográfica de numerosos trabajos acerca de cómo los diferentes estreses ambientales condicionan la funcionalidad de la encina (*Q. ilex*). Como especie perennifolia que vive en un clima con marcados periodos de estreses térmicos e hídricos, la encina ha tenido que desarrollar una serie de adaptaciones fisiológicas que le permitan ocupar dichos hábitats. Además, se incorporan datos nuevos que, junto con un metaanálisis de datos ya publicados en otros artículos, se concluye que se pueden apreciar diferencias significativas entre las dos subespecies de encina, siendo *Q. ilex* subsp. *ballota* más resistente tanto a la sequía como al frío en comparación a la subespecie ligeramente más mésica *Q. ilex* subp. *ilex*.

El **capítulo III**, titulado "*Oak leaf morphology may be more strongly shaped by climate than by phylogeny*" ha sido publicado en la revista *Annals of Forest Science* (Q1 en *Forestry*) en 2024. Este capítulo se enfoca en el segundo objetivo específico, analizando diversos parámetros de la morfología foliar en 141 especies de *Quercus*. En la correlación de dichas variables con el clima, y teniendo en cuenta la filogenia de las especies, se puede apreciar como, a grandes rasgos, la morfología responde generando síndromes foliares a los diferentes macroclimas en los que habitan las especies. Con la excepción del LMA, los parámetros morfológicos están más modulados por el clima que por la filogenia.

El **capítulo IV**, titulado "*Xylem y phloem in petioles are coordinated with leaf gas exchange in oaks with contrasting anatomical strategies depending on leaf habit*" ha sido publicado en la revista *Plant Cell y Environment* (D1 en *Plant Science*) en 2024. En este estudio se explora la anatomía del peciolo en diversas especies de *Quercus*. Como único nexo entre la hoja y el resto de la planta, el peciolo debe presentar una anatomía de sus tejidos conductores (xilema y floema) acorde a las necesidades de la lámina foliar, tanto de importación de agua para la transpiración como de exportación de azúcares procedentes de la fotosíntesis. Se cumpliría así con el tercer objetivo específico al demostrar como caducifolios y perennifolios siguen diferentes estrategias a la hora de producir y empaquetar sus conductos.

El **capítulo V**, titulado "*Trade-offs among leaf toughness, constitutive chemical defense, y growth rates in oaks are influenced by the level of leaf mass per area*" ha sido publicado en la revista *Annals of Forest Science* (Q1 en *Forestry*) en 2023. Este capítulo aborda el cuarto y último objetivo específico. Se analiza la contribución relativa de las tres principales defensas contra la herbivoría en hojas: la dureza, la producción de compuestos químicos disuasorios y la tasa de crecimiento. Se observa como las especies apuestan principalmente por una sola estrategia, siendo endurecer las hojas la preferida por las esclerófilas, mientras que las especies malacófilas optan por una de las otras dos estrategias.

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Capítulo II

Summer and winter can equally stress holm oak (*Quercus ilex* L.) in Mediterranean areas: A physiological view

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Summer and winter can equally stress holm oak (*Quercus ilex* L.) in Mediterranean areas: A physiological view

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ABSTRACT

Q. ilex is a circum-Mediterranean species which must deal with two stressful periods throughout the year: summer and winter. However, the intensity of these abiotic stresses is very variable depending on the specific area of its distribution range. Besides, two subspecies are usually distinguished, even sometimes recognised as two different true species. Nevertheless, differences in the response to both summer and winter stresses among populations overstep the purely taxonomic question. Regarding to temperature, this species has shown a high resistance to both extremes. On the one hand, critical temperature at leaf level has been estimated in 50.2 °C for a provenance in the Iberian Peninsula (Soria, Spain), a temperature which even in the hottest areas of its distribution range is hardly ever reached. On the other hand, minimal temperature for the survival of the leaves has been estimated in −19.8 °C for Italian provenances and -26.6 °C for one of the coldest provenances (Soria, Spain). Similarly, these temperatures are quite rarely reachable in their respective provenances. Concerning to summer-drought, this may compromise xylem stability in severe cases of scarcity of water in the soil, inducing cavitation when water soil potential is too negative. In this sense, *Q. ilex* has demonstrated to have a high resistance to cavitation, represented by quite negative values of P_{50} and P_{88} when compared to other species. Besides, Iberian provenances (i.e. the most continental ones) have shown a higher resistance to cavitation than French or Italian ones. Likewise, holm oak may suffer cavitation during winter, but in this case by a completely different factor: freezing-thawing cycles. Winter-cavitation seems to be related to vessel size, with wider vessels being more vulnerable to cavitate than narrower ones. In spite of its paleotropical origin, *Q. ilex* has achieved to develop multiple adaptations and physiological strategies that has enabled it not only to withstand the hard climatic conditions imposed by Mediterranean climate, but to colonize and even dominate great part of the Mediterranean landscape.

1. Introduction

As a result of its geological history and its current geographical situation between 30° and 40° latitude, the Mediterranean Basin has been recognized to have its own climate, the Mediterranean one, which is shared with only other four and less-extensive areas at similar latitudes in both hemispheres. This genuine climate is characterized by dry summers and cold or mild moist winters (Walter, 1985; Lionello et al., 2006). Consequently, the vegetative period is mainly split into two seasons: spring and autumn, since plant growth is constrained by drought during summer and low temperatures during winter (Mitrakos, 1980). This particularity has led to consider that the Mediterranean climate presents a highly adapted vegetation type. In this respect, Schimper (1903), who firstly synthesized the worldwide geobotanical information in his book “Plant-geography upon a physiological basis”, stated that “the mild temperature districts with winter rain and prolonged summer drought are the home of evergreen xerophilous woody plants, which, owing to the stiffness of their thick, leathery leaves, may be termed as sclerophyllous woody plants”. Later, Walter (1985) summarized the climates in the world and assigned to the Mediterranean

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region its own Zonobiome: the Zonobiome IV or "Zonobiome of Sclerophyllic Woodlands". In this regard, Breckle (2002) claimed that the original zonal vegetation of this Zonobiome was evergreen sclerophyllous forest and woodlands with *Q. ilex*.

Holm oak is widely considered a circum-Mediterranean tree species that covers a large geographic range in the Mediterranean Basin, from the Iberian Peninsula and northwestern Africa to Greece and northern Anatolian Peninsula. Besides, there are remarkable differences in the altitude that this species occupies within the Mediterranean Basin, from coastal forests of *Q. ilex* in the Italian part of Adriatic Sea and Tyrrhenian Sea (Gratani, 1997), the Turkish Black Sea coast or the southeastern Mediterranean coast of France up to 2500–2800 m a.s.l (metres above sea level) in the Moroccan western part of High Atlas (Barbero et al., 1992). For these reasons, holm oak can be found in very different habitats with a quite ample range of rainfall regime, from semiarid to perhumid (Niinemets, 2015). However, there is no doubt that this species has its optimum in the western Mediterranean, and it begins to lose importance as we approach to Middle East, where holm oak is often substituted by *Quercus calliprinos* Webb in coastal and milder habitats (Barbero et al., 1992). In the transitional areas, towards other climatic regimes, the presence of *Q. ilex* can be seriously restricted due to the strong competition with other species, many of them congeneric ones. Thus, when both aridity and mean annual temperature decreases (i.e. the transition to Zonobiome VI or "Zonobiome of Deciduous Forests"), *Q. ilex* would be substituted by winter deciduous submediterranean *Quercus* species such as *Quercus faginea* Lam. and *Quercus pyrenaica* Willd. in Western Mediterranean, or *Quercus cerris* L. and *Quercus frainetto* Ten. in the Eastern Mediterranean. Furthermore, when summer drought disappears as far as temperate climates are reached, forests are dominated by species like *Quercus robur* L., *Quercus petraea* (Matt.) Liebl. or *Fagus sylvatica* L. On the other hand, holm oak can also be restricted by the increase in climatic hardness. In this sense, *Quercus coccifera* L. substitutes *Q. ilex* in the transition towards Zonobiome III or "Zonobiome of Hot Deserts", where deserts and semi-arid areas are found. And, finally, when the mean annual temperature decreases but the average rainfall keeps similar (i.e. towards the Zonobiome VII or "Zonobiome of Steppes and Cold Deserts"), it can be assumed that only one of the recognized subspecies, *Q. ilex* subsp. *ballota* (Desf.) Samp., that has been also considered a separate species (*Quercus rotundifolia* Lam. Encycl. 1: 723 (1785)) can withstand the cold conditions of the innermost part of the Iberian Peninsula. In the eastern part of its distribution, and under the same circumstances, holm oak would be substituted by *Quercus baloot* Griff. (Gil-Pelegrín et al., 2017).

Therefore, it is reasonable to identify *Q. ilex* as a paradigm species of the Mediterranean climate. However, is *Q. ilex* a truly Mediterranean species originated under Mediterranean conditions? Or, conversely, is it an older taxon that presents pre-adaptations to Mediterranean climate? Genus *Quercus* is currently divided into eight sections grouped in two subgenera: subgenus *Quercus* and subgenus *Cerris*, the latter comprising the three most ancient sections, e.g. section *Ilex,* to which *Q. ilex* belongs (Jiang et al., 2019). Section *Ilex* was originated in Asia, as suggested by the existence of macrofossils with a clear affinity to species of this group, being the oldest one dated approximately 34 Ma (early Oligocene) (Su et al., 2019a). Phylogenetic analyses supported this fact and estimated the divergence of sections *Ilex* and *Cerris* some million years before, during Eocene (ca. 48 Ma) (Jiang et al., 2019). For that epoch, ancestor of section *Ilex* species occupied tropical and subtropical forests in the Himalaya-Hengduan mountains. The clade of Mediterranean species diverged during Oligocene ca. 28 Ma ago and migrated via a humid, forested Tibet-Himalaya corridor (Jiang et al., 2019).

This migration continued during Miocene, and the convulse environmental changes that Europe underwent during Neogene were likely to facilitate both speciation and colonization of Europe (Jiang et al., 2019). This fact is supported by fossil evidence of *Quercus drymeja* Unger and *Quercus mediterranea* Unger, morphotype complexes of several fossil taxa that resemble the extant Himalayan, East Asian, and Southeast Asian species of *Quercus* section *Ilex*, which may indicate subtropical, relatively humid conditions (He et al., 2014; Denk et al., 2017) that were maintained up to middle-Miocene. In Himalaya-Hengduan mountains, during Pliocene until present, these humid tropical and subtropical conditions have remained, and despite that, some species exhibit xeromorphic-like traits as is evidenced by fossil records such as *Quercus yangyiensis* He, suggesting that xeromorphic traits do not necessarily imply dry o xeric conditions (He et al., 2014). Whereas in the Mediterranean region, during the middle and late-Miocene, a progressive decrease in rainfall and variation in temperature started due to tectonic movements (e.g., closure of communication between Mediterranean Sea and Indian Ocean) and glaciations (Pons et al., 1995). By 14 Ma ago, all tropical elements in the north-western Mediterranean Basin disappeared and a seasonally contrasted temperature regime was initiated (Suc, 1989). Although the dryness seems to be characteristic of Mediterranean Basin since Miocene, it was not until Pliocene (ca. 3.2 Ma ago) when temperature regimes dropped once again (Suc, 1984) and induced drier conditions, establishing the thermally contrasted seasons throughout this area. This phenomenon can be considered the origin of the current Mediterranean climate (Pons et al., 1995). These climatic changes are supported by palynological evidences: *Quercus* species became predominant from 3.2 Ma onwards and other xerophytic taxa became more frequent (*Phillyrea, Olea, Cistus, Pistacia*) (Suc, 1984).

Therefore, according to the evolutionary history of *Q. ilex* and the *Ilex* section it belongs to, we can finally conclude that this species was not originated under Mediterranean conditions, but in tropical and subtropical forests in Asia under humid conditions with the absence of a summer-dry season. This fact contrasts with the palaeobotanical origin of *Q. faginea*, an oak species of the *Quercus* group co-occurring with *Q. ilex* in the inland areas of the Iberian Peninsula, whose fossil records coincided with the development of the Mediterranean seasonality during the Pliocene (Roiron, 1983). Interestingly, other Himalayan-Hengduan oak species of the *Ilex* group have parallel-developed xeromorphic traits quite similar to Mediterranean species, especially in terms of sclerophylly (Alonso-Forn et al., 2020). This fact could be associated with the existence of a period in the Himalayas during the pre-monsoon months (March to May) with scarce rainfall and increased temperatures that can potentially induce water stress in these plant species (Singh and Zobel, 1995; Shrestha et al., 2007). In fact, several sclerophyllous oak species occurring in this region showed the minimum water potential values during this period (Singh et al., 2000; Poudyal et al., 2004; Poudyal, 2013). This fact leads to think that *Ilex* section presents pre-adaptations to cope with drought that have subsequently been developed by natural selection in different parts of the world in response to similar climatic conditions, i.e. the existence of a hot-dry season.

Regardless of its palaeobotanical origin, another issue that should not go unnoticed is that holm oak currently shows a huge morphological and ecophysiological variability (Peguero-Pina et al., 2014). According to the leaf morphology, botanists usually distinguish two subspecies of holm oak, *Q. ilex* subsp. *ballota* (= *Q. rotundifolia* Lam.) and *Q. ilex* subsp. *ilex* L. On one hand, *Q. ilex* subsp. *ilex*, which presents larger and elongated leaves, is restricted to the milder, coastal and humid or subhumid areas of the northern and eastern Mediterranean region. On the other hand, *Q. ilex* subsp. *ballota*, which has smaller and thicker rounded leaves than *Q. ilex* subsp. *ilex*, occupies the driest and most continental parts of the western Mediterranean region, especially in the inner areas of the Iberian Peninsula. Thus, the analysis of the ecophysiological variability of this species is a matter of special interest that will be discussed hereunder, as both subspecies live under different climatic conditions but must cope with two potentially stressful periods throughout the year, i.e. summer drought and winter cold. Specifically, throughout this review, we will describe how holm oak is affected by both stressful periods, underscoring the mechanisms developed by this species to cope with them, mainly in terms of i/ resistance of the xylem to cavitation and ii/ tolerance to drought and extreme temperatures by photoprotection

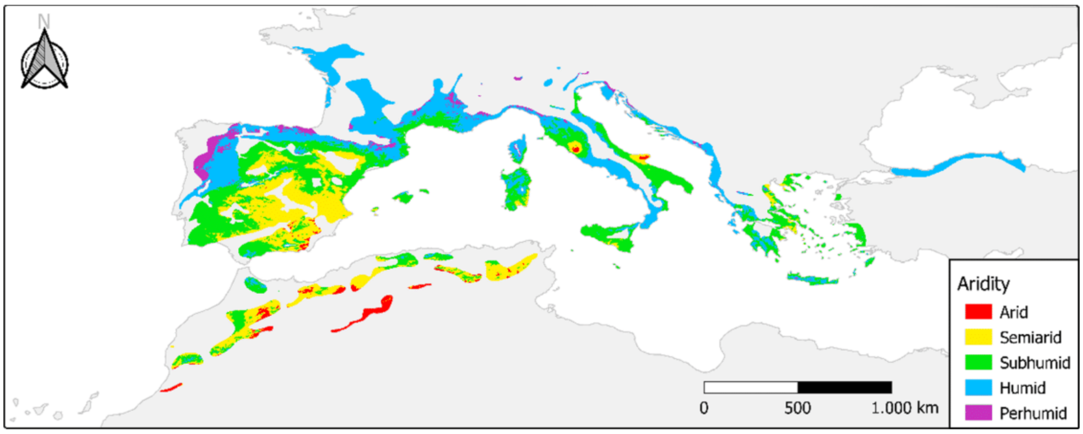

Fig. 1. Aridity index of the distribution area of *Quercus ilex* calculated according to Emberger index (Emberger, 1930). Information about distribution was taken from EUFORGEN (European Forest Genetic Resources Programme, http://www.euforgen.org/) and modified according to the map of provenances for *Quercus ilex* in the Iberian Peninsula (MITECO, https://www.miteco.gob.es) and the "Climatic Modelling of Distribution Ranges of Plant Species" Research project of the Martin-Luther-University Halle-Wittenberg (2006).

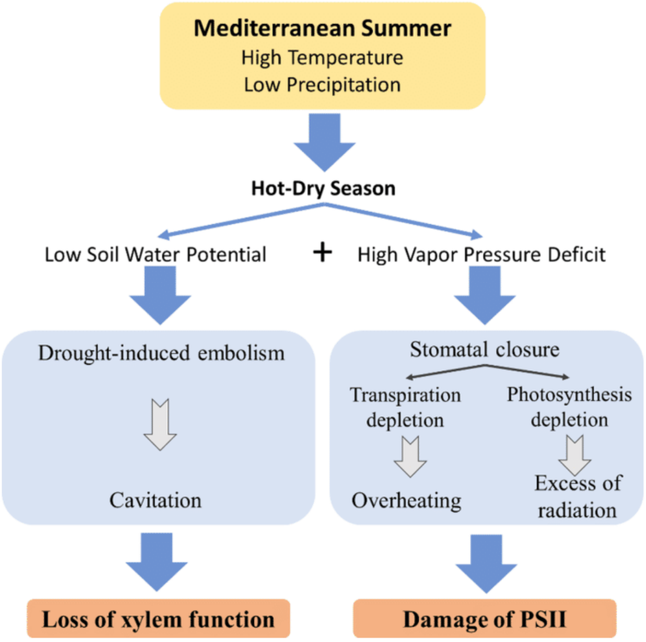

Fig. 2. Schematical representation about the effects of summer stresses on the functionality of the plant.

mechanisms at cell level.

2. Summer stresses: drought and heat

Summer drought is one of the main constraints for the growth and distribution of plant species subjected to Mediterranean climate conditions (Gil-Pelegrín et al., 2017). However, even though holm oak is widely considered a circum-Mediterranean tree species, there is no consensus about its resistance degree to high levels of water stress. To analyze the palaeobotanical origin of holm oak may help to shed light on this aspect.

The uncertainty on the *Q. ilex* capacity to withstand severe water stress is due to the duality between its current distribution and the evolutionary history of the section *Ilex*. Indeed, *Q. drymeja* complex is intimately related to the current Asian tropical and humid-subtropical oak species, the so-called *Heterobalanus* species (e.g. *Quercus floribunda* Lindl. ex Camus, *Quercus engleriana* Seemen, *Quercus tarokoensis* Hayata or *Quercus setulosa* Hickel & A.Camus). Conversely, *Q. mediterranea* complex is more similar to current Mediterranean sclerophyllous oak species from section *Ilex* occurring in the most arid parts of the Mediterranean Basin (Denk et al., 2017), which share a common origin with these fossil taxa (Denk and Grimm, 2010). Both floras, fossil and present, are sclerophyllous, with similar leaf morphology and some xerophytic features, but fossil-taxa occupied, migrated and evolved under humid subtropical conditions (Alonso-Forn et al., 2020). These fossil-taxa are remnants of laurophyllous vegetation, humid warm-temperate forests occupying subtropical or temperate climates, but all of them inhabiting with the absence of summer-drought (Axelrod, 1975). In short, part of the current Mediterranean flora, *Q. ilex* included, has a pre-Mediterranean origin and has not originated and evolved under Mediterranean conditions (Verdú et al., 2003). Therefore, the presence of these fossil-taxa does not imply mediterraneity, mainly because this climate did not even exist by then. It is not until the truly Mediterranean climate arises 5 Ma ago when its characteristic summer-drought periods started to appear (Suc, 1984). Nowadays, *Q. ilex* occupies some areas where drought is not severe or is even absent, especially in the northern and eastern extremes of its distribution area (Fig. 1). On the other hand, this species must definitely cope with drought in its southernmost distribution range (Fig. 1). Hence, holm oak should have developed mechanisms and strategies to survive under situations of soil water deficit and high atmospheric dryness (i.e. an elevated vapor pressure deficit, VPD) that can affect plant functioning in several ways (Fig. 2), and which will be further analysed below in this section. Thus, both dualities, the presence of xeromorphism despite of humid subtropical conditions during Tertiary and the occupation of current areas with and without summer-drought, needs to be well investigated to better clarify at what extent *Q. ilex* adapts to drought stress.

2.1. Xylem resistance to drought-induced cavitation

The long-distance transport of water through the xylem from roots to leaves is a basic function for plant growth and survival in terrestrial environments. The decrease in soil water availability is one of the main factors that can induce embolism formation (i.e. the formation of air or water vapor bubbles inside a xylem conduit), which can cause the loss of xylem functionality by cavitation. Besides, the bigger the vessels are, the more susceptible to cavitate they are. In angiosperms, an ample loss of functionality (between 80% and 90%) by cavitation of the xylem vessels seems to lead irretrievably to plant death (Urli et al., 2013). Drought-induced cavitation at moderate levels below this critic point

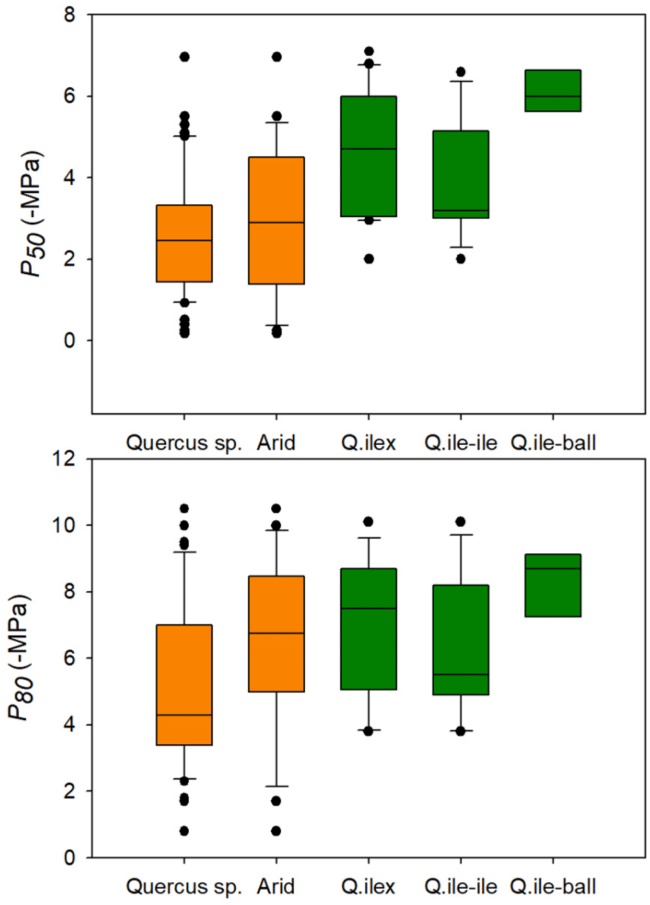

Fig. 3. Box-plots of stem water potential at 50% (P_{50}) (upper panel) and 80% (P_{80}) (lower panel) loss of conductivity for 24 *Quercus* species (*Quercus* sp., n = 67, in orange), from which 13 species occupy arid areas (Arid, n = 42, in orange), besides *Q. ilex* (n = 19) as a single species, and distinguishing between both subspecies: *Q. ilex* subsp. ilex and *Q. ilex* subsp. *ballota* (in green). References and values used in the analysis for *Quercus* species are shown in Table A2, and references, values and methodology used for its calculation in the case of *Q. ilex* are shown in Table A1.

does not provoke the death of the plant, but it will mainly affect its hydraulic function and secondarily its photosynthetic capacity, as plants close their stomata under drought conditions in order to minimize water depletion. Stomatal closure in response to soil water depletion is a widespread mechanism that has been described for holm oak by several authors (e.g. Peguero-Pina et al., 2009, 2018; Alonso-Forn et al., 2021).

Xylem resistance to drought-induced cavitation is considered a good indicator of the ability of the plants to withstand water stress. Specifically, the values of xylem tension (MPa) inducing 50% and 88% loss of hydraulic conductivity in the stems (P_{50} and P_{88}, respectively) are considered proxies of the tolerance to drought-induced embolism (Urli et al., 2013). However, how much is known about this issue in holm oak? Tyree and Cochard (1996) were among the first to elaborate vulnerability curves for some *Quercus* species, including holm oak. In the subsequent years, several studies have been published about the resistance of different provenances of holm oak to drought-induced cavitation (Table A1). Choat et al. (2012) published a meta-analysis where they compiled P_{50} and P_{88} values for many plant species, including several *Quercus* species. We took advantage of these data in order to i/ compare the resistance to drought-induced cavitation of *Q. ilex* with congeneric species, and ii/ analyze the within-species variability between both subspecies of holm oak. In a first instance, we could appreciate the high heterogeneity of genus *Quercus* in terms of cavitation resistance (Fig. 3), with species living in drier habitats showing higher values of both P_{50} and P_{88} (Fig. 3), as previously reported by Maherali et al. (2004). Regarding holm oak, we could appreciate at a glance the high degree of within-species variation in P_{50} and P_{88}, very similar to that found for a representative sample of the whole genus (Fig. 3). In spite of this, it can be concluded that holm oak was on average more resistant to cavitation than the rest of the species of the genus, especially concerning to P_{50} values.

Within *Q. ilex*, we could observe two tendencies that clearly corresponded with each subspecies (Fig. 3). *Q. ilex* subsp. *ballota* tended to present higher values for both P_{50} and P_{88}, except in the study accomplished by Pinto et al. (2012) in Portugal, where oddly low values for P_{50} were obtained (−3.21 MPa). Nevertheless, provenance does not seem to be responsible of the result, since Tyree and Cochard (1996) give a P_{50} value of −5.54 MPa for *Q. ilex* subsp. *ballota* also in Portugal (Martin-StPaul et al., 2014). Regarding *Q. ilex* subsp. *ilex*, we found lower values for both P_{50} and P_{88} when analysed French, Italian or Spanish provenances, albeit there was more heterogeneity than that showed by *Q. ilex* subsp. *ballota* (Fig. 3). The high variability in the resistance to drought-induced cavitation among provenances of *Q. ilex* subsp. *ilex* could be attributed to the high values measured by Peguero-Pina et al. (2014) for three Italian provenances measured in Zaragoza (Spain). Thus, these authors reported values of P_{50}= −5.6, −5.8 and −6.6 MPa and values of P_{88}= −8.2 and −10.1 MPa for Italian provenances, which are quite more typical of *Q. ilex* subsp. *ballota*. Notwithstanding this, the differences found in P_{50} and P_{88} between both subspecies were statistically significant ($P < 0.05$).

The fact that *Q. ilex* subsp. *ilex* provenances are able to develop a resistance to drought-induced cavitation similar to those for *Q. ilex* subsp. *ballota* provenances under climatic conditions typical of this last subspecies (Peguero-Pina et al., 2014) leads to think about the plasticity of this species in terms of xylem vulnerability. According to Cochard et al. (1997), the xylem vulnerability is not only species- or organ-specific, but can also depend on the acclimation of the plants to environmental conditions. Besides, Hacke et al. (2000) claimed that the resistance to cavitation may adjust to the amount of drought experienced in situ. In this sense, several studies have found plasticity in xylem vulnerability to embolism. For instance, Beikircher and Mayr (2009) found that forest plants of *Ligustrum vulgare* L. and *Viburnum lantana* L. were significantly less resistant to drought-induced cavitation than drought-treated garden plants. Besides, they are among the first in proposing the hypothesis that species do not develop high resistance to drought-induced embolism a priori, but are able to acclimate when extended drought periods occur. In line with this, Awad et al. (2010) found that severely water-stressed plants of *Populus x canescens* (Aiton) Sm. showed more negative P_{50} values than well-watered plants. In addition, Corcuera et al. (2011) found that *Pinus pinaster* Ait. under severe drought achieved a better resistance to xylem embolism (P_{50}). Finally, Stojnić et al. (2018) proved how marginal populations of *F. sylvatica* exhibited significant variation in xylem vulnerability to embolism. By contrast, it should be noted that some studies have not found this plasticity. Maherali and DeLucia (2000), for example, did not find significant changes in P_{50} when comparing populations of *Pinus ponderosa* Douglas ex C.Lawson growing in montane and desert conditions. Likewise, Cornwell et al. (2007) did not find evidence of plasticity in cavitation resistance when comparing wet and dry sites of Hawaiian *Metrosideros polymorpha* Gaudich. Concerning *Q. ilex*, no significant differences in the ability of branches to resist xylem cavitation along a climatic gradient were found either (Limousin et al., 2010; Martin-St-Paul et al., 2013). Therefore, the scope of plasticity in xylem resistance to drought-induced cavitation as a main feature of plants to cope with drought is still under discussion.

The resistance to cavitation seems to be related to xylem anatomy and especially vessel size. Awad et al. (2010) postulated that the decrease in vulnerability to cavitation in plants growing under soil

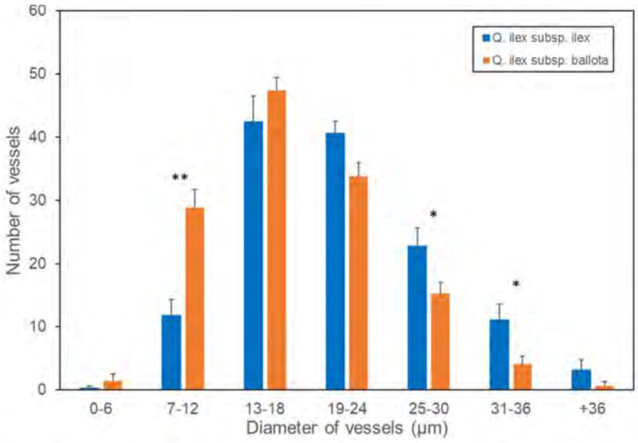

Fig. 4. Average number of vessels for each diametrical class in one year-old seedlings of *Q. ilex* subsp. *ilex* (blue bars) and *Q. ilex* subsp. *ballota* (orange bars) (unpublished results). Values were calculated from 24 seedlings corresponding to three provenances of *Q. ilex* subsp. *ilex* (Veneto, Lazio and Sardinia, Italy) and 32 seedlings corresponding to four provenances of *Q. ilex* subsp. *ballota* (Cuenca, Ciudad Real, Jaen and Soria, Spain). Data are mean ± SE. Asterisks indicate significant differences between both subspecies for each diametrical class (Mann–Whitney U test) (*, $0.05 < P < 0.1$; **, $0.001 < P < 0.05$).

drought conditions was correlated with decreased vessel diameter, increased vessel wall thickness and a stronger bordered pit field. Hence, plants experiencing low (i.e. more negative) water potential values need to reinforce their vessel walls to avoid cell collapse before suffering cavitation (Hacke and Sperry, 2001; Nardini et al., 2014). Several studies have corroborated this hypothesis, showing that lower vulnerability to drought was associated with larger wood density (Hacke and Sperry, 2001; Jacobsen et al., 2007; Pratt et al., 2007). Thus, diffuse-porous species like *Q. ilex* would be more resistant to drought-induced cavitation than ring-porous species, such as *Q. robur* (Cochard et al., 1992; Bréda et al., 1993). Besides, holm oak presents an intraspecific plasticity regarding to vessel size (Campelo et al., 2010). To evaluate this within-species variation in vessel size in *Q. ilex*, we measured this trait in one-year old seedlings from three provenances of *Q. ilex* subsp. *ilex* (Veneto, Lazio and Sardinia; Italy) and four provenances of *Q. ilex* subsp. *ballota* (Cuenca, Ciudad Real, Jaen and Soria; Spain). To do this, middle segments of seedling stems were transversally sectioned with a sliding microtome (Microm HM 350S, Thermo Scientific, Walldorf, Germany). Sections with a thickness of 20 µm were dehydrated by means of ethanol series and stained with safranin (Corcuera et al., 2004). The cross-sections were viewed with a Leica DM LB microscope (Leica Microsystems, Wetzlar, Germany) and photographed with a digital camera (Moticam 2300, Motic, Hong Kong, China). The resulting micrographs were analysed using Image-J software (http://rsb.info.nih.gov/nih-image/) to measure vessel number and diameter. The results obtained from these measurements suggest that *Q. ilex* subsp. *ilex* tended to have wider vessels, having on average more vessels over 18 µm than *Q. ilex* subsp. *ballota*, which seemed to have narrower vessels (Fig. 4).

Another issue that should be considered when analyze both intraspecific and interspecific differences in resistance to cavitation may be related to safety margin. Brodribb and Hill (1999) defined the safety margin as the difference between soil water potential inducing stomatal closure (namely turgor loss point, TLP) and P_{50}. Alternatively, Meinzer et al. (2009) defined the safety margin as the difference between the

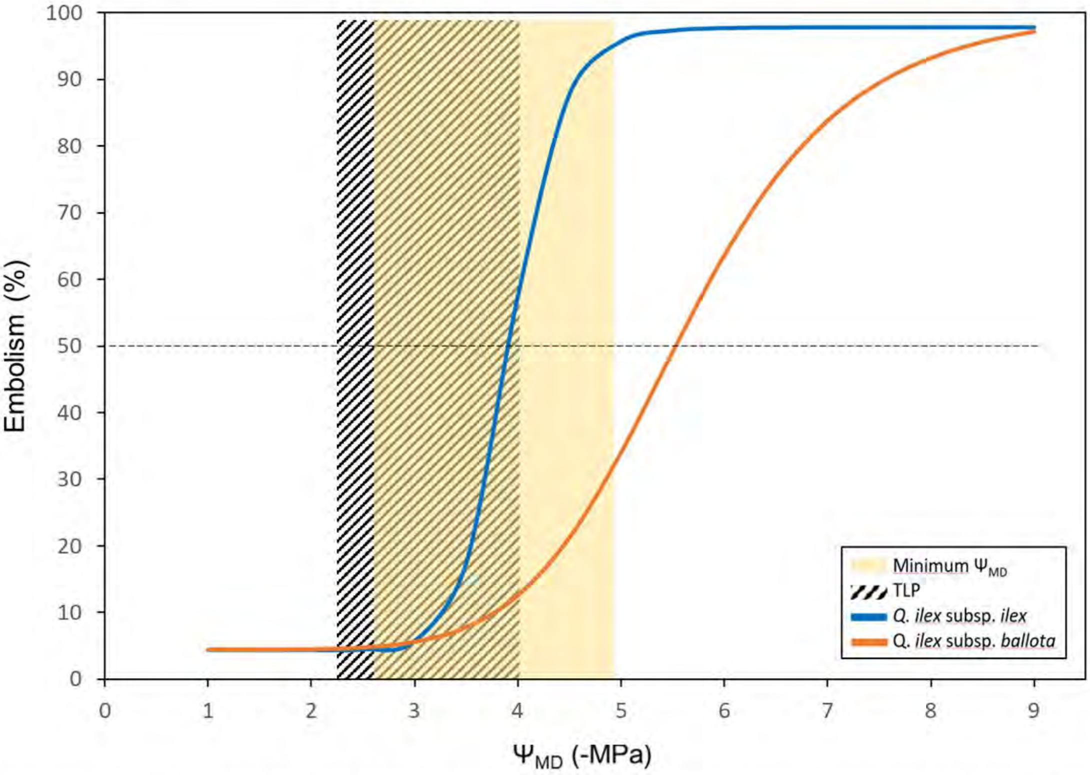

Fig. 5. Embolism type-curves of *Q. ilex* subsp. *ilex* (blue) and *Q. ilex* subsp. *ballota* (orange) and the range of water potentials values found in the literature (Table A3) for the two criteria of safety margin calculation: i) minimum water potential measured in the field at midday (Ψ_{MD}, in yellow, from −2.6 to −4.91 MPa) and ii) turgor loss point (TLP, with dashed lines, from −2.23 to −4 MPa). The horizontal dotted line represents the P_{50} values for both subspecies. Type-curves for each subspecies are extracted from Alonso-Forn et al. (2020) for *Q. ilex* subsp. *ballota* and from our own unpublished data for *Q. ilex* subsp. *ilex*.

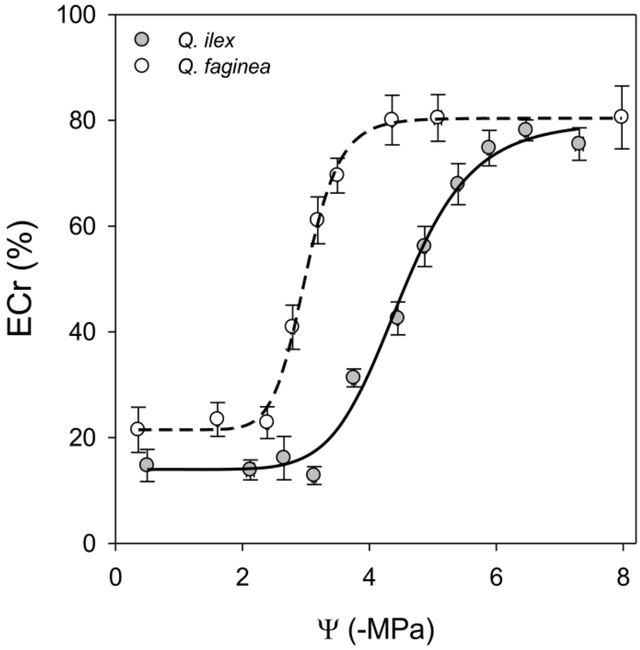

Fig. 6. Cell membrane injury (% of relative conductivity, ECr) measured by means of the electrolyte leakage technique as a function of water potential for *Q. ilex* (gray symbols) and *Q. faginea* (white symbols) (unpublished results). Data were adjusted to a four-parameter logistic curve, both for *Q. ilex* (solid line, $R^2 = 0.99$, $P < 0.0001$) and *Q. faginea* (dashed line, $R^2 = 0.99$, $P < 0.0001$). Data are mean ± SE.

minimum water potential experienced by the tree in the field during the dry season and the water potential inducing 12%, 50% and 88% loss of hydraulic conductivity. In the case of holm oak, values of safety margin were quite similar regardless of the criterion used, evidencing meaningful differences between both subspecies (Fig. 5). Thus, turgor loss point and minimum water potential recorded in the field oscillated from −2.23 to −4 MPa and from −2.6 to −4.91 MPa, respectively. If we notice that *Q. ilex* subsp. *ilex* has on average a less negative P_{50} than *Q. ilex* subsp. *ballota*, we can infer that the former will have lower safety margin that the latter. Indeed, Martin-StPaul et al. (2013) reported a safety margin value of just 0.3 MPa for a population of *Q. ilex* subsp. *ilex* in northern Greece. Similarly, Savi et al. (2015) found very low (0.01 and 0.04 MPa) or even negative (−0.43 and −0.72 MPa) safety margin values for *Q. ilex* subsp. *ilex* in Croatia.

Finally, the overall plant resistance to cavitation not only depends on stem vulnerability but it is also related to the capacity of other plant organs to withstand xylem embolism (Skelton et al., 2018). In this sense, the most expendable organs (e.g. the leaves) would be more vulnerable to cavitation than the most important ones (e.g. the stem), which is known as “vulnerability segmentation” (Hochberg et al., 2017). Peguero-Pina et al. (2015) reported this phenomenon in the winter-deciduous Mediterranean oak *Quercus subpyrenaica* E.H. del Villar, which explained the premature leaf withering as a response to a severe-drought period. Similarly, Alonso-Forn et al. (2021) also demonstrated the existence of vulnerability segmentation in *Q. faginea*, another winter-deciduous Mediterranean oak. However, both studies did not find evidence of vulnerability segmentation in *Q. ilex* subsp. *ballota*, which exhibited high resistance to drought-induced cavitation in both leaves and stem. This conservative leaf strategy could be related to the longer leaf life span of holm oak, which allows this species to assimilate carbon during more than one vegetative period (Corcuera et al., 2005).

2.2. Resistance to summer drought and high temperatures at leaf cell level

The implementation of a conservative leaf strategy, such as that shown by *Q. ilex* not only relies on leaf hydraulic resistance, but also in an increased resistance to drought at cell level. Thus, when water potential becomes more negative and reaches a critical threshold, water stress can induce the collapse of the cells, which may be associated to the physical damage of the cell membrane during cell buckling and electrolyte leakage (Farrant, 2000). Therefore, the resistance of the leaf to drought at cell level can be explored through the assessment of cell membrane injury by measuring electrolyte leakage in response to decreases in leaf water potential (Ψ) (Vilagrosa et al., 2010). To assess this issue in holm oak, we compared the relationships between Ψ and relative electrical conductivity (ECr,%) for *Q. ilex* and the co-occurring winter-deciduous *Q. faginea* following the methodology described by Vilagrosa et al. (2010). We used five leaves ($n = 5$) from three-year old seedlings in which we had previously measured Ψ with a Scholander pressure chamber. From each leaf, three leaf disks (0.2 cm^2) were collected and incubated in 3 mL of distilled water at 10 °C for 5 h (ECi). The disks were removed from the vial, plunged into liquid nitrogen and placed in another vial to be stored for 12 h at −25 °C. We then added 3 mL of distilled water and again stored the disks in the dark at 10 °C for 5 h (ECf). Initial (ECi) and final (ECf) electrical conductivities were measured at 25 °C with a conductivity meter (Crison CM 2202, Barcelona, Spain). Relative electrical conductivity (ECr,%) of each sample was calculated as: $ECr = (ECi/ECi + ECf) \times 100$. According to our data, ECr showed better membrane stability at lower water potentials in *Q. ilex* than in *Q. faginea*. Specifically, *Q. faginea* displayed ECr values at −4 MPa that reflected a high degree of membrane destabilization (ca. 80%), whereas ECr reached only ca. 30% at the same Ψ in *Q. ilex* (Fig. 6). From this Ψ value, *Q. ilex* showed a strong increase in ECr, reaching ca. 80% when Ψ was ca. −6 MPa (Fig. 6).

Besides the stability of cell membrane, other factors should also be considered when analysing the resistance to summer drought at cell level. As stated above, stomatal closure under drought conditions is a well-known mechanism that minimizes water depletion at the expense of a reduction in photosynthetic activity, which has been widely described for *Q. ilex* (Acherar and Rambal, 1992; Mediavilla and Escudero, 2003, 2004; Peguero-Pina et al., 2009, 2018; Alonso-Forn et al., 2021). Overall, these studies reported that stomatal closure occurs in this species at leaf water potential values (ca. −3 MPa) much higher than P_{50} and P_{88} (see above in this manuscript). Thus, this species is able to withstand severe drought periods with leaf water potentials lower than those inducing stomatal closure, but much higher than cavitation limit (i.e. a wide hydraulic safety margin, according to Martin-StPaul et al., 2017). Under such circumstances, since the light-harvesting complexes of both photosystems continue collecting light, excitation energy cannot be directed to the photosynthetic electron transport chain and may exceed the amount that can be used for photosynthesis (Demmig-Adams and Adams, 2006). Electrons not consumed in CO_2 fixation may react with O_2 generating reactive oxygen species and increasing the possibility of oxidative damage (Hernández et al., 2012). To cope with this situation, plants have developed different mechanisms that allow the protection of the photosynthetic apparatus. Regarding *Q. ilex*, it is widely acknowledged its great capacity for the implementation of photoprotective mechanisms. For example, the presence of trichomes in the adaxial leaf surface could contribute to a decrease in susceptibility to photodamage through the increase in leaf reflectance (Morales et al., 2002). Moreover, a significant number of studies have analysed the role of thermal dissipation of the excess energy in response to water scarcity in this species, such as the non-photochemical quenching (NPQ) of chlorophyll fluorescence and the de-epoxidation of the xanthophyll cycle pigments (García-Plazaola et al., 2017).

In this sense, Méthy et al. (1996) stated that an increase in NPQ in *Q. ilex* subsp. *ilex* when leaf predawn water potential was lower than −4 MPa implied the onset of photoprotective mechanisms associated with

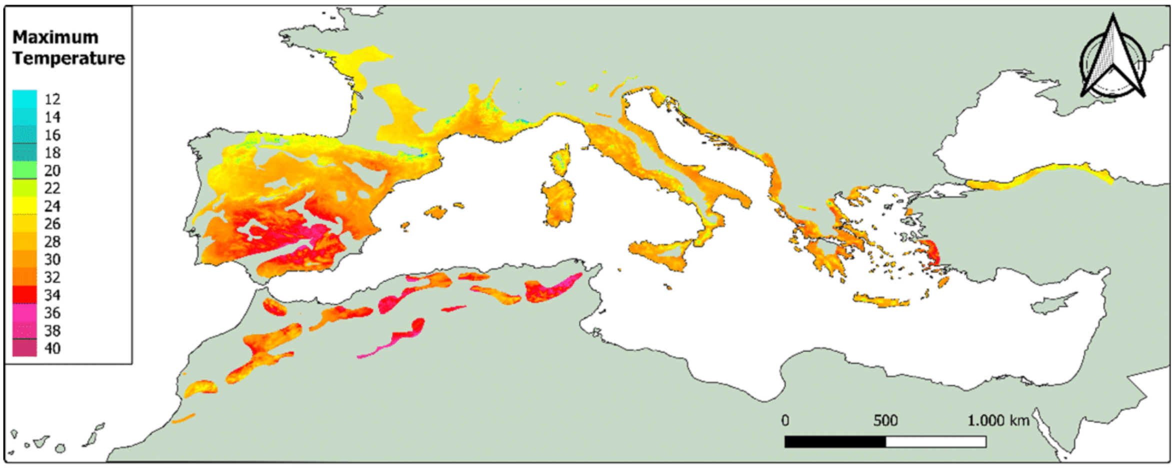

Fig. 7. Average maximum temperature of the warmest month that *Quercus ilex* endures in its natural distribution area (represented by coloured map). Information about distribution was taken from EUFORGEN (European Forest Genetic Resources Programme, http://www.euforgen.org/) and modified according to the map of provenances for *Quercus ilex* in the Iberian Peninsula (MITECO, https://www.miteco.gob.es) and the "Climatic Modelling of Distribution Ranges of Plant Species" Research project of the Martin-Luther-University Halle-Wittenberg (2006).

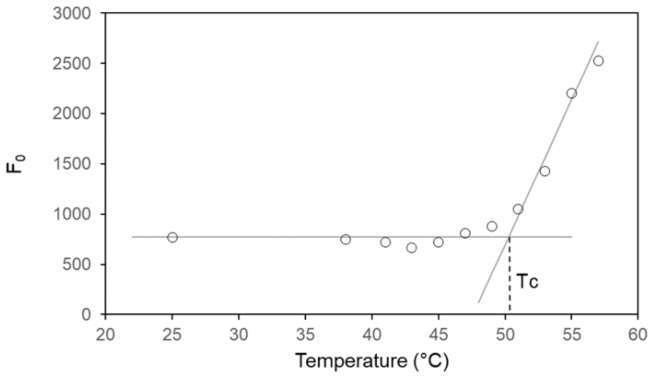

Fig. 8. Relationship between temperature and minimum chlorophyll fluorescence in the dark-adapted state (F_0) for leaves of *Q. ilex* subsp. *ballota* ($n = 4$) (unpublished results). Critical temperature (Tc) was calculated following the procedure described by Schreiber and Berry (1977) and estimated at 50.2 °C.

the dissipation of excessive energy in the photosynthetic apparatus. Moreover, these authors concluded that photoprotection is probably an important component of water-stress tolerance in *Q. ilex* as could allow the recovery of this species after an intense drought period. Corcuera et al. (2005) firstly reported the association of the down-regulation of photosynthesis during summer in *Q. ilex* subsp. *ballota* with the thermal energy dissipation and NPQ through the increase of de-epoxidated forms of the xanthophyll cycle pigments, antheraxanthin (A) and zeaxanthin (Z). In line with this, Peguero-Pina et al. (2009) also showed that *Q. ilex* subsp. *ballota* experienced a drought-mediated increase in the thermal dissipation of the energy excess (through an increase in A and Z) coupled with an increase in the amount of absorbed energy that was dissipated in the PSII antenna ($1{-}\Phi_{exc}$). Furthermore, these authors evidenced that plants of *Q. ilex* subsp. *ballota* subjected to intense water stress retained overnight the de-epoxidated forms A and Z accumulated during the day, which might indicate that photoprotective mechanisms of this species could be related in some way to the persistence of a trans-thylakoid proton gradient (ΔpH). In addition, the persistence of A and Z at pre-dawn coincided with the decrease of dark-adapted, maximum quantum efficiency of PSII (F_V/F_M), which seemed to be related to an additional

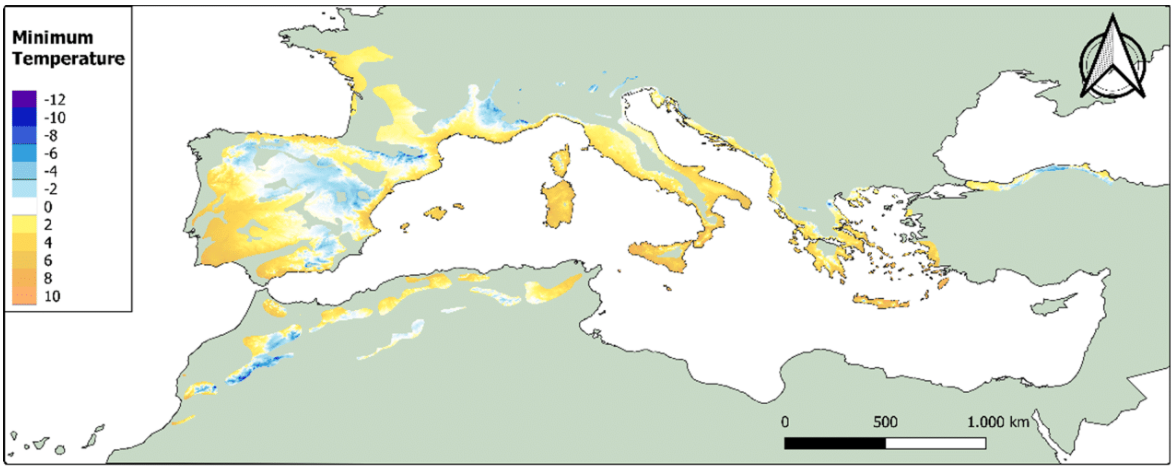

Fig. 9. Average minimum temperature of the coldest month that *Quercus ilex* endures in its natural distribution area (represented by coloured map). Information about distribution was taken from EUFORGEN (European Forest Genetic Resources Programme, http://www.euforgen.org/) and modified according to the map of provenances for *Quercus ilex* in the Iberian Peninsula (MITECO, https://www.miteco.gob.es) and the "Climatic Modelling of Distribution Ranges of Plant Species" Research project of the Martin-Luther-University Halle-Wittenberg (2006).

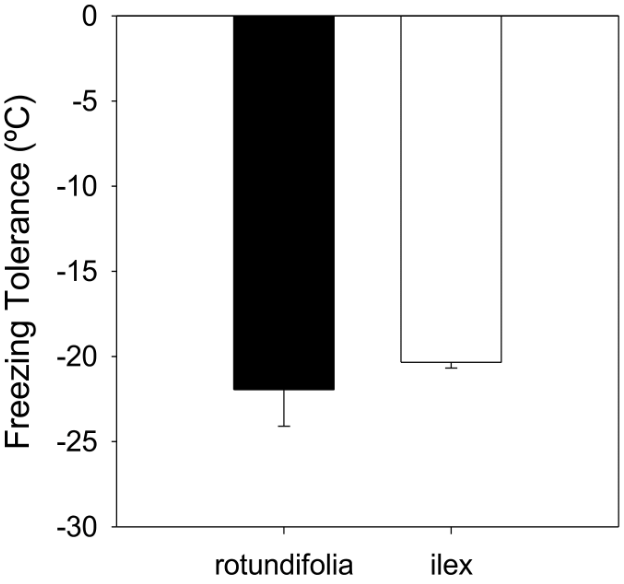

Fig. 10. Freezing tolerance values (°C) for *Q. ilex* subsp. *ballota* (black bar) and *Q. ilex* subsp. *ilex* (white bar) estimated through the changes in F_V/F_M as an indicator of photoinhibition induced by low temperatures (Peguero-Pina et al., 2008b) (unpublished results). Data are mean ± SE of four provenances per subspecies.

photoprotective mechanism that preserved an intact photosynthetic pigment machinery. According to these authors, this mechanism may play an important role in the survival of species such as *Q. ilex* subsp. *ballota* in sites with long and intense summer drought periods. These findings were further confirmed for *Q. ilex* subsp. *ilex* by Chiatante et al. (2015), who found that drought-induced a decrease in F_V/F_M and in the actual PSII efficiency (Φ_{PSII}) that was reversible when plants recovered from water stress, which confirmed the resilience of the photosynthetic apparatus of *Q. ilex* subsp. *ilex* in response to water scarcity. More recently, Sancho-Knapik et al. (2018) showed that *Q. ilex* subsp. *ballota* displayed an early decrease in Φ_{PSII} in response to drought, down-regulating the photosynthetic electron transport when CO_2 assimilation was severely limited. These authors also reported a strong increase in NPQ that was closely related to changes in the physiological reflectance index (PRI), as both parameters reflect in many cases photoprotection processes related to ΔpH and/or de-epoxidation of the xanthophyll cycle (Peguero-Pina et al., 2008a, 2013). Variations in PRI with changes in water status of holm oak have been also reported by Tsonev et al. (2014) and Zhang et al. (2017).

Therefore, drought triggers a decrease in Φ_{PSII} and a rise in NPQ related to the de-epoxidation of xanthophylls that efficiently reduce the risks of photodamage under drought conditions for both *Q. ilex* subsp. *ilex* and *Q. ilex* subsp. *ballota*. In addition, both subspecies down-regulate F_V/F_M when leaf water potential drops below the value inducing stomata to close. The evidences of an ample safety margin in *Q. ilex* subsp. *ballota* would be consonant with this ability to preserve photoprotective mechanisms since both, leaves and branches, are able to tolerate water potential levels where photosynthetic activity is impaired due to stomatal closure but are far from the cavitation threshold. This conservative leaf strategy based on the high resilience of the photosynthetic apparatus plays an important role in the rapid recovery of this species after a long summer drought period (Vaz et al., 2010), and would contribute to maintain the leaves functional for more than one vegetative period (Corcuera et al., 2005).

Besides drought, leaves of holm oak must deal with high temperatures during summer, especially in its southernmost and westernmost distribution range (Fig. 7), where maximum temperatures during the hottest days of summer usually exceed 40 °C. It should be noted that this area is occupied by *Q. ilex* subsp. *ballota*, whose leaves are much smaller than those of *Q. ilex* subsp. *ilex* (Peguero-Pina et al., 2014). The smaller the leaves are, the thinner the boundary layer is, which facilitates a sensible heat loss in summer since they tend to be better coupled to air temperature and can avoid overheating through a faster convective cooling (Peguero-Pina et al., 2020). In any case, the resistance of the leaf to high temperatures should be analysed for holm oak, especially for *Q. ilex* subsp. *ballota*, as thermal stress may jeopardize several processes occurring in the leaves at cell level, especially those related to photosynthetic activity. Thus, when leaves are exposed to extreme temperatures, they accumulate damage to photosystem II (PSII) (Arnold et al., 2021). Temperature-dependent changes in minimum chlorophyll fluorescence in the dark-adapted state (F_0) can be used to identify the critical temperature at which PSII is irreversibly damaged. In this sense, we performed an essay for this review where we calculated this critical temperature according to Schreiber and Berry (1977) for four leaves of one-year old seedlings of *Q. ilex* subsp. *ballota*. This procedure consisted on subjecting leaves to an increasing ramp of temperature and measuring F_0 with a FMS II modulated fluorometer (Hansatech Instruments, Norfolk, UK) at regular time intervals. Initially, F_0 remained constant up to a certain temperature where it suddenly increased due to thermal damage in PSII. The critical temperature for *Q. ilex* subsp. *ballota* was of 50.2 °C (Fig. 8), which was similar to those measured in other species (Ranney and Peet, 1994; Krause et al., 2013). In any case, it is far above enough from maximum temperatures usually reached in circum-Mediterranean areas where holm oak lives.

However, although high temperatures themselves do not normally compromise the survival of the plant, the combination of hot temperatures and reduced moisture in the air during summer leads to an increase in VPD (Fig. 2). A higher evaporative demand by the atmosphere implies a higher transpiration rate when stomata are opened during gas exchange (McAdam and Brodribb, 2015; Kimm et al., 2020). In this sense, the plant risks to significant water losses under a very negative VPD. For this reason, most plants respond to VPD closing stomata, either passively, i.e. induced by leaf water status (e.g. gymnosperms) or even actively, through a local increase in abscisic acid (e.g. in angiosperms) (McAdam and Brodribb, 2015) when a certain value of VPD is reached, even though they have available water in the ground. These plants are said to follow a conservative strategy, that is, water-saver plants (Levitt, 1980). Mediavilla and Escudero (2003) showed that *Q. ilex* exhibits this conservative strategy, presenting significant reduction of stomatal conductance at approximately 2.0 kPa. Holm oak was the species with the lowest VPD threshold when compared with other three co-occurring *Quercus* species (*Quercus suber* L., *Q. faginea* and *Q. pyrenaica*), where deciduous oaks showed the highest VPD at which they begin to close stomata. This water-saver strategy used by *Q. ilex* and *Q. suber* is likely to be related to being evergreen, since they need to avoid irreversibly damage at leaf level in order to preserve leaves in the best possible condition during its life span (Mediavilla and Escudero, 2003).

Finally, the response of stomata closure is common to high VPD and scarcity of water in the soil due to drought. Thus, the additive effect of these two phenomena, which are often linked and even prolonged in time (Perez-Martin et al., 2009), means that the plant is only able to open stomata and photosynthesise for a few hours mainly at sunrise on many summer days. To the aforementioned effects of closing stomata, the fact of having a substantial reduction of photosynthesis has consequences in carbon fixation, where a lower carbon fixation is related to a slower growth rate and, consequently, a minor productivity at ecosystem level (Grossiord et al., 2020).

3. Winter cold

With reference to winter cold, less negative soil water potentials are expected. However, low temperatures (Fig. 9), commonly below zero during many hours daily, followed by a continuous freeze-thawing cycles may compromise xylem stability due to freeze-induced embolism.

Table A1
Compilation of studies that have proportionated the values of xylem tension (MPa) inducing 50% and 88% loss of hydraulic conductivity in the stems (P_{50} and P_{88}, respectively) for *Quercus ilex* or have published an embolism curve from which P_{50} and P_{88} values have been inferred (values marked with an asterisk). The kind of plant material used in the study, subspecies (when is specified) and the geographical provenance of that material are also indicated. Besides, the method used in each experiment is shown according to the terminology of Martin-StPaul et al. (2014).

Source	Plant material	Provenance	Method	P_{50} (-MPa)	P_{88} (-MPa)
Lo Gullo and Salleo (1993)	*Q. ilex*. Potted plants grown at 800 m. altitude	Sicily (Italy)	Native embolism throughout a drying cycle in summer. Dehydration *in planta*	2.95 (53%)	3.84 (85%)
Tyree and Cochard (1996)	*Q. ilex*. Branches	Non specified	Bench drying	3*	3.8*
Tyree and Cochard (1996)	*Q. ilex*. Branches	Non specified	Bench drying	5.52	6.5*
Tognetti et al. (1998)	*Q. ilex*. Branches from trees in the field	Siena (Italy) 43°17′ N, 11°35′ E and 350 m.a.s.l.	Bench drying	3	5
Martínez-Vilalta et al. (2002)	*Q. ilex*. Branches from trees in the field	Prades (Spain) 41°13′N, 0°55′E and 1000 m a.s.l.	Bench drying	2	5.5*
Gartner et al. (2003)	*Q. ilex*. Seedlings grown in a greenhouse	Acorns from Nîmes (France)	Air injection (double-ended pressure chamber)	3.2	5.1
Corcuera et al. (2004) a	*Q. ilex* subsp. *ilex*. Potted seedlings growing in Zaragoza (Spain) 41°50′20″ N, 0°51′07″W	Mallorca (Spain)	Air injection (double-ended pressure chamber)	3	5.5*
Corcuera et al. (2004) b	*Q. ilex* subsp. *ballota*. Potted seedlings growning in Zaragoza (Spain) 41°50′20″ N, 0°51′07″W	Cubel (Spain) 41°00′07″ N, 1°03′09″ W, 1177 m a.s.l.	Air injection (double-ended pressure chamber)	5.6	9*
Cochard et al. (2005)	*Q. ilex*. Branches from trees in the field	Montpellier (France) 3°35′45″ E, 43°44′29″ N, 270 m a.s.l.	Centrifugue	4.13	
Limousin et al. (2010)	*Q. ilex*. Trees in the field	Montpellier (France) 3°35′45″ E, 43°44′29″ N, 270 m a.s.l.	Air injection (double-ended pressure chamber)	3.8	8*
Nardini et al. (2012)	*Q. ilex*. Trees in the field	Cernizza (Italy) 45°46′ 49″ N, 13°35′31″ E; 25 m a.s.l.	Bench drying	3.5	
Pinto et al. (2012)	*Q. ilex* subsp. *ballota*. Trees in the field	Evora (Portugal) 38°32′ N, 8°00′ E	Bench drying	3.21	8.5*
Urli et al. (2013)	*Q. ilex*. Seedlings grown in a greenhouse (4–5 years old)	Non specified	Cavitron	5.52	7.08
Martin-StPaul et al. (2013)	*Q. ilex*. Trees in the field. From three forests with different level of xericity	1) Les Mages 44°13′ N, 4°08′ E,360 m a.s.l. 2) Puechabon 43°44′ N, 3°35′ E, 270 m a.s.l. 3) Vic la Gardiole 43°29′ N, 3°45′ E 170 m a.s.l.	Air injection (double-ended pressure chamber)	3.8	
Martin-StPaul et al. (2014)	*Q. ilex*. Current year resprouts from trees planted in 1963. CNRS campus of the CEFE at Montpellier (France)	Acorns from Puéchabon forest 3°35′45″ E, 43°44′29″ N	Bench drying	4.7	6*
Peguero-Pina et al. (2014)	*Q. ilex* subsp. *ilex*. Trees planted in a common garden in Zaragoza (Spain) 41°50′20″ N, 0°51′07″W	Veneto (Italy) 45°44′ N, 10°48′ E, 617 m a.s.l.	Bench drying	5.6	8.2
Peguero-Pina et al. (2014)	*Q. ilex* subsp. *ilex*. Trees planted in a common garden in Zaragoza (Spain) 41°50′20″ N, 0°51′07″W	Lazio (Italy) 41°13′ N, 13°03′ E, 29 m a.s.l.	Bench drying	5.8	8.2
Peguero-Pina et al. (2014)	*Q. ilex* subsp. *ilex*. Trees planted in a common garden in Zaragoza (Spain) 41°50′20″ N, 0°51′07″W	Sardinia (Italy) 39°21′ N, 08°34′ E, 627 m a.s.l.	Bench drying	6.6	10.1
Peguero-Pina et al. (2014)	*Q. ilex* subsp. *ballota*. Trees planted in a common garden in Zaragoza (Spain) 41°50′20″ N, 0°51′07″W	Cazorla (Spain) 38°06′ N, 02°33′ W, 1236 m a.s.l.	Bench drying	6	9.5
Peguero-Pina et al. (2014)	*Q. ilex* subsp. *ballota*. Trees planted in a common garden in Zaragoza (Spain) 41°50′20″ N, 0°51′07″W	Ciudad Real (Spain) 39°27′ N, 04°24′ W, 724 m a.s.l.	Bench drying	7.1	
Peguero-Pina et al. (2014)	*Q. ilex* subsp. *ballota*. Trees planted in a common garden in Zaragoza (Spain) 41°50′20″ N, 0°51′07″W	Soria (Spain) 41°46′ N, 02°29′ E, 1074 m a.s.l.	Bench drying	6.2	8.9
Urli et al. (2015)	*Q. ilex*. Trees in the field.	Natural Reserve of Hourtin (France) 45° 1114N, 1°0323W 18 m a.s.l.	Cavitron	5.52	7.08
Peguero-Pina et al. (2018)	Potted plants growing (8 years old) growing in Zaragoza (Spain) 41°50′20″ N, 0°51′07″W	Soria (Spain) 41°46′ N, 02°29′ E, 1074 m a.s.l.	Native embolism throughout a drying cycle in summer. Dehydration *in planta*	6	
Alonso-Forn et al. (2021)	Potted plants growing (9 years old) growing in Zaragoza (Spain) 41°50′20″ N, 0°51′07″W	"Alcarria-Serranía de Cuenca" (Spain) 40°19′ N, 2°15′ W, 950 m a.s.l.	Native embolism throughout a drying cycle in summer. Dehydration *in planta*	6.8	7.5
Alonso-Forn et al. (unpublished data)	Potted plants growing (9 years old) growing in Zaragoza (Spain) 41°50′20″ N, 0°51′07″W	Lazio (Italy) 41°13′ N, 13°03′ E, 29 m a.s.l.	Native embolism throughout a drying cycle in summer. Dehydration *in planta*	3.93	5.5

Additionally, low winter temperatures affect the photosynthetic activity of *Q. ilex* by reducing or even nullifying it in the coldest areas (Crescente et al., 2002). Compared to very high temperatures, low ones are worse for the same level of light, since photosynthesis levels are minimal and, therefore, the combination of temperatures below 0 °C and the excess of radiation can trigger damages in the photosynthetic apparatus (Corcuera et al., 2005).

3.1. Xylem resistance to cavitation induced by low temperatures

Holm oak surviving throughout the cold winters is not only a matter of leaf tolerance to damages induced by frost, as above expressed.

Table A2

Values of xylem tension (MPa) inducing 50% and 88% loss of hydraulic conductivity in the stems (P_{50} and P_{88}, respectively) for 22 *Quercus* species and references where they have been extracted.

Species	P_{50} (-MPa)	P_{88} (-MPa)	Reference
Q. agrifolia	2	5	Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. Nature. 491, 752–755.
Q. alba	1.37	2.6	Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. Nature. 491, 752–755.
Q. berberidifolia	5.1	9.5	Jacobsen, A.L., Pratt, R.B., Ewers, F.W., & Davis, S.D., 2007. Cavitation resistance among 26 chaparral species of southern California. Ecol.l Monogr. 77 (1), 99–115.
Q. berberidifolia	–	6.5	Venturas, M.D., MacKinnon, E.D., Dario, H.L., Jacobsen, A.L., Pratt, R.B., Davis, S.D., 2016. Chaparral Shrub Hydraulic Traits, Size, and Life History Types Relate to Species Mortality during California's Historic Drought of 2014. PLoS ONE. 11(7): e0159145.
Q. berberidifolia	1.5	–	Jacobsen, A.L., Pratt, R.B., Davis S.D., Ewers, F.W., 2007. Cavitation resistance and seasonal hydraulics differ among three arid Californian plant communities. Plant Cell Envir. 30: 1599–1609.
Q. berberidifolia	2.6	9.4	Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. Nature. 491, 752–755.
Q. coccifera	6.96	10	Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. Nature. 491, 752–755.
Q. cornelius-mulleri	3.17	5.5	Paddock, III, W.A., Davis, S.D., Pratt, R. B., Jacobsen, A.L., Tobin, M.F., López-Portillo, J., Ewers, F.W., 2013. Factors determining mortality of adult chaparral shrubs in an extreme drought year in California. Aliso. 31(1), 49–57.
Q. faginea	3.9	5.65	Esteso-Martínez, J., Camarero, J.J., Gil-Pelegrín, E., 2006. Competitive effects of herbs on Quercus faginea seedlings inferred from vulnerability curves and spatial-pattern analyses in a Mediterranean stand (Iberian System, northeastern Spain). Ecoscience. 13(3), 378–387.
Q. falcata	0.92	1.8	Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. Nature. 491, 752–755.
Q. frainetto	4.5	8.38	Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. Nature. 491, 752–755.
Q. gambelii	0.25	0.8	Sperry, J.S., Christman, M.A., Torres-Ruiz, J.M., Taneda, H., Smith, D.D., 2012. Vulnerability curves by centrifugation: is there an open vessel artefact, and are 'r'shaped curves necessarily invalid? Plant Cell Environ. 35(3), 601–610.
Q. gambelii	0.18	–	Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. Nature. 491, 752–755.
Q. gambelli	1.08	–	Christman, M.A., Sperry, J.S., Smith, D. D., 2012. Rare pits, large vessels and extreme vulnerability to cavitation in a ring-porous tree species. New Phytol. 193, 713–720.
Q. laevis	1.89	3.6	Esteso-Martínez, J., Camarero, J.J., Gil-Pelegrín, E., 2006. Competitive effects of herbs on Quercus faginea seedlings inferred from vulnerability curves and spatial-pattern analyses in a Mediterranean stand (Iberian System, northeastern Spain). Ecoscience. 13(3), 378–387.
Q. nigra	1.31	2.7	Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. Nature. 491, 752–755.
Q. oleoides	3.03	3.9	Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J.,

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Table A2 (*continued*)

Species	P_{50} (-MPa)	P_{88} (-MPa)	Reference
			Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. Nature. 491, 752–755.
Q. petraea	1.9	–	Nardini, A., Pedà, G., La Rocca, N., 2012. Trade-offs between leaf hydraulic capacity and drought vulnerability: morpho-anatomical bases, carbon costs and ecological consequences. New Phytol. 196, 788–798.
Q. petraea	3.5	4.2	Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. Nature. 491, 752–755.
Q. petraea	3.46	4.58	Urli, M., Porté, A.J., Cochard, H., Guengant, Y., Burlett, R., Delzon, S., 2013. Xylem embolism threshold for catastrophic hydraulic failure in angiosperm trees. Tree Physiol. 33, 672–683.
Q. petraea	3.4	4.2	Tyree, M.T., Cochard, H., 1996. Summer and winter embolism in oak: impact on water relations. Ann. For. Sci. 53, 173–180.
Q. phellos	1.42	2.3	Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. Nature. 491, 752–755.
Q. prinus	1.7	–	Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. Nature. 491, 752–755.
Q. pubescens	2.75	–	Nardini, A., Pedà, G., La Rocca, N., 2012. Trade-offs between leaf hydraulic capacity and drought vulnerability: morpho-anatomical bases, carbon costs and ecological consequences. New Phytol. 196, 788–798.
Q. pubescens	1.7	3.2	Tognetti, R., Longobucco, A., Raschi, A., 1999. Seasonal embolism and xylem vulnerability in deciduous and evergreen Mediterranean trees influenced by proximity to a carbon dioxide spring. Tree Physiol. 19, 271–277.
Q. pubescens	3.3	5.5	Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. Nature. 491, 752–755.
Q. pubescens	3.5	> 4.5	Cochard, H., Bréda, N., Granier, A., Aussenac, G., 1992. Vulnerability to air embolism of three European oak species (Quercus petraea (Matt) Liebl, Q pubescens Willd, Q robur L). Ann. Sci. For. 49, 225–233.
Q. pubescens	3	–	Tyree, M.T., Cochard, H., 1996. Summer and winter embolism in oak: impact on water relations. Ann. For. Sci. 53, 173–180.
Q. robur	2.8	3.46	Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. Nature. 491, 752–755.
Q. robur	2.8	3.5	Cochard, H., Bréda, N., Granier, A., Aussenac, G., 1992. Vulnerability to air embolism of three European oak species (Quercus petraea (Matt) Liebl, Q pubescens Willd, Q robur L). Ann. Sci. For. 49, 225–233.
Q. robur	2.7	3.3	Tyree, M.T., Cochard, H., 1996. Summer and winter embolism in oak: impact on water relations. Ann. For. Sci. 53, 173–180.
Q. robur	2.83	3.41	Urli, M., Porté, A.J., Cochard, H., Guengant, Y., Burlett, R., Delzon, S., 2013. Xylem embolism threshold for catastrophic hydraulic failure in angiosperm trees. Tree Physiol. 33, 672–683.
Q. robur	1.36	–	Venturas, M.D., Rodriguez-Zaccaro, F. D., Percolla, M.I., Crous, C.J., Jacobsen, A.L., Pratt, R.B., 2016. Single vessel air injection estimates of xylem resistance to cavitation are affected by vessel network characteristics and sample length. Tree Physiol. 36, 1247–1259.
Q. robur	1.67	4.3	Tobin, M.F., Pratt, R.B., Jacobsen, A.L., Guzman, M.E., 2013. Xylem vulnerability to cavitation can be accurately characterised in species with long vessels using a centrifuge method. Plant Biol. 15(3): 496–504.
Q. robur	2.26	4.3	Tobin, M.F., Pratt, R.B., Jacobsen, A.L., Guzman, M.E., 2013. Xylem vulnerability to cavitation can be accurately characterised in species with long vessels using a centrifuge method. Plant Biol. 15(3): 496–504.
Q. rubra	2.5	3.4	Cochard, H., Tyree, M.T., 1990. Xylem dysfunction in Quercus: vessel sizes, tyloses, cavitation and seasonal changes in embolism. Tree Physiol. 6, 393–407.
Q. rubra	1.61	3.1	Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. Nature. 491, 752–755.
Q. rubra	2.4	3.7	

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Table A2 (*continued*)

Species	P_{50} (-MPa)	P_{88} (-MPa)	Reference
			Cochard, H., Bréda, N., Granier, A., Aussenac, G., 1992. Vulnerability to air embolism of three European oak species (Quercus petraea (Matt) Liebl, Q pubescens Willd, Q robur L). Ann. Sci. For. 49, 225–233.
Q. rubra	2.3	4.1	Tyree, M.T., Cochard, H., 1996. Summer and winter embolism in oak: impact on water relations. Ann. For. Sci. 53, 173–180.
Q. stellata	1.44	2.5	Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. Nature. 491, 752–755.
Q. suber	5.02	7	Vaz, M., Cochard, H., Gazarini, L., Graça, J., Chaves, M.M., Pereira, J.S., 2012. Cork oak (Quercus suber L.) seedlings acclimate to elevated CO2: Photosynthesis, Growth, Wood Anatomy and Hydraulic Conductivity. Trees - Struct. Funct. 26, 1145–1157.
Q. suber	5.3	5.9	Tyree, M.T., Cochard, H., 1996. Summer and winter embolism in oak: impact on water relations. Ann. For. Sci. 53, 173–180.
Q. suber	2.89	7.3	Pinto, C.A., David, J.S., Cochard, H., Caldeira, M.C., Henriques, M.O., Quilhó, T., Paço, T.A., Pereira, J.S., David, T.S., 2012. Drought-induced embolism in current-year shoots of two Mediterranean evergreen oaks. For. Ecol. Manag. 285, 1–10.
Q. suber	2.94	7.8	Pinto, C.A., David, J.S., Cochard, H., Caldeira, M.C., Henriques, M.O., Quilhó, T., Paço, T.A., Pereira, J.S., David, T.S., 2012. Drought-induced embolism in current-year shoots of two Mediterranean evergreen oaks. For. Ecol. Manag. 285, 1–10.
Q. subpyrenaica	5	8.7	Peguero-Pina, J.J., Sancho-Knapik, D., Martín, P., Saz, M.Á., Gea-Izquierdo, G., Cañellas, I., Gil-Pelegrín, E., 2015. Evidence of vulnerability segmentation in a deciduous Mediterranean oak (Quercus subpyrenaica EH del Villar). Trees 29, 1917–1927.
Q. wislizenii	1.37	5	Tobin, M.F., Pratt, R.B., Jacobsen, A.L., Guzman, M.E., 2013. Xylem vulnerability to cavitation can be accurately characterised in species with long vessels using a centrifuge method. Plant Biol. 15(3): 496–504.
Q. wislizenii	1.72	7	Tobin, M.F., Pratt, R.B., Jacobsen, A.L., Guzman, M.E., 2013. Xylem vulnerability to cavitation can be accurately characterised in species with long vessels using a centrifuge method. Plant Biol. 15(3): 496–504.
Q. wislizenii	1.39	7	Tobin, M.F., Pratt, R.B., Jacobsen, A.L., Guzman, M.E., 2013. Xylem vulnerability to cavitation can be accurately characterised in species with long vessels using a centrifuge method. Plant Biol. 15(3): 496–504.

Table A3
Values of Turgor Loss Point (TLP) and Minimum water potential recorded in the field at midday (Ψ_{MD}) for *Quercus ilex* and references from which data have been extracted.

Criterion	Value (Mpa)	Reference
TLP	−2.85 −3.2 −3.4 −4	Sala, A., Tenhunen, J.D., 1994. Site-specific water relations and stomatal response of Quercus ilex in a Mediterranean watershed. Tree physiol. 14(6), 601–617. https://doi.org/10.1093/treephys/14.6.601.
	−2.95	Nardini, A., Salleo, S., Gullo, M.A.L., Pitt, F., 2000. Different responses to drought and freeze stress of Quercus ilex L. growing along a latitudinal gradient. Plant ecol. 148 (2), 139–147. https://doi.org/10.1023/A:1009840203569 .
	−2.43	Villar-Salvador, P., Planelles, R., Oliet, J., Peñuelas-Rubira, J.L., Jacobs, D.F., González, M., 2004. Drought tolerance and transplanting performance of holm oak (Quercus ilex) seedlings after drought hardening in the nursery. Tree physiol. 24(10), 1147–1155. https://doi.org/10.1093/treephys/24.10.1147.
	−2.95	Gullo, M.A.L., & Salleo, S., 1993. Different vulnerabilities of Quercus ilex L. to freeze-and summer drought-induced xylem embolism: an ecological interpretation. Plant, Cell Environ. 16(5), 511–519. https://doi.org/10.1111/j.1365–3040.1993.tb00898.x.
	−2.23	Serrano, L., Penuelas, J., 2005. Contribution of physiological and morphological adjustments to drought resistance in two Mediterranean tree species. Biol. Plant. 49(4), 551–559. https://doi.org/10.1007/s10535–007–0087–8.
Ψ_{MD}	−2.6 −2.8	Urli, M., Lamy, J.B., Sin, F., Burlett, R., Delzon, S., Porté, A. J., 2015. The high vulnerability of Quercus robur to drought at its southern margin paves the way for Quercus ilex. Plant Ecol. 216(2), 177–187. https://doi.org/10.1007/s11258–014–0426–8.
	−3.5	Pinto, C.A., David, J.S., Cochard, H., Caldeira, M.C., Henriques, M.O., Quilhó, T., Paço, T.A., Pereira, J.S., David, T.S., 2012. Drought-induced embolism in current-year shoots of two Mediterranean evergreen oaks. For. Ecol. Manag. 285, 1–10. https://doi.org/10.1016/j.foreco.2012.08.005.
	−2.6	Baquedano, F.J., Castillo, F., 2007. Drought tolerance in the Mediterranean species Quercus coccifera, Quercus ilex, Pinus halepensis, and Juniperus phoenicea. Photosynthetica 45, 229–238. https://doi.org/10.1007/s11099–007–0037-x.
	−4.2	Pesoli, P., Gratani, L., Larcher, W., 2003. Responses of Quercus ilex from different provenances to experimentally imposed water stress. Biol. Plant. 46(4), 577–581. https://doi.org/10.1023/A:1024823830225.
	−2.7	Bussotti, F., Bettini, D., Grossoni, P., Mansuino, S., Nibbi, R., Soda, C., Tani, C., 2002. Structural and functional traits of Quercus ilex in response to water availability. Environ. Exp. Bot. 47(1), 11–23. https://doi.org/10.1016/S0098–8472(01)00,111–3.
	−4.91	Limousin, J.M., Longepierre, D., Huc, R., Rambal, S., 2010. Change in hydraulic traits of Mediterranean Quercus ilex subjected to long-term throughfall exclusion. Tree Physiol. 30, 1026–1036. https://doi.org/10.1093/treephys/tpq062.

Freezing and thawing of the xylem water, in single or multiple cycles, may induce hydraulic conductivity losses and limit the survival of a tree species and its geographical distribution (Pockman and Sperry, 1996; Willson and Jackson, 2006). In the absence of refilling mechanisms during the spring, that have been reported in some tree species associated to the development of positive pressures (Cochard et al., 2001; Améglio et al., 2002), the loss of conductivity after the winter colds can be almost complete, especially in the wide vessels of ring-porous oaks (Cochard and Tyree, 1990; Corcuera et al., 2006). Cavitation induced during winter has been largely explained in different studies (Feng et al., 2015 and references therein). Basically, bubbles produced during the freezing of the xylem water expand when thawing, assuming a certain tension in the xylem. The wider the bubbles are the more likely will be the process of cavitation, being the bubble diameter proportional to the dimension of the xylem conduit concerned (Ameglio et al., 2002). In fact, Davis et al. (1999) proposed 44 μm as the conduit diameter above which the vessel will be very prone to be cavitated due to this process. Of course, the water tension in the xylem when thawing also affects the process, relating both stresses (drought and frost) in the final induction of hydraulic loss, which is especially critical in evergreen species that maintain their transpiration area during the winter (Willson and Jackson, 2006).

Does this process of frost-induced cavitation affect holm oak survival in its natural areas? Unfortunately, the number of published studies concerning this process is really limited, especially if compared to those related to drought-induced embolism. First data about frost effect on hydraulic conductivity in *Q. ilex* can be found in LoGullo and Salleo (1993), when comparing the relative importance of both factors inducing cavitation – drought and frost – in this species in Sicily (Italy). The widest vessels in one-year old stem that they measured in this population – diameter class from 40 μm to 60 μm – were highly vulnerable to freezing temperatures in terms of hydraulic conductivity losses, while narrowest – fewer than 30 μm – were not affected at any of the temperatures experienced by the plants in their experiment.

Comparison of native embolism in plants from two contrasted climates in Italy, namely at Sicily (Southern Mediterranean Basin) and Friuli Venezia Giulia (Northeastern Italy), evidenced that cavitation during the winter in this last location was clearly higher than the measured during the summer, and also higher than that experienced in the location in Sicily (Nardini et al., 2000). The authors of this study concluded that *Q. ilex* is more limited by frost than by drought in its geographical distribution, confirming the assumption that holm oak is confined to freeze-free areas due to its overall susceptibility to frost injuries (Nardini et al., 1998).

However, this is not applicable to most of the populations in other territories of the distribution area of the species. Corcuera et al. (2004) studied a population of holm oak growing under a typical continental subtype of the Mediterranean-type climate, with a short freeze-free period (May to October) at Cubel (Zaragoza, Spain). The mean vessel diameter in one-year old stem in this population was below 20 μm, with a few vessels above 30 μm. No data of vulnerability to frost-induced cavitation were reported in this area, but the much narrower vessels in the studied population could indicate that the stems are probably free of risk concerning this process. In fact, the perfusion with phloxine of branches in this population revealed that functional vessels (dye-stained when perfused at low pressure) were found in three year-old growth rings (unpublished results).

3.2. *Resistance to low temperatures at leaf cell level*

Evergreen species living under Mediterranean-type climates such as holm oak are often subjected to freezing temperatures during winter months that impair the enzymatic processes involved in photosynthesis. Hence, under these conditions, overwintering species need mechanisms to protect the photosynthetic apparatus, which are mainly based on sustained energy dissipation and antioxidants (see García-Plazaola et al., 2017 and references therein).

Sustained thermal energy dissipation during winter is characterized by reductions in F_V/F_M together with overnight retention of the de-epoxidized forms of the VAZ cycle (A and Z). This mechanism protects the photosynthetic apparatus from excess excitation pressure when plants are exposed to high light and low temperatures during winter (Verhoeven, 2014). Several studies have described the implementation of this photoprotective mechanism in response to low temperatures in *Q. ilex*. Thus, the occurrence of chronic winter photoinhibition in *Q. ilex* subsp. *ballota* was firstly reported by Martínez-Ferri et al. (2004), who registered low predawn F_V/F_M values (ranging from 0.4 to 0.7) due to cumulative effect of low temperatures and high excitation energy throughout winter. These authors also suggested that the association of this phenomenon with the overnight retention of Z and A might be attributed to the inhibitory effect of chilling temperatures on the enzymatic conversion of Z and A to violaxanthin (V) (Adams and Demmig-Adams, 1995; Adams et al., 1995). In line with this, Corcuera et al. (2005) demonstrated the presence of A and Z at predawn in leaves of *Q. ilex* subsp. *ballota* after cold nights due to an increase in NPQ. This study also confirmed that this species showed long-term PSII down-regulation (i.e. low predawn F_V/F_M values) during winter, which could be partly caused by changes in the chlorophyll pigment bed associated with the presence of A and Z in the PSII antenna (Gilmore and Ball, 2000). Similar results were further obtained by Baquedano and Castillo (2007), who reported chronic photoinhibition and increased de-epoxidation of xanthophyll pigments at predawn for this species during winter. However, contrary to previous studies, these authors did not find high NPQ values in winter. Regarding *Q. ilex* subsp. *ilex*, Arena et al. (2008) put in evidence the essential role of thermal dissipation as the main process to dissipate the excess absorbed light during winter. These authors concluded that the drawdown of F_V/F_M and the increased NPQ in the early morning at the end of the winter constituted an effective photoprotective mechanism to avoid photo-oxidative damage to chlorophylls, which provided remarkable stability to the photosynthetic apparatus when subjected to winter stress conditions (Gratani et al., 1998). In line with this, Sperlich et al. (2014) also stated that increased NPQ and lower F_V/F_M values in *Q. ilex* subsp. *ilex* could be related to several photoprotective mechanisms (i.e. re-organization of the thylakoid membrane, closure of reaction centres and/or reduced antennal size) that allowed this species to keep the photosynthetic apparatus intact in response to low temperatures.

Besides sustained energy dissipation, several studies have also analysed the role of antioxidants in holm oak as a mechanism to avoid the risk of oxidative damage associated with the potential generation of reactive oxygen species (ROS) under low winter temperatures. With respect to non-enzymatic antioxidants, García-Plazaola et al. (1999) registered a high synthesis of ascorbate during winter when compared to spring in *Q. ilex*. However, García-Plazaola et al. (2003) found that the most highly induced non-enzymatic antioxidant in *Q. ilex* for the coldest winter was α-tocopherol. On the other hand, regarding enzymatic antioxidants, García-Plazaola et al. (1999) reported an induction in glutathione reductase (GR) and monodehydroascorbate reductase (MDAR) activity in *Q. ilex* during winter, reducing the over-excitation of electron transport chain and ROS production. In line with this, Corcuera et al. (2005) registered a high activity of superoxide dismutase (SOD) and GR in leaves of *Q. ilex* subsp. *ballota* during winter, which implied an increased ability to withstand oxidative stress at low temperature (Kingston-Smith and Foyer, 2000). In fact, these authors did not observe leaf photo-oxidative stress, in accordance with the absence of changes in chlorophyll concentration during winter.

In summary, holm oak has developed drastic antioxidant and photoprotective mechanisms in response to winter stress that could be of adaptive importance. Thus, this species could improve the carbon balance under favourable winter conditions, re-adjusting and protecting its photosynthetic machinery when these relatively favourable conditions changed (García-Plazaola et al., 2017). The full recovery of photosynthetic capacity and PSII efficiency when temperature increased in spring also evidences the adaptive capacity of holm oak to withstand low temperatures during winter, both for *Q. ilex* subsp. *ilex* (Gratani et al., 1998) and *Q. ilex* subsp. *ballota* (Corcuera et al., 2005).

Throughout this review we have shown that photoprotection mechanisms described for *Q. ilex* in response to low temperatures are very similar to those implemented by this species to cope with summer drought (see above in this manuscript). With regard to this issue, Verhoeven et al. (2018) noted that the ability of plants to withstand dehydration stress is closely related to the tolerance to freezing temperatures, and the similarities in response at the cellular level are marked. Moreover, these authors claimed that maintenance of cellular structure is critical to tolerance of desiccation and low temperature conditions.

To examine this issue, we have taken advantage of our own unpublished data where it was examined freezing tolerance in leaves of *Q. ilex* subjected to different temperatures by measuring F_V/F_M changes as an indicator of photoinhibition induced by low temperatures, as firstly proposed by Peguero-Pina et al. (2008b). Specifically, leaves of four provenances of *Q. ilex* subsp. *ballota* from Spain (Cazorla, Cuenca, Soria and Extremadura) and four provenances of *Q. ilex* subsp. *ilex* from Spain (Gerona) and Italy (Veneto, Lazio and Sardinia) were exposed to various freezing temperatures between −5 °C and −40 °C and F_V/F_M was subsequently measured with a FMS II modulated fluorometer (Hansatech Instruments, Norfolk, UK) following the methodology described by Sangüesa-Barreda et al. (2018). The results obtained revealed that all studied provenances showed high resistance to freezing (Fig. 10), ranging from −19.8 °C (Veneto and Sardinia) to −26.6 °C (Soria provenance) (data not shown). It should be noted that these temperatures were much lower than those typically occurring in the geographical location of each provenance (Fig. 9).

4. Conclusions

Holm oak has been considered as a genuine representative species of the Mediterranean flora by different geobotanical synthesis. The hard (sclerophyllous) and evergreen leaves developed by this species have been interpreted as an evolutive response to the stressful situations potentially imposed by the Mediterranean-type climate, i.e. hot-dry summers and cold winters. Nevertheless, the evolutionary origin of this taxon can be traced back to pre-Mediterranean lineages living in humid, tropical or subtropical climates. In fact, most of the species of the section *Ilex* inhabit now in territories under tropical, subtropical or temperate climates, while showing similar leaf morphology and phenology than *Q. ilex* itself. The existence of an extensive amount of information about the ecophysiological response of *Q. ilex* from different territories, supplemented with some unpublished results, allowed us to perform this metanalysis.

The analysis of previous published studies revealed that *Q. ilex* showed a considerable degree of within-species variability in the ability to cope with water stress in terms of xylem vulnerability to drought-induced cavitation, one of the main constraints for plant growth or survival in Mediterranean areas. As an example, P_{50} values from ca. −2 to −7 MPa have been reported, with a similar variation range when P_{88} is considered. A deeper analysis of this information allows concluding that these high P_{50} and P_{88} values are mostly found in studies concerning populations from the subspecies found in the western distribution range (*Q. ilex* subsp. *ballota*). Concerning the effect of freeze-thaw cycles on the xylem cavitation, only a few data are available. However, while *Q. ilex* is assumed to be highly vulnerable to this process, occupying coastal areas in the central and eastern locations, the populations in the western area (Iberian Peninsula and North of Africa) can occupy continental-type climates where freeze-thaw events are probable during more than three months in the winter. So, in terms of the vulnerability to cavitation in the stem, two contrasting performances can be observed within holm oak, corresponding to both different subspecies.

Contrastingly, the analysis of the response of holm oak at leaf-level to summer drought and winter cold indicated that both subspecies developed effective antioxidant and photoprotective mechanisms that minimized the risk of photodamage, preserving the leaves when photosynthetic activity is impaired. Moreover, leaves of both subspecies showed a high resistance at cell level to extremely low and high temperatures. Overall, the analysis of these results revealed that holm oak is characterized by a conservative leaf strategy, irrespective of the subspecies and/or the climatic conditions. The maintenance of the photosynthetic machinery after a stress period is of paramount importance for improving the carbon balance in an overwintering species such as *Q. ilex*. Therefore, this species can be considered a paradigm of tolerance to extreme temperatures at leaf level, in spite of its evolutionary origin under tropical or subtropical climates.

Finally, when atmospheric dryness is considered, VPD can impose very restrictive limitations for the plant to open stomata and photosynthesise in the case of *Q. ilex* due to its water-saver strategy. Thus, especially during summer, the stressful conditions imposed by Mediterranean summers derives in a trade-off between growth and survival.

Author contributions

R.M.-S., J.J.P.-P. and E.G.-P. conceived the review and prepared the original draft; all authors contributed to the writing and review of the final version of the manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix

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Capítulo III

Oak leaf morphology may be more strongly shaped by climate than by phylogeny

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RESEARCH PAPER **Open Access**

Oak leaf morphology may be more strongly shaped by climate than by phylogeny

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Abstract

Key message Despite been grown under the same climate, oak species are able to correlate with looser, but still identifiable, leaf morphological syndromes, composed by morphological traits with an ecological role in their respective macroclimates.

Context Environmental restrictions imposed by climate have been shown to modulate leaf morphology. A reduction of leaf area in hot and dry climates reduces overheating because of a thinner boundary layer. Lobed shapes enhance hydraulic conductivity and faster cooling. Elongated leaves drain more quickly under high precipitation. Trichomes may help to reduce the effects of excessive sun exposure in hot and dry environments. Leaf mass per area (LMA) increases in stressful environments.

Aim To assess the influence of global climate and clade on oak leaf syndromes comprising morphological traits with ecological roles.

Methods We analyzed seven morphological traits in 141 oak species grown in a botanical garden, characterized into five macroclimates, and explored the partial effects of clade and climate.

Results We found significant associations between macroclimate and every morphological trait measured. Temperate species tend to have large, obovate, lobed, malacophyllous leaves. Species occurring in dry habitats usually present small, rounded, pubescent, sclerophyllous leaves. Warm and wet climates induce the development of slender, lanceolate, glabrous leaves with an acuminate apex.

Conclusion The functional roles of the different morphological traits are partially confirmed in genus *Quercus* as a response to the different macroclimates, where different leaf syndromes can be distinguished.

Keywords *Quercus*, Macroclimate, Functional traits, Factor Analysis of Mixed Data, Leaf syndrome

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1 Introduction

Leaves are the primary photosynthetic organs of plants. Their morphology should be adapted to environmental conditions (Traiser et al. 2005), as seemingly fine differences in leaf form may strongly affect the capacity of plants to grow and survive under different environments (Givnish 1987; Desmond et al. 2021). It has long been recognized that natural selection by climatic restrictions may influence leaf morphology (Stahl 1880, 1883; Schimper 1903). In the past two decades, numerous studies have found correlations between leaf morphological traits (e.g. leaf size and shape) and environmental factors (mainly annual mean temperature and precipitation) from global to local scales (Royer et al. 2005; Traiser et al. 2005; Adams et al. 2008; Peppe et al. 2011; Li and Wang 2021). Leaf area, shape, pubescence, and dry mass per area in particular have proven to be of high functional significance (Alonso-Forn et al. 2020; Cavender-Bares and Ramírez-Valiente 2017; Du et al. 2022; Sancho-Knapik et al. 2021).

Leaf area varies and evolves in response to water availability, temperature, and other aspects of climate (Webb 1968; Dolph and Dilcher 1980; Givnish 1984; Peppe et al. 2011; Li et al. 2020). Small leaves have been linked to harsh conditions such as hot, dry and light-saturating environments (Peguero-Pina et al. 2020; Nicotra et al. 2011) due to the thinner boundary layer of small leaves (Yates et al. 2010), which facilitates heat loss (Roth-Nebelsick 2001). Leaf temperature tends to be higher in large leaves due to their thicker boundary layers (Vogel 1970; Parkhurst and Loucks 1972; Givnish 1987; Vogel 2009).

Leaf shape—e.g. length–width ratio, toothing and lobation, apex structure—interacts with leaf area in adapting plants to climate and habitat. Leaf shape has various functional roles, including thermoregulation, hydraulic constraints, and leaf expansion patterns in deciduous species (Nicotra et al. 2011). Plants in cool regions tend to have broader leaves with lower length–width ratios to increase the thickness of their boundary layers and prevent wind cooling, maintaining higher leaf temperatures for photosynthesis (Wright et al. 2017; Li et al. 2020). In contrast, plants in warm and humid regions often have elliptic leaves with higher length–width ratios to avoid overheating and enhance water transport (Wright et al. 2017; Hu and Hu 2015), analogous to the effect of having smaller leaves. Mean annual temperature has long been shown to influence leaf toothing and lobation, with narrow and deeply lobated leaves better adapted to and more frequent in warmer climates due to thinner boundary layers and enhanced hydraulic efficiency (Sisó et al. 2001; Sack and Tyree 2005; Leigh et al. 2017). However, toothed leaves are more common in cool climates, where they are considered an adaptation that enhances sap flow and carbon gain early in the growing season (Bailey and Sinnott 1916; Baker-Brosh and Peet 1997; Royer and Wilf 2006; Nicotra et al. 2011; Peppe et al. 2011; MacKee et al. 2019; Li and Wang 2021).

Leaf dry mass per unit area (LMA) is a leaf morphological trait related to shape widely used as a proxy for sclerophylly (see Alonso-Forn et al. 2020 and references therein). LMA commonly varies along environmental gradients (Niinemets 2001; Poorter et al. 2009). It is widely recognized that an increase in LMA is a general response to environmental stress (Onoda et al. 2011; Alonso-Forn et al. 2020), such as drought (Turner 1994; Reich et al. 1999; Jordan et al. 2005) or low winter temperatures (Ogaya and Peñuelas 2007; González-Zurdo et al. 2016; Niinemets 2016). Recently, Sancho-Knapik et al. (2021) showed that LMA in deciduous oak species may be modulated by aridity, whereas diversification of LMA within evergreen oaks seems modulated by both aridity and winter cold.

The functions of leaf pubescence are more ambiguous. Functions that have been demonstrated include providing resistance against biotic or abiotic damages, absorbing nutrients and water, and protecting against excessive solar radiation (Ehleringer et al. 1976; Sakai et al. 1980; Ehleringer 1981; Werker 2000; Morales et al. 2002; Agrawal et al. 2009; Fernández et al. 2014). Adaxial trichomes may increase leaf reflectance, which could be advantageous in dry and hot climates by reducing heat load and leaf temperature without increasing water loss through transpiration (Ehleringer and Björkman 1978; Ehleringer and Mooney 1978; Pérez-Estrada et al. 2000; Abdulrahaman and Oladele 2011; Bickford 2016). Additionally, trichomes have been explored for their role in repelling water from the leaf surface to keep stomata dry and ensure free gas exchange (Brewer and Smith 1997; Pierce et al. 2001). However, some studies have found conflicting results regarding the role of trichomes in regulating leaf transpiration and coping with aridity, indicating that further research is needed to understand better their functional significance and possible context-dependence of different roles (Aronne and DeMicco 2001; Haworth and McElwain 2008).

There are few studies within Fagaceae genera that analyze the relationships between morphological traits and their response to climatic variables in a broad set of species and climatic conditions. For example, Chen and Kohyama (2022) studied in 91 *Lithocarpus* species how fruit and vegetative traits correlate with temperature and wetness, however, *Lithocarpus* is a genus with little morphological and climatic variation compared to other Fagaceae genera. Most of the research focuses on other proxies, such as how a reduced set of species, or even a single species, responds to a climatic gradient or to climate change, typically using the most common species (e.g. *Fagus sylvatica* or *Castanea sativa*). *Quercus* (oaks) is the genus with more research in this matter since it is an excellent system for investigating how co-variation in leaf traits reflects complex adaptations to varying environments (Ramírez-Valiente et al. 2020; Cavender-Bares 2019). Oaks show considerable variation among-species in leaf morphological traits, climatic niches and geographical range (Gil-Pelegrín et al. 2017; Cavender-Bares 2019; Jiang et al. 2019; Sancho-Knapik et al. 2021). Although some studies have explored the relationships between climate and morphology within oaks (Kaproth et al. 2023; Ramírez-Valiente et al. 2020) they are limited by the number of species or the biogeographical range. To our knowledge, there is not any study taking into account a great number of species, representing almost every biome occupied by oak species and involving species from each infrageneric clade.

The genus is divided into two monophyletic subgenera with eight monophyletic sections: subgenus *Quercus* (Sect. *Lobatae*, *Protobalanus*, *Ponticae*, *Virentes* and *Quercus*), with a hypothesized Nearctic origin (Cavender-Bares 2019; Hipp et al. 2020); and subgenus *Cerris* (Sect. *Cyclobalanopsis*, *Cerris* and *Ilex*), which likely evolved in warm-temperate east Asia (Denk et al. 2023) and the northern Paleotropics (Deng et al. 2018; Jiang et al. 2019).

The study aims to assess in a wide range of oak species (141) to what extent leaf morphological traits (leaf area, leaf mass per area, length–width ratio, index of lobation, leaf shape, leaf apex, leaf margin, pubescence and leaf habit) are associated with the prevailing macroclimate within the species distribution. The study also includes clade as a covariate to investigate the partial effects of clade and climate in the evolution of oak leaf syndromes. These analyses help illuminate the relative importance of phylogenetic niche conservatism and adaptation in the broad evolution of oak leaf syndromes.

2 Material and methods

2.1 Plant material

One hundred and forty-one oak species (*Quercus* spp.) (Martín-Sánchez et al. 2024, Table S1) were sampled during midsummer from living collections in the Jardín Botánico de Iturrarán (43°130N, 02°010W, 70 m asl, Gipuzkoa, Spain, www.iturraran.org). Using of the botanical garden as a quasi-common garden in our study enables interspecific comparisons of traits that result from long-term adaptation to the species' native climate without conflating that adaptive history with the short-term plastic response of each tree to the climate where it occurs naturally. Note that some variation we observe is plastic in response to site conditions within the botanic garden, but this variation is essentially irrelevant to our question about among-biome adaptation. Ten fully developed leaves per species (3–4 leaves per tree × 3 different trees) were collected from mature trees (15–25 years old) in midsummer. Leaves were sampled from south-exposed branches during early morning (07:00–09:00 h, solar time) and they were stored in sealed plastic bags and carried to the laboratory in portable coolers to measure both quantitative and qualitative morphological traits.

2.2 Morphological traits

In this study, we considered five qualitative variables: overall shape, apex shape, margin, pubescence and leaf habit. We also measured four quantitative variables: leaf area (LA), leaf mass per area (LMA), length–width ratio (LW) and index of lobation (ILB). Leaf shape, apex and margin were classified according to Ellis et al. (2009) as one of six shapes (obovate, elliptic, oblong, ovate, lanceolate or circular), one of four apex shapes (rounded, acute, acuminate or straight), and one of six margin types (entire, serrate, dentate, crenate, undulate or spinose). Although quantitative variables are always preferred, we decided to include qualitative traits to support the continuous traits. The variability in nominal margin is gathered by the continuous ILB. In the same way, LW strongly overlaps with the shape. However, nominal traits may explain nuances that are not achieved with these continuous traits. Regarding pubescence, we followed Gil-Pelegrín et al. (2017), classifying species into three categories: glabrous (or with scattered trichomes), pubescent, and densely pubescent, according to the botanical description of the species in their respective floras (Flora of North America, Nixon 1993; Flora of China, Huang et al. 1999; and Flora Europaea, Schwarz 1964). The same floras were taken into account to classify species as deciduous or evergreen.

Leaves were digitized and processed using *ImageJ* (http://rsb.info.nih.gov/nih-image/) to measure LA, length and width (for LW) and perimeter (for ILB, calculated as perimeter / square root of LA) (Valladares et al. 2000). Afterwards, leaves were oven dried for 3 d at 70 °C and weighed using an analytical balance (Sartorius AG Model BP221S; Sartorius AG, Goettingen, Germany). LMA is calculated as the ratio of the foliage dry mass to foliage area. For 85 of our 141 species, LA and LMA data were already published (Sancho-Knapik et al. 2021; Kattge et al. 2020; http://doi.org/https://doi.org/10.17871/TRY.64.).

2.3 Climatic and geographic data

Climate analysis for each studied species were carried out following a similar procedure used by Gil-Pelegrín et al. (2017). We first obtained geographical distribution coordinates for each species individually from GBIF (https://www.gbif.org/) taking into account their distribution ranges (when available, see Euforgen and US Tree Atlas databases) or the geographical descriptions in the beforementioned floras to crop the areas from where points were downloaded. Then, the downloaded datasheets were thinned using SDMtune R package so that redundant points within a square kilometre were removed (Vignali et al. 2022). The resultant information (see Martín-Sánchez et al. 2024, Table S2) was used to obtain the values of each climatic variable from the WorldClim version 2.1 database (Fick and Hijmans 2017. WorldClim 2. https://www.worldclim.org/) for each presence point using Raster R package. We calculated the annual precipitation range (bio20) as BIO16 minus BIO17 (see Martín-Sánchez et al. 2024, Table S3 for the meaning of each bioclimatic variable). Points were also overlaid on a climatic Köppen map (Köppen 1936) to extract their Köppen climate (Martín-Sánchez et al. 2024, Table S2). Köppen climates inhabited by *Quercus* were grouped into five macroclimates: Arid, Mediterranean, Winter-dry temperate to Tropical, Warm Temperate and Cold Temperate, similar to the schema used by Gil-Pelegrín et al. (2017) (Fig. 1). Arid macroclimate (ARID) is defined by climates where annual precipitation is lower than evapotranspiration (BSk and BSh primarily, with some peripheral populations in BWk or BWh). Mediterranean macroclimate (MED) comprises climates with the dry period in summer (Csa, Csb, Csc, Dsa and Dsb). Winter-dry temperate to Tropical macroclimate (WdT-TROP) includes warm and temperate climates with a dry season other than summer (Aw, Am, Cwa and Cwb). Warm Temperate macroclimate (WARM_T) comprises Cfa Köppen climates, since it represents a climate widely colonized by *Quercus* species, especially in southeastern regions of the United States and China. The Cold Temperate (COLD_T) macroclimate comprises Dfa, Dfb, Dwa, Dwb, Cfb, and Cfc primarily, with some peripheral populations in Dsc, Dsd, Dwc, Dwd, Dfc or Dfd. Several species of oaks, especially some *Cyclobalanopsis* species, live in an equatorial climate (Af), which was not treated in this study as these last species are not considered in this study (for a more detailed definition of the Köppen climate categories see Martín-Sánchez et al. 2024, Table S4). Species were assigned to each macroclimate according to where most of their occurrences fall compared with the rest of the other macroclimates (Appendix Fig. 8).

2.4 Statistical analysis

ANOVAs were performed to quantify the variance explained by macroclimate, subgenus, section—nested into subgenus—on each quantitative variable (LA, LMA, LW and ILB) (Table 1). We also performed ANOVAs to compare quantitative traits among macroclimates. Multiple comparisons were carried out among macroclimates for LA, LMA, LW and ILB using the post-hoc Tukey's honestly significant difference test. Heteroscedasticity and normality of the data were checked throughout analyses of the residuals, and Kolmogorov–Smirnov tests were carried out using the DHARMa R package (Hartig 2022). In addition, contingency tables based on pairwise χ^2 tests—corrected by Holm-Bonferroni method for multiple comparisons—were used to test the correlation between macroclimate and the qualitative traits: margin, apex, shape, pubescence and leaf habit.

Factor analysis of mixed data (FAMD) was carried out to summarize the multivariate relationships among

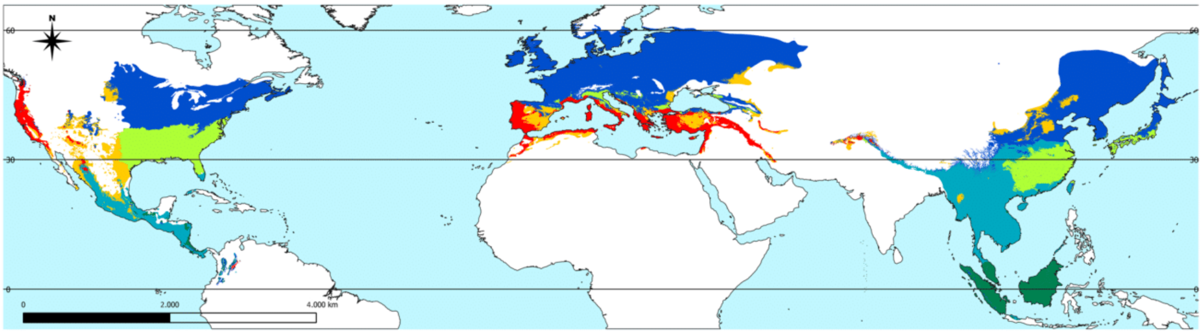

Fig. 1 Map of the five macroclimates considered in this study: Arid in yellow (BSk, BSh, according to Köppen Classification), Mediterranean in red (Csa, Csb, Csc, Dsa, Dsb), Winter-dry to Tropical in cyan (Aw, Am, Cwa, Cwb), Warm Temperate in light green (Cfa) and Cold Temperate in dark blue (Cfb, Cfc, Dfa, Dfb, Dwa, Dwb). Colored areas represent the regions where *Quercus* species are present. The Equatorial climate (not considered in this study) is represented by Tropical Rainforest Climate (Af) and coloured with dark green

Table 1 Summary of percentage of variance explained by ANOVAs performed for each variable separately: Leaf area (LA), length–width ratio (LW), index of lobation (ILB), leaf mass per area (LMA) and altogether (PC1 scores from FAMD). Each row represents a separate ANOVA. All models include all factors: macroclimate, subgenus and section nested in subgenus. Number of asterisks represents the level of significance ($P<0.001$ ***, $P<0.01$ **, $P<0.05$ *). The proportion of variance is calculated dividing the sum squares for each factor by the total sum of squares

	Macroclimate	Subgenus	Section	Residuals
LA	30.0 ***	1.47	3.70	64.8
LW	18.1 ***	1.71	9.64 **	70.6
ILB	11.3 **	0.25	5.49	83.0
LMA	6.64 *	6.61 ***	16.4 ***	70.3
Altogether	29.9 ***	4.79 **	3.90	61.4

morphological traits. FAMD has been proved to be an accurate statistical method used to analyze a data set containing both quantitative and qualitative variables (Pagès 2004) since they are normalized during the analysis to balance the influence of each set of variables. It was performed using FactoExtra and FactoMineR R packages (Josse and Husson 2008; Kassambara and Mundt 2020). First dimension scores (PC1 Morphology) were treated as a generalized leaf morphological response variable influenced by all four quantitative traits together. An additional ANOVA was performed to quantify the variance explained by macroclimate, subgenus, and section when all traits are taken into account altogether (Table 1). Principal components analysis (PCA) was performed with climatic variables extracted from Worldclim database, with a previous selection of those that accounted for more variance in the first two principal components via the FactoExtra R package (Kassambara and Mundt 2020). Every data included in both FAMD and climatic PCA represent the mean value for each variable and species.

Finally, we did cross-correlations between the first and second dimensions of both FADM and climatic PCA to check if there is any significance between both analyses and to explore the existence of relationships between morphological traits and climatic variables. We first tested if there was a phylogenetic signal performing phylogenetic generalized least squares (PGLS) regressions using the most complete published phylogenetic tree for *Quercus* (Hipp et al. 2020). Section and subgenus names correspond to named clades in Hipp et al. (2020). Ninety-nine species intersected between phylogenetic and trait datasets. We first performed a PGLS with all species. The results do not allow us to refuse the lack of phylogenetic signal. However, since the slopes of both regressions: ordinary least squares (OLS) and PGLS, are minimally different (Appendix Fig. 9) and we want to include all taxa, we decided to present the results of the OLS regression. We also performed additional PGLS within the different clades: both subgenera and the two sections with more species, i.e. *Quercus* and *Lobatae* (Martín-Sánchez et al. 2024, Table S5).

3 Results

ANOVAs reveal that subgenus and macroclimate significantly ($P<0.001$) account for 6.9% and 35.4% of the morphological variance when traits are analyzed altogether (Table 1). The taxonomic section is a non-significant predictor of 3.2% of the variance ($P=0.18$). 54.5% of variance was unexplained. By contrast, when traits are analysed separately, we find that macroclimate explains more variance for LA (29%), LW (20.3%) and ILB (14.4%) than any other factor. However, in the case of LMA, not only macroclimate is significant, but also subgenus and section, with this last one explaining the highest percentage of variance (16.4%) (Table 1).

All quantitative traits differ significantly among macroclimates according to Tukey tests (Fig. 2). LMA shows the least variation among climates, reaching its highest values in drier climates, but only showing a significant difference in its smaller values in Cold Temperate species (8.33 ± 1.47 g m^{-2}) compared with the rest of the macroclimates (Fig. 2b). By contrast, LA is the most variable trait among macroclimates, ranging from a mean value of 7.62 ± 3.86 cm^2 in Arid species to 30.38 ± 28.17 cm^2 in Warm Temperate species, doubling to 64.71 ± 40.64 cm^2 in Cold Temperate species (Fig. 2a). LW varies from rounder in Cold Temperate (1.81 ± 0.41), Mediterranean (1.79 ± 0.34) and Arid (1.96 ± 0.49) species to more elongate in Warm Temperate (2.25 ± 0.97) and Winter-dry temperate to Tropical species (2.56 ± 0.76) (Fig. 2c). Cold Temperate species display the highest lobation ($ILB = 6.43 \pm 1.44$), Winter-dry temperate to Tropical species the lowest (5.11 ± 0.56) (Fig. 2d).

Contingency tables show significant associations between the five qualitative morphological traits and macroclimate. In terms of shape, Obovate leaves relate to Cold Temperate ($P=0.003$) and Warm Temperate ($P<0.001$) species; besides, elliptic leaves to

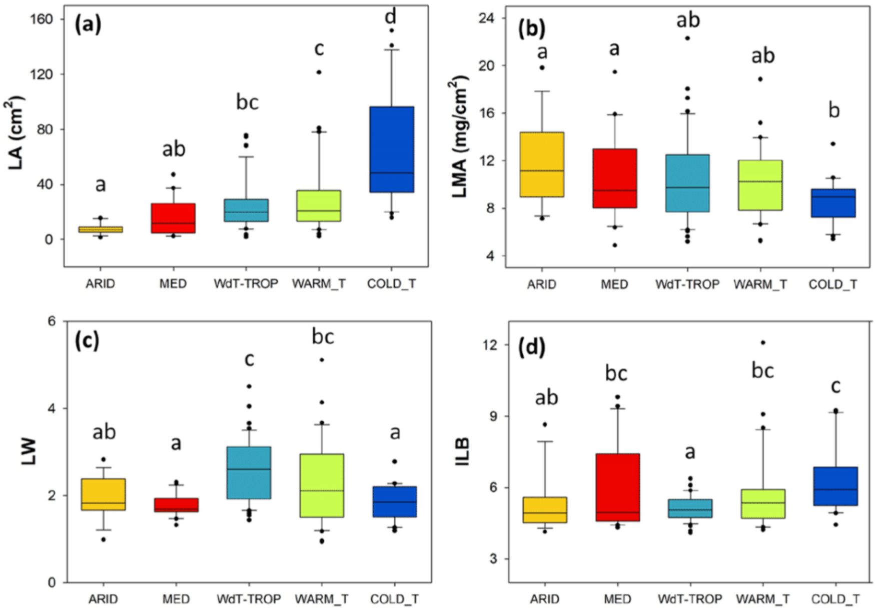

Fig. 2 Box-plot representation of the four quantitative traits measured: Leaf area (LA) (**a**), leaf mass per area (LMA) (**b**), length–width ratio (LW) (**c**) and index of lobation (ILB) (**d**) in the five macroclimates considered: Arid (ARID), Mediterranean (MED), Winter-dry to Tropical (WdT-TROP), Warm Temperate (WARM_T) and Cold Temperate (COLD_T). Letters indicate statistically significant differences across macroclimates (Tukey test, $P<0.05$)

Winter-dry temperate to Tropical species ($P<0.001$) (Fig. 3a). For apex, acute ($P<0.001$) and acuminate ($P=0.01$) apices are linked to Winter-dry temperate to Tropical species (Fig. 3b). Lobated margins are associated with Cold Temperate ($P=0.014$) and Warm Temperate ($P<0.001$) species, entire margins with Warm Temperate ($P=0.014$) and Winter-dry temperate to Tropical species ($P<0.014$) and serrate margins with Winter-dry temperate to Tropical species ($P<0.001$) (Fig. 3c). Glabrous leaves are associated with Winter-dry temperate to Tropical species ($P<0.001$) (Fig. 3d). Finally, deciduousness is significantly correlated with Cold Temperate ($P=0.008$) and Warm Temperate species ($P<0.001$) (Fig. 3).

FAMD on the combined morphological groups species together by macroclimate, but with strong overlap (Fig. 5a). Traits cluster loosely by climate into three groups: (1) obovate, lobated leaves with large areas corresponding mainly to species from Cold Temperate climates, with some Warm Temperate and deciduous Mediterranean species; (2) elongate leaves with acuminate apices, lacking abaxial trichomes tend to be associated with Winter-dry temperate to Tropical and Warm Temperate species; and (3) oblong and undulate leaves with dentate margins, rounded apices, a dense tomentum abaxially and a high LMA are concentrated in Arid and evergreen Mediterranean species (Fig. 5a).

Climatic PCA clusters species into well-defined groups, with the most overlap between Arid and Mediterranean species (Fig. 5b). Cold Temperate species, are defined by a high temperature annual range (BIO3, BIO7), with very cold winters (BIO6). By contrast, Winter-dry temperate to Tropical species are characterized by warmer winters (BIO6) and more temperature-stable climates (BIO3 and BIO4) but pronounced seasonality in precipitation regimes (BIO15, annual precipitation range), with high rainfall during the wet season (BIO16, BIO18, annual precipitation range). Warm temperate species do not suffer a great reduction in precipitation during their driest month. Arid and Mediterranean species occupy climates with a very dry season during the warmest period of the year (BIO18, annual precipitation range) and low annual precipitation (BIO12). This relationship between climate and morphology is supported as well by the OLS regression between morphology PCA axis 1 (PC1-Morph) and climate PCA axis 1 (PC1-Clim), which is significant ($r=0.543$, $P<0.001$) (Fig. 6). The same correlation performing a PGLS shows a similar slope, significance ($P<0.001$) and a low phylogenetic signal ($\lambda=0.28$) (Appendix Fig. 9). The additional PGLS within the different clades: subgenus *Cerris*, subgenus *Quercus*, section *Lobatae* and section *Quercus* (clades named as in Hipp et al. 2020) revealed that we cannot claim that both subgenera are

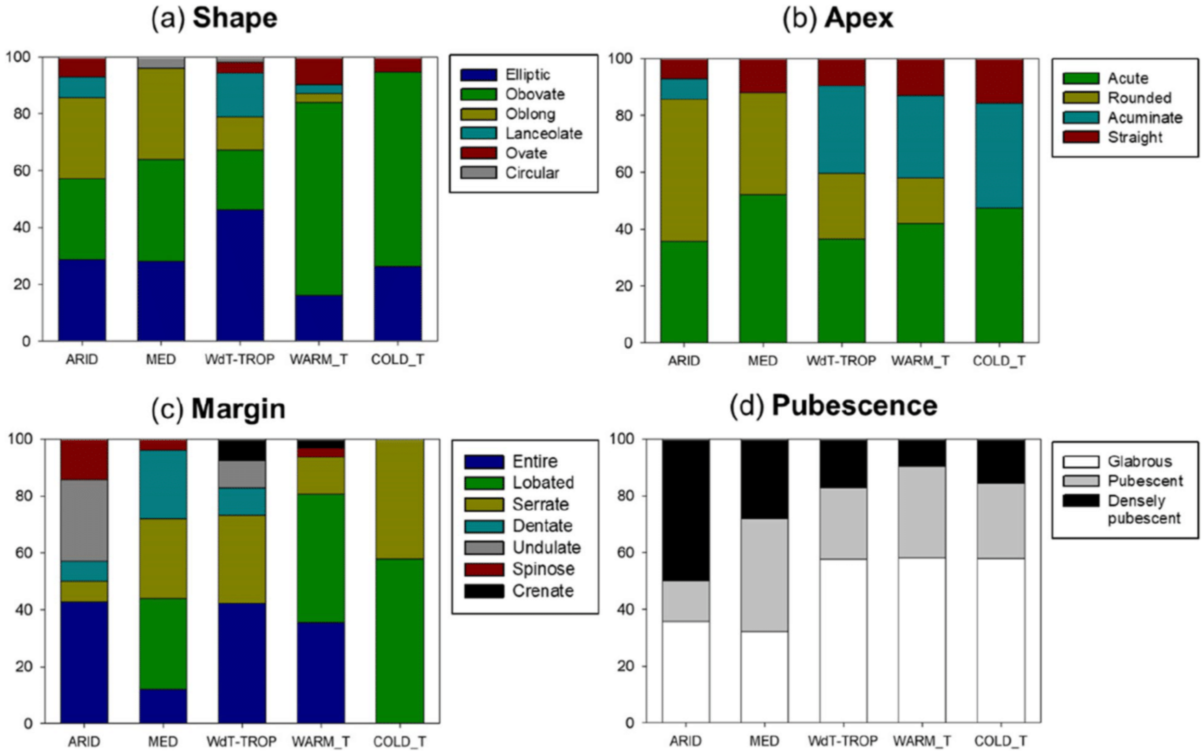

Fig. 3 Relative percentage of species presenting the different qualitative traits in the five macroclimates considered: Leaf shape (**a**), apex (**b**), margin (**c**) and abaxial pubescence (**d**) in Arid (ARID), Mediterranean (MED), Winter-dry Temperate to Tropical (WdT-TROP), Warm Temperate (WARM_T) and Cold Temperate (COLD_T) macroclimates

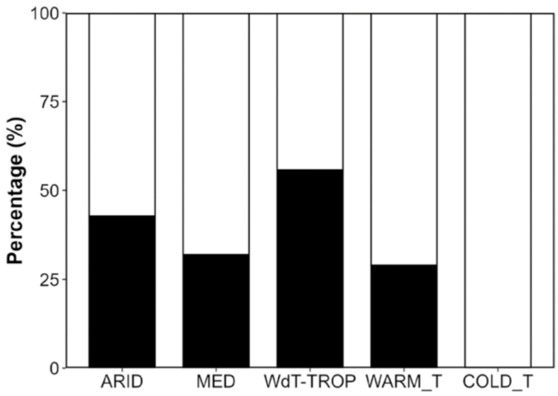

Fig. 4 Relative percentage of species according to their leaf habit in the five macroclimates considered: Deciduous (white) and evergreen (black) in Arid (ARID), Mediterranean (MED), Winter-dry Temperate to Tropical (WdT-TROP), Warm Temperate (WARM_T) and Cold Temperate (COLD_T) macroclimates

significantly different, since the confidence intervals of the slope overlap. However, at section level significant differences can be found between sections *Quercus* and *Lobatae*. (Martín-Sánchez et al. 2024, Table S5).

4 Discussion

4.1 Individual leaf traits correlate with climate

Leaf area exhibits high evolutionary lability in some clades, with high potential to evolve in response to climate variation (Jones et al. 2009; Peppe et al. 2011). Our work shows a strong negative correlation between leaf size, growing season temperature, and aridity. Thus, the smallest leaves are found in Arid and Mediterranean climates, the largest ones in Cold Temperate climates (Fig. 2a). This may be a result of oaks reducing LA to decrease their boundary layer, enhancing heat loss (Vogel 1970; 2009; Givnish 1987). A reduced LA also contributes to a higher leaf-specific conductivity (LSC) of the shoot which is advantageous in dry habitats (Peguero-Pina et al. 2014, 2016). Conversely, larger leaves confer to species a greater photosynthetic rate when compared with smaller leaves. However, many small-leaf species usually compensate for this reduction of photosynthetic tissue with a higher ability to photosynthesize throughout a higher carboxylation rate, mesophyll and stomatal conductance (Peguero-Pina et al. 2016). Of leaf shape traits, lobation, length–width ratio, and leaf apex exhibit the strongest correlation with climate. Lobing assessed qualitatively and higher ILB, both

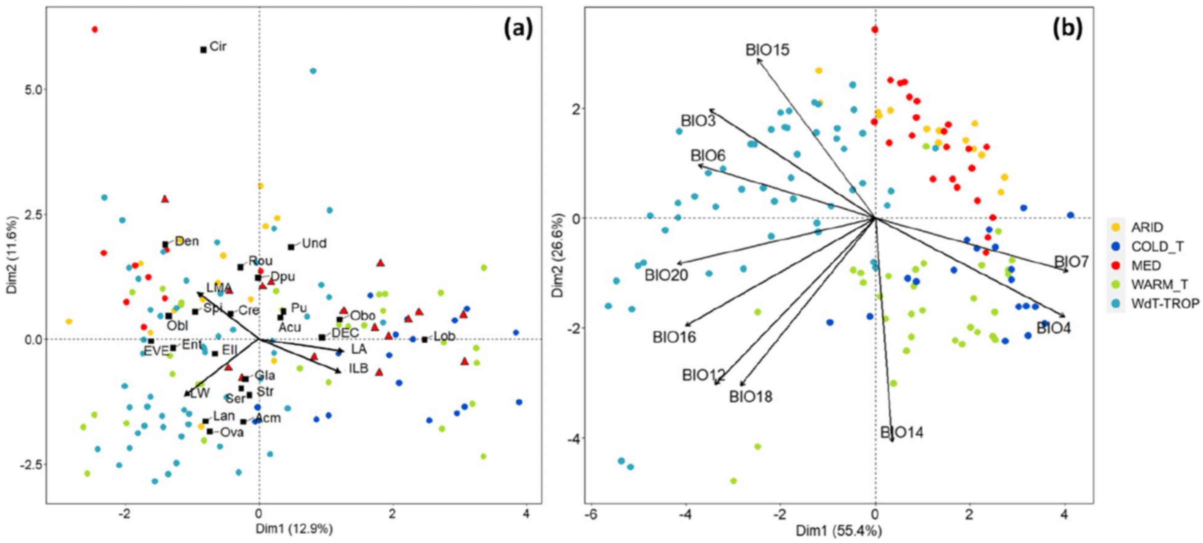

Fig. 5 Factor Analysis of Mixed Data (FAMD) performed with morphological traits (**a**) and Principal component analysis (PCA) performed with climatic data (**b**), showing the first two principal components (Dim1 and Dim2). In FAMD (**a**), Dim1 and Dim2 account for 12.9% and 11.6% of the variance respectively. The FAMD was elaborated with the four quantitative traits: Leaf area (LA), leaf mass per area (LMA), length–width ratio (LW), index of lobation (ILB) and the qualitative traits regarding shape (Cir = circular, Ell = elliptic, Lan = lanceolate, Obl = oblong, Obo = obovate, Ova = Ovate), apex (Acm = acuminate, Acu = Acute, Rou = rounded, Str = straight), margin (Den = dentate, Cre = crenate, Ent = entire, Lob = lobated, Ser = Serrate, Spi = spinose, Und = undulate), pubescence (Gla = glabrous, Pu = pubescent, Dpu = densely pubescent) and leaf habit (DEC = deciduous, EVE = evergreen). In climatic PCA (b), Dim1 and Dim2 account for 55.4% and 26.6% of the variance respectively. The PCA was elaborated with the next variables: BIO3, BIO4, BIO6, BIO7, BIO12, BIO14, BIO15, BIO16, BIO18 and annual precipitation range (see Martín-Sánchez et al. 2024, Table S3 for meaning). Individual species are represented by dots and their colour represent the macroclimate which they have been classified: Arid (yellow), Mediterranean (red, with triangles deciduous Mediterranean species and dots for evergreen Mediterranean, see discussion for further details), Winter-dry Temperate to Tropical (cyan), Warm Temperate (light green) and Cold Temperate (dark blue)

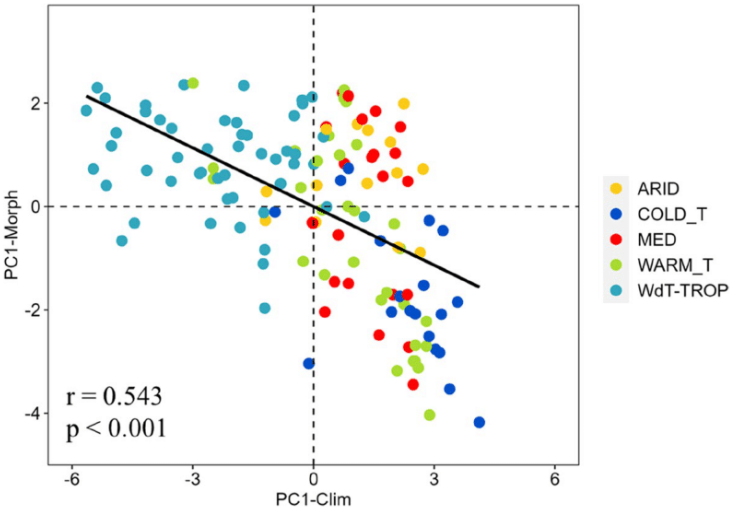

Fig. 6 Correlations between PC1 scores resulting from FAMD (PC1-Morph) and climatic PCA (PC1-Clim). Individual species are represented by dots and their colour represent the macroclimate which they have been classified: Arid (yellow), Mediterranean (red), Winter-dry temperate to Tropical (cyan), Warm Temperate (light green) and Cold Temperate (dark blue). A linear model was adjusted and showed a significant *p*-value ($P < 0.001$)

were associated significantly with temperate climates (Fig. 2d, Fig. 3c). Lobation is also exhibited in sub-Mediterranean species, which occupy a climate transitional between Mediterranean and temperate (Abadía et al. 1996; Himrane et al. 2004; Gil-Pelegrín et al. 2017).

Cold Temperate, sub-Mediterranean and some of the Warm Temperate (primarily American) species gather under the same group in the FAMD (Fig. 5a), which is defined by four traits: high ILB, high LA and obovate and lobated shapes. Leaf lobation may enhance hydraulic efficiency by reducing the ratio of mesophyll to conductive tissue (Sisó et al. 2001; Sack and Tyree 2005) and decreasing the boundary layer to speed leaf cooling (Vogel 1970, 2009; Givnish 1987). Both potential benefits of lobation are especially relevant in sub-Mediterranean species, which experience very high, dry temperatures during summer compared to temperate species. The sub-Mediterranean species in our study also compensate for the reduction of photosynthetic tissue in their relatively small and / or lobated leaves by increasing net CO_2 assimilation per unit leaf area, as Peguero-Pina et al. (2016) showed when comparing the sub-Mediterranean *Q. faginea* with the cold temperate *Q. robur*. In temperate environments, by contrast, leaf lobation may play a role in bud packing, which is crucial for competitive growth in winter-freezing environments (Edwards et al. 2016).

By contrast, Winter-dry temperate to Tropical and some Warm Temperate species exhibit a high LW (Fig. 2c), besides a significant percentage of lanceolate shapes (Fig. 3a) and acuminate apices (Fig. 3b). These three traits associate in the FAMD with Winter-dry temperate to Tropical and Warm Temperate species (Fig. 5a). Together with a tilted leaf position, these traits have been hypothesized to limit rainfall damage (Wang et al. 2020). High rains in the tropics and Warm Temperate regions can slow photosynthesis (Ishibashi and Terashima 1995), leach minerals from the leaves (Minorsky 2019), damage leaves mechanically (Wang et al. 2020), and promote pathogens (Burd 2007). The morphological mechanisms we document, which evacuate water from the leaf surface, are particularly needed in the Warm Temperate and Winter-dry temperate to Tropical climates where these traits dominate. These climates, in the *Quercus* distribution, are most characterized by high precipitation (Fig. 5b).

LMA follows a completely different pattern in our study. Although it is significantly modulated by climate (Table 1), the major clade in which a species occurs plays a more significant role in explaining differences in LMA among species (Table 1) (cf. Sancho-Knapik et al. 2021). The effect of clade at section level is likely explained by the LMA-homogeneous sections, i.e., *Ilex*, *Protobalanus* and *Virentes*. After accounting for the clade, Arid and Mediterranean species tend to have a higher LMA than the rest of the species, although not significant (Fig. 2b). This relatively weak effect of climate is particularly remarkable given the plasticity that LMA presents in response to several environmental factors, such as drought (Niinemets 2001), exposure to light (Poorter et al. 2006) or herbivory (Mediavilla et al. 2018; Onoda et al. 2011). For example, Sancho-Knapik et al. (2021) demonstrated that oak species with long cold periods and/or humid climates have lower LMA.

Despite the poor correlation between climate and LMA, we find a significant correlation between sclerophylly—which is strongly correlated with LMA—and continuous climatic variables (Alonso-Forn et al. 2020). Species with low LMA are usually deciduous and malacophyllous. In contrast, species with a high LMA tend to be evergreen and sclerophyllous (Mediavilla et al. 2008) (Appendix Fig. 10). Thus, Arid, evergreen Mediterranean, most of Winter-dry temperate to Tropical and some Warm Temperate species are located at low Dim1 values in the FAMD (Fig. 5a). By contrast, deciduous Mediterranean, Cold Temperate and the rest of Warm Temperate species are located at high Dim1 values.

The increment in LMA, both via increasing leaf density or leaf thickness, is a major resource investment for the plant. Species which make a higher investment per leaf must keep their photosynthetic tissues active for longer to recover the resources expended according to the worldwide leaf economics spectrum (Wright et al. 2004). However, there are several factors within an individual to take into account in the construction costs and payback time, such as light exposure of the leaves, chemical compounds and leaf habit. Poorter et al. (2006) showed how shade leaves present longer payback times than light leaves, in spite of the fact that these long times can be partially compensated by decreasing the LMA. Differences in payback time can be found between deciduous and evergreen species too (Villar and Merino 2001). Thus, species with a higher LMA—correlated with sclerophylly and longer payback times—tend to be evergreen. Winter-dry temperate to Tropical and some Warm Temperate species can develop sclerophyllous leaves increasing their LMA more than temperate deciduous species but less than Arid and Mediterranean (Alonso-Forn et al. 2020), as the high contribution of LMA to Dim2 of FAMD reflects (Appendix Fig. 11b). This might be related to the two kinds of sclerophyllous leaves: pycnophylls and pachyphylls (Grubb 1986). Differences between them are found in the mesophyll since sclerophylly is mainly defined by an investment to reinforce the epidermis, which is shared by both types. Pycnophyll leaves present a very compact mesophyll, usually small cells with little space among them, i.e., an increase in LMA via investment in leaf density. By contrast, pachyphyll leaves are characterized by a larger, laxer and spongier mesophyll, i.e., an increase in LMA via leaf thickness (Grubb 1986). Thereby, Winter-dry temperate to Tropical and Warm Temperate species might be mainly pachyphylls, exhibiting thick leaves with low density; meanwhile, Arid and evergreen Mediterranean might be pycnophylls.

Leaf water relations relations are also strongly influenced by pubescence, which appears in our study to vary independently of macroclimate, except glabrous leaves associated with Winter-dry temperate to tropical climates ($P < 0.001$). Previous studies have suggested that abaxial pubescence in oaks could be a xeromorphic trait in response to drier climates (Fig. 3d) (Hardin 1979). This trend is observed in a major presence of pubescent species in Arid and Mediterranean species, although not significant ($P = 0.41$). (Fig. 3d). The functional roles of trichomes in the genus may be broader, including protection against a range of biotic and abiotic factors (Zvereva et al. 1998; Sato and Kudoh 2015; Ehleringer et al. 1976; Coltharp et al. 2021). A trichome layer may act as a protection against some environmental factors, especially excessive solar radiation, by modifying the leaf's optical properties, lowering heat load, and reducing leaf temperature and transpiration (Ehleringer et al. 1976; Ehleringer 1981; Sandquist and Ehleringer 1997; Pérez-Estrada et al. 2000; Abdulrahaman and Oladele 2011; Bickford 2016). Nonetheless, the hydrophobic nature of some trichomes may also be advantageous under wet climates, since they can keep the stomata dry and ensure the free exchange of CO_2 (Brewer and Smith 1997; Pierce et al. 2001). However, trichomes do not necessarily have to be hydrophobic, as Fernández et al. (2014) demonstrated in *Quercus ilex* subsp. *rotundifolia*, whose adaxial trichomes seem to help leaves to rehydrate during summer rainfall events. This double behaviour of trichomes and the lack of significance in our results indicate that their role as xeromorphic trait cannot be taken for granted, and further research to clarify the role of pubescence is needed.

4.2 Multivariate leaf syndromes associate with macroclimate

Our study demonstrates that the world's oak species assemble into well-defined climatic groups that correlate with loose but still identifiable leaf morphological syndromes. The Arid and Mediterranean species are an exception, as they mainly overlap in climatic space (Fig. 5b). Because species were classified into their macroclimate according to their presence within a specific Köppen climate, the climatic separation is a result of the Köppen system itself: PC1 and PC2 together account for 82% of the explained variance, which suggests that Köppen climates are well defined by WorldClim climatic variables.

A more interesting finding is the significant correlation of climatic PC1 with morphological PC1 (Fig. 6). Morphological PC1 is explained most strongly by lobation (ILB and lobated shapes), LA and Obovate shapes (Appendix Fig. 11a). In contrast, climatic variables with the highest scores are the difference in precipitation between the wettest month and the driest one (annual precipitation range), temperature annual range (BIO7), temperature seasonality (BIO4) and precipitation of the wettest quarter (BIO16) (Appendix Fig. 12a). Climatic PC1 thus separates most strongly between Winter-dry temperate to Tropical (with negative values) and the rest of the species (with positive ones) (Fig. 5b). The segregation of species along this climatic gradient represents both an ecological split and a phylogenetic split replicated twice in the Americas (Hipp et al. 2018) and between the primarily Mediterranean to temperate sections *Cerris* and *Ilex* of Eurasia versus section *Cyclobalanopsis* of the southeast Asia's broad-leaved evergreen forests (Deng et al. 2018).

Mediterranean, Arid, and temperate species live in climates with a marked thermal seasonality. The stressful dry or cold periods that mark these climates are much reduced in Winter-dry temperate to Tropical climates. Winter-dry temperate to Tropical, Arid and Mediterranean species—three macroclimates with higher temperatures, on average, than temperate climates—are represented with negative values in PC1 of FAMD (Fig. 4a). Simultaneously, temperate and sub-Mediterranean species appear together and are characterized by a high lobation, and large, obovate leaves (Fig. 5a), which are the four variables with highest loadings in PC1 (Appendix Fig. 11a). Thus, we see once again a correlation between area and lobation with temperature.

The significant regression between climate and morphology PC2 (Appendix Fig. 13) shows the correlation of glabrous, slender leaves with drip-tips with higher-precipitation climates and pubescent rounded leaves with dry climates (Appendix Fig. 11b and 12b). As morphological and climatic PC2 represents a water-availability gradient, Mediterranean, Arid, and Winter-dry temperate to Tropical with monsoon-dry season cluster in the FAMD separate from most Warm Temperate and the rest of the Winter-dry temperate to Tropical species (Fig. 5a).

Cold Temperate species are generally characterized by large, obovate, deciduous leaves with complex—generally lobated—margins and low LMA (Fig. 7). Winter-dry temperate to Tropical species are defined by long, lanceolate and acuminate leaves, generally glabrous, evergreen or tardily-deciduous and sclerophyllous, likely pachypyll-type (anatomical studies in further researches are needed) (Fig. 7). Warm Temperate species tend to share or present

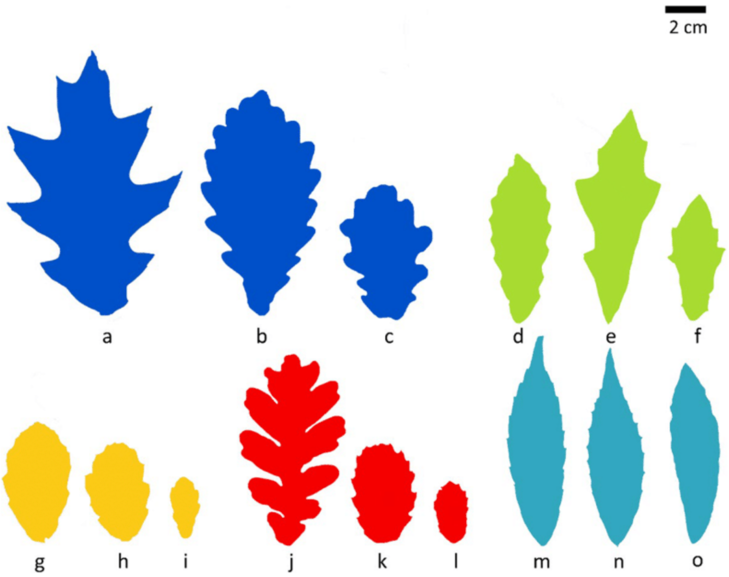

Fig. 7 Type-leaves of three different species as representatives of the different macroclimates studied. Cold Temperate (in dark blue): **a** *Quercus rubra*, **b** *Q. mongolica*, **c** *Q. robur*. Warm Temperate (in light green): **d** *Q. fabri*, **e** *Q. nigra*, **f** *Q. virginiana*. Arid (in yellow): **g** *Q. hintoniorum*, **h** *Q. hypoxantha*, **i** *Q. grisea*. Mediterranean: **j** *Q. pyrenaica* (sub-Mediterranean), **k** *Q. faginea*, **l** *Q. rotundifolia*. Winter-dry temperate to tropical (in cyan): **m** *Q. stenophylloides*, **n** *Q. leucotrichophora*, **o** *Q. pinnativenulosa*

intermediary traits between Cold Temperate and Winter-dry temperate to Tropical species (Fig. 7). Arid and Mediterranean species exhibit the smallest leaves, short, generally with a LW ratio near 1, and entire or relatively simple margins. They are usually evergreen or semi-evergreen, with the highest values of LMA probably due to their hypothetical sclerophyll pycnophyll-type condition (also further researches are needed) (Fig. 7). Finally, sub-Mediterranean species (e.g. *Q. pyrenaica*, *Q. frainetto* or *Q. cerris*) are characterized by larger leaves than their genuine Mediterranean relatives, with deeply lobated margins, usually obovate, deciduous and pubescent (Fig. 7). Overall, our work shows that we can relate leaf syndromes in oaks to broad-scale patterns in global climate.

Finally, although climate explains most of the variance found in oak leaf morphology, clade seems to have a minor but still significative effect since we cannot reject the presence of a phylogenetic signal in these morphological traits, especially in LMA. This phylogenetic signal could be appearing due to some morphologically homogeneous clades, e.g. Sect. *Protobalanus*, *Virentes* or *Ilex*.

5 Conclusion

In this study we analysed 141 *Quercus* species across a broad range of northern hemisphere biomes, ca. 25% of the species in the genus. Although we cannot refuse the presence of a phylogenetic signal, our results strongly suggest that leaf morphological traits of oaks are adapted to their macroclimates, as previous work has demonstrated for leaf anatomy (Sancho-Knapik et al. 2021; Alonso-Forn et al. 2023). Our study demonstrates that even in a common garden, growing under the same climatic conditions, a wide sample of the world's oak species develop a set of morphological traits with different ecological roles that conclude into recognisable leaf syndromes according to the climate that these species occupy. Except for Mediterranean species that, on the one hand, tend to develop a common leaf syndrome with Arid species. On the other hand, another group composed by sub-Mediterranean species gathers into a temperate syndrome.

Even though climate explains most of the variance found in leaf morphology, the partial effects of clade cannot be disregarded, as we see within the LMA, which resulted to be an outlier trait in this study. LMA is still explained significantly by climate; however, section explains twice variance as much as the climate for this trait.

Finally, pubescence did not show significant enough results to be considered a xeromorphic trait, as many authors have proposed.

Appendix

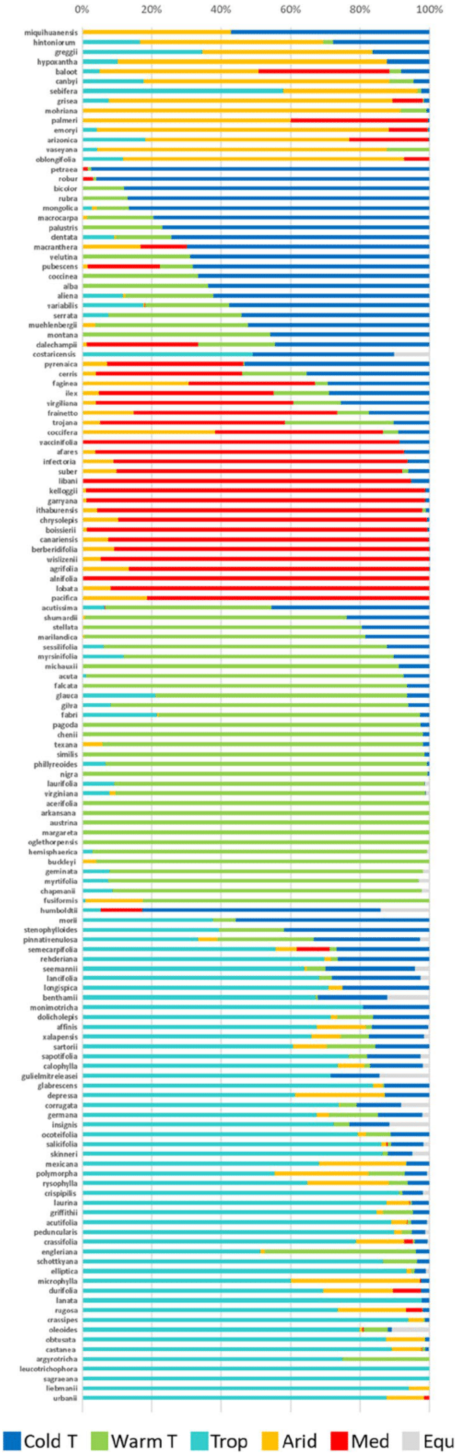

Fig. 8 Percentage of presence points in each of the five macroclimates for each species: Arid (yellow), Mediterranean (red), Winter-dry temperate to Tropical (cyan), Warm Temperate (light green) and Cold Temperate (dark blue). Equatorial climate, not considered in this study, is represented in grey

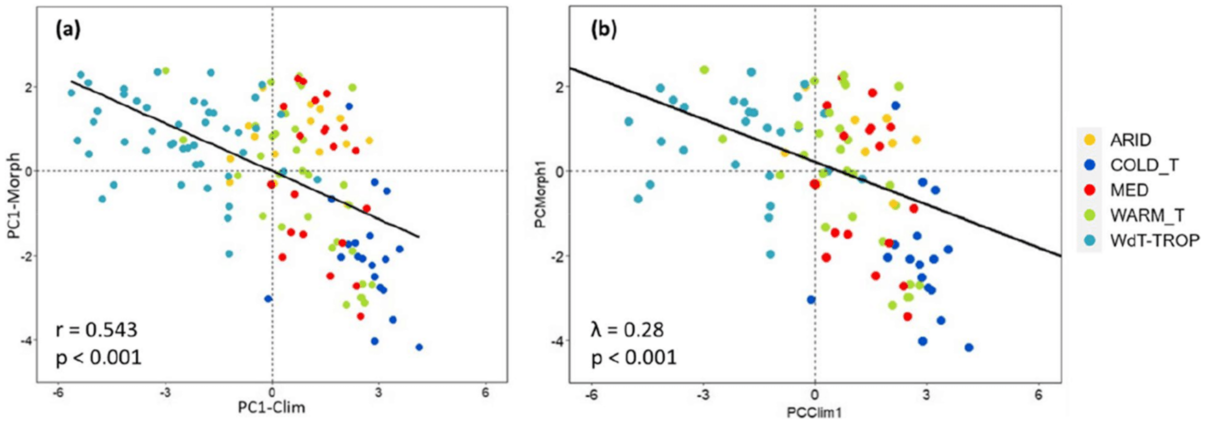

Fig. 9 Comparison between ordinary least squares regression ($N = 138$, Fig. 5 in the main text), and the phylogenetic generalized least square regression ($N = 99$). Phylogenetic signal showed with lambda (λ)

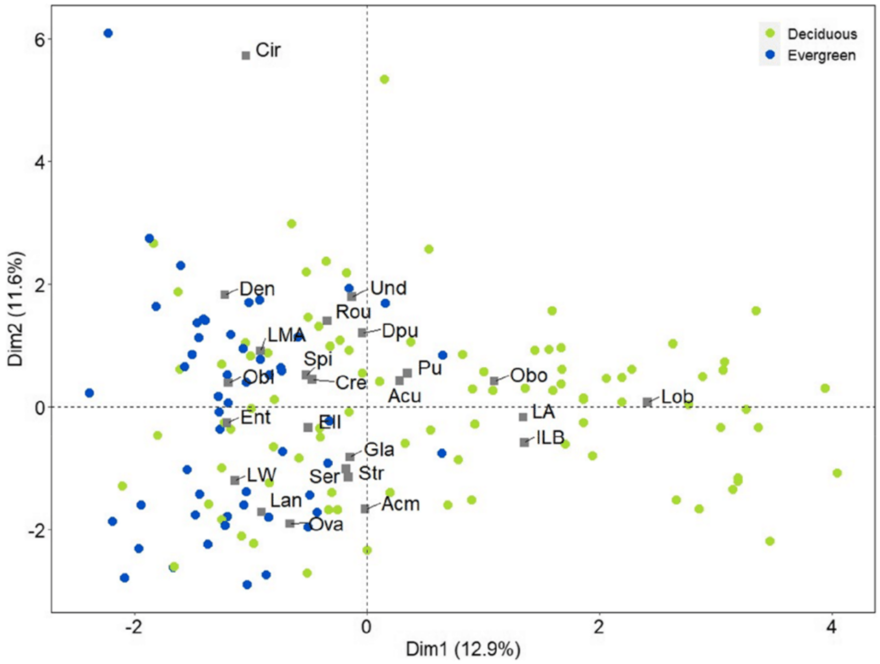

Fig. 10 Factor Analysis of Mixed Data (FAMD) performed with morphological traits. Same FAMD that Fig. 5a, but colouring species according to their leaf habit: Evergreen (dark blue) or deciduous (green)

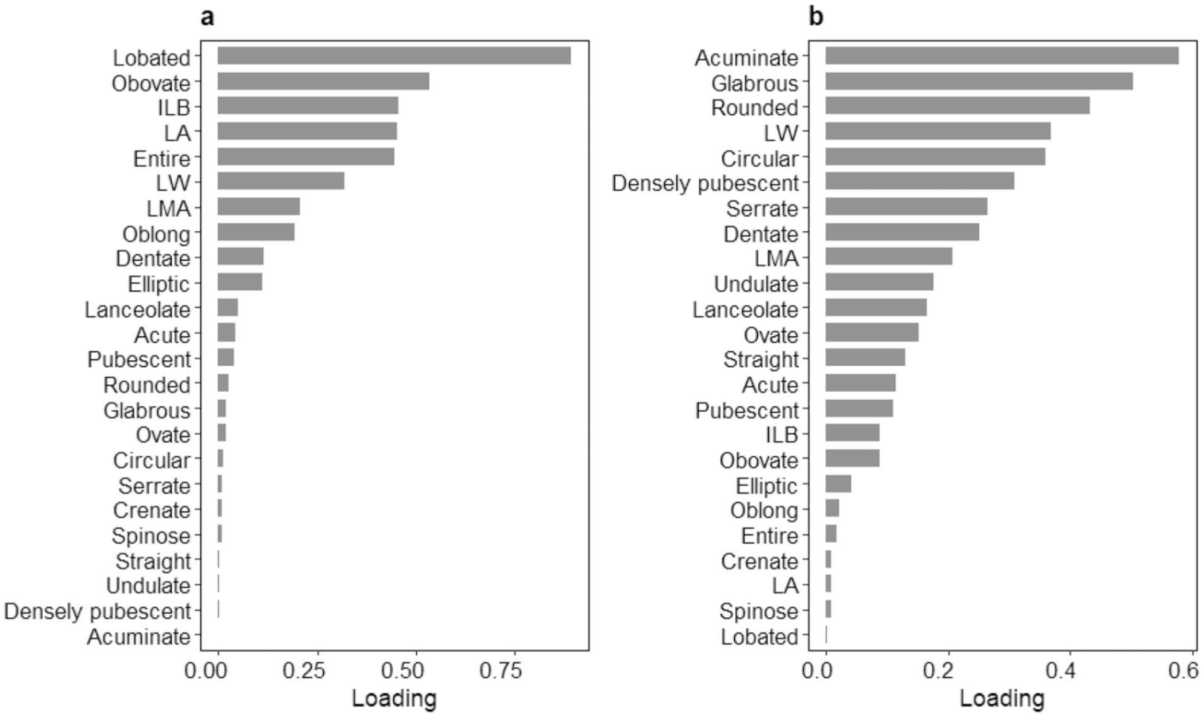

Fig. 11 Bar plots of the loadings of the first (a) and second (b) principal components computed via Factor Analysis of Mixed Data (FAMD) for all morphological traits

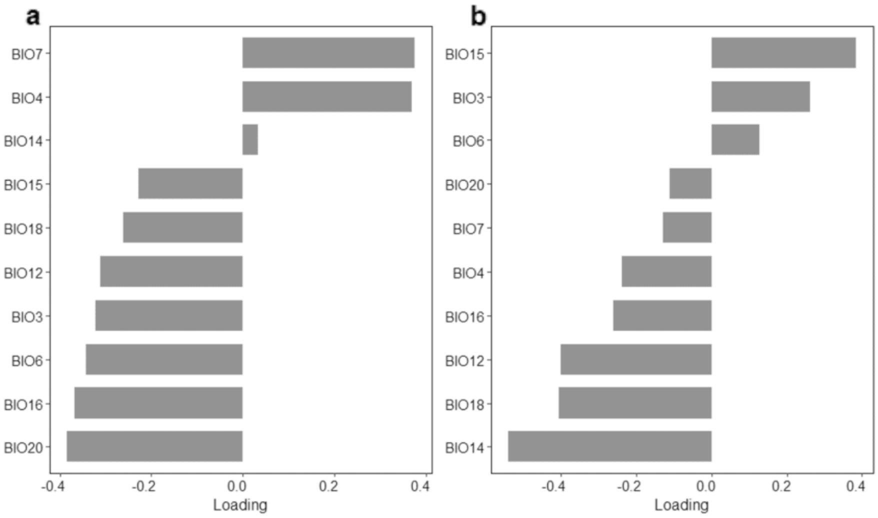

Fig. 12 Bar plots of the loadings of the first (a) and second (b) principal components computed via Principal Component Analysis (PCA) for climatic variables with a total number of 10 variables

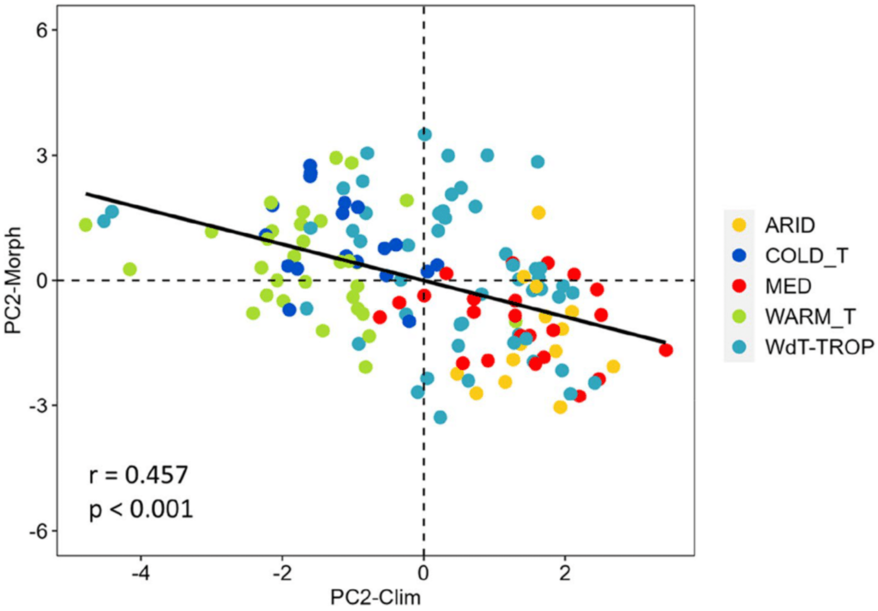

Fig. 13 Correlations between PC2 scores resulting from FAMD (PC2-Morph) and climatic PCA (PC2-Clim). Individual species are represented by dots and their colour represent the macroclimate which they have been classified: Arid (yellow), Mediterranean (red), Winter-dry temperate to Tropical (cyan), Warm Temperate (light green) and Cold Temperate (dark blue). A linear model was adjusted and showed a significant *p*-value ($P < 0.001$)

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Conceptualization: Rubén Martín-Sánchez, José Javier Peguero-Pina, Domingo Sancho-Knapik and Eustaquio Gil-Pelegrín; sampling: Domingo Sancho-Knapik; methodology: Rubén Martín-Sánchez, David Alonso-Forn, Domingo Sancho-Knapik; formal analysis and investigation: Rubén Martín-Sánchez, José Javier Peguero-Pina, Ana López-Ballesteros, Juan Pedro Ferrio and Andrew L. Hipp; writing—original draft preparation: Rubén Martín-Sánchez, José Javier Peguero-Pina and Andrew L. Hipp; writing – review and editing: Rubén Martín-Sánchez, José Javier Peguero-Pina, Domingo Sancho-Knapik, Juan Pedro Ferrio, Ana López-Ballesteros, Andrew L. Hipp and Eustaquio Gil-Pelegrín; funding acquisition: Juan Pedro Ferrio, Domingo Sancho-Knapik; supervision: Eustaquio Gil-Pelegrín The authors read and approved the final manuscript.

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Availability of data and materials
The datasets generated and/or analyzed during the current study are available at https://doi.org/https://doi.org/10.5281/zenodo.10633924.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors gave their informed consent to this publication and its content.

Competing interests
The authors declare no conflict of interest.

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Publisher's Note

Capítulo IV

Xylem and Phloem in Petioles Are Coordinated With Leaf Gas Exchange in Oaks With Contrasting Anatomical Strategies Depending on Leaf Habit

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ORIGINAL ARTICLE

Xylem and Phloem in Petioles Are Coordinated With Leaf Gas Exchange in Oaks With Contrasting Anatomical Strategies Depending on Leaf Habit

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ABSTRACT

As the single link between leaves and the rest of the plant, petioles must develop conductive tissues according to the water influx and sugar outflow of the leaf lamina. A scaling relationship between leaf area and anatomical traits of xylem and phloem is expected to improve the efficiency of these tissues. However, the different constraints compromising the functionality of both tissues (e.g., risk of cavitation) must not be disregarded. Additionally, deciduous and evergreen plants may have different strategies to produce and package their petiole conduits to cope with environmental restrictions. We explored in 33 oak species the relationships between petiole anatomical traits, leaf area, stomatal conductance, and photosynthesis rate. Results showed allometric scaling between anatomical structure of xylem and phloem with leaf area. We also found correlations between photosynthesis rate, stomatal conductance, and anatomical traits in the petiole. The main novelty is how oaks present a different strategy depending on the leaf habit. Deciduous species tend to increase their diameters to achieve greater leaf-specific conductivity. By contrast, evergreen oaks develop larger xylem conductive areas for a given leaf area than deciduous ones. This trade-off between safety-efficiency in petioles has never been attributed to the leaf habit of the species.

1 | Introduction

Petioles, besides accomplishing a structural function, link the main photosynthetic organs—that is, leaf laminae—with the rest of the plant. In this regard, they may act as a bridge in the soil–plant–atmosphere continuum for water transport and the translocation of photosynthates (Brocious and Hacke 2016). Thus, transpiration has been traditionally related to transport capacity of xylem and photosynthesis rate to export capacity of phloem in petioles (Salisbury 1913; Brocious and Hacke 2016). However, this correlation has been questioned within angiosperms (Gleason et al. 2016). These authors reported a weak or even absent coordination between hydraulic capacity and gas exchange capacity.

Petioles must contain enough xylem vessels and sieve tubes to, respectively, supply water to the leaf lamina and export assimilates from the leaf to the rest of the plant. For this reason, a scaling

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relationship between leaf area and both xylem and phloem structures (area of conductive tissues and size of the conduits) in petioles is expected (Ray and Jones 2018). The number and size of the conduits ultimately reflects the transport ability—namely, the hydraulic conductivity (K_h)—of the conductive tissues according to the Hagen–Poiseuille law (Tyree and Zimmermann 2002; Hirose et al. 2005; Woodruff 2014). Nonetheless, the K_h only shows how much fluid is potentially able to be moved along a pathway whereas two petioles with the same K_h can support different leaf areas. For this reason, the leaf-specific conductivity (LSC) of a petiole provides a more physiological explanation of a leaf's efficiency as LSC is the capacity to supply water (K_h) per leaf area. LSC can also be expressed as the product of the specific conductivity (K_s, i.e., K_h per conductive area ratio) and the Huber value (H_v, i.e., conductive area per leaf area ratio) (Mencuccini et al. 2019). Therefore, the same LSC can be achieved in different ways by modulating both K_s and H_v. Indeed, Mencuccini et al. (2019) found in a wide range of plant species a negative relationship between K_s and H_v in stems. What are the implications of increasing each variable?

Increasing the Huber value, that is, allocating more cross-sectional area to a conductive function (i.e., xylem), would imply a reduction in the availability of space for structural support. This could result in a possible trade-off between both kind of tissues (Zwieniecki et al. 2004). By contrast, as the specific conductivity mainly depends on the diameter of the conduits, the hydraulic capacity of the petiole is compromised by the same factors (e.g., drought or cold) that determine its vulnerability, as in any other parts of the plant (Hacke and Sauter 1996) In fact, it has been suggested that petioles may act as hydraulic fuses for the plant through higher vulnerability to embolism than stems, thereby ensuring resilience to extreme drought events (Peguero-Pina et al. 2015; Alonso-Forn et al. 2021). Throughout their lifespan, leaves can be affected by climatic stresses (e.g., aridity or cold) that may influence the size of the xylem conduits (Gil-Pelegrín et al. 2017). In this sense, many studies showed a higher vulnerability to cavitation in species with wider vessels (Hacke, Sperry, and Pittermann 2000; Tyree 2003; Hacke et al. 2006; Cai and Tyree 2010; Jacobsen et al. 2019; Blackman et al. 2024). Embolized xylem conduits leads to hydraulic failure, which is the main cause of plant mortality in response to drought (Tyree and Sperry 1989). By contrast, many species inhabiting very stressful habitats reduce the diameter of their conduits, achieving a higher resistance to cavitation. This reduction in the conduit diameter results in a lower water transport efficiency (Giordano et al. 1978). However, this decrease may be partially compensated by increasing the number of conduits (Nardini, Pedà, and Rocca 2012). When cavitation is caused by freeze-thaw cycles, the same arguments arise. Wider conduits are likewise more vulnerable than small ones because they contain greater dissolved air which can form larger bubbles causing cavitation at lower tensions (Cochard and Tyree 1990; Sperry and Sullivan 1992; Lo Gullo and Salleo 1993; Lemoine, Granier, and Cochard 1999; Sevanto, Holbrook, and Ball 2012; Zanne et al. 2014; Ni et al. 2022). Finally, the 'rare pit' hypothesis also explains how vessels with a large area of inter-vessel pits are more likely to present a pit with relatively low air-seeding pressure, reducing the threshold pressure for cavitation of the whole conduit (Wheeler et al. 2005; Christman, Sperry, and Adler 2009; Plavcová et al. 2013).

Regarding the phloem, other factors rather than climatic stresses might influence the size of its conduits. Sugars, amino acids and other organic metabolites in the sap make sieve tubes a target for some phytophagous insects like aphids (Will, Furch, and Zimmermann 2013). When an aphid stings a sieve tube with its stylet, the plant responds by occluding the sieve plates with callose, turning it into a non-functional conduit (Will and van Bel 2006). Thus, building more but smaller sieve elements is safer than building a few large conduits (Ewers and Fisher 1991). Besides, wider sieve elements would need to load more sugars in the source (i.e., the leaf), to generate enough turgor pressure gradient for sap to flow towards the sink organs (Hölttä, Mencuccini, and Nikinmaa 2009; Sevanto 2014).

The scaling relationship between xylem and phloem areas has been explored in several species (Jyske and Hölttä 2015; Carvalho et al. 2017a; Carvalho et al. 2017b; Kiorapostolou and Petit, 2019; Ray and Jones 2018; Losada, He, and Holbrook 2022). Most of the studies focus on stems, although Ray and Jones (2018) analysed petioles in several *Pelargonium* species. Albeit plants tend to present more xylem than phloem, an isometrical scaling has been found in these studies. Nonetheless, most research focuses on a single species. To our knowledge, they do not conduct comparative analyses across different leaf longevities, even in genus level studies. Deciduousness and evergreenness offer different solutions to cope with climatic stresses, which are, in turn, closely related to specific leaf area (SLA) (Sancho-Knapik et al. 2021) (Figure 1). Similarly, these climatic stresses have been demonstrated to limit the growth of the conduits, even in petioles (Blackman et al. 2024). Since the LSC ultimately depends on the leaf area, the Huber value and the diameter of the conduits, we wonder if the two different leaf habits could develop two different strategies (Figure 1, Models A and B) to reach similar values of LSC.

The genus *Quercus* (oaks) offers an excellent system to study the scaling between both conductive tissues, xylem and phloem, besides its ecophysiological implications. Oaks represent a single monophyletic clade with over 400 species occupying very different habitats around the northern hemisphere, from tropical rainforests to cold temperate forests through semideserts and chaparrals. Additionally, oak species exhibit a much broader range not only in terms of leaf area, but also in leaf lifespan compared to other widely studied genera such as *Populus*, *Pelargonium* or *Eucalyptus* (Brocious and Hacke 2016; Ray and Jones 2018; Blackman et al. 2024). The variability in leaf area can suppose a difference of 70 times between the species with the largest and the smallest leaves (Sancho-Knapik et al. 2021). Finally, phenology ranges from deciduous with a lifespan of just 5 months up to evergreen species whose leaves can persist over several years (Mediavilla et al. 2008; Harayama, Ishida, and Yoshimura 2016).

In this study we explore the scaling relationships among the different xylem and phloem traits in petiole cross-sections, leaf area, stomatal conductance, photosynthesis rate and climatic variables in 33 oak species (16 deciduous and 17 evergreen) covering the full range of variation in oaks in terms of leaf area and leaf habit. Based on the information presented thus far (Figure 1), four aims were addressed: (1) to explore the scaling relationships between

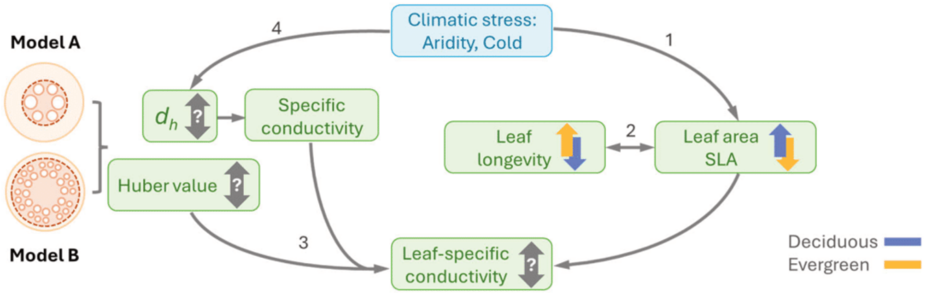

FIGURE 1 | Scheme of the traits that ultimately modulate the leaf-specific conductivity (LSC) of the petiole. Thick coloured arrows show the tendency of each leaf habit (deciduous in blue and evergreen in orange) to have larger (upward arrow) or smaller (downward arrow) values for leaf longevity, leaf area and specific leaf area (SLA). Thick grey arrows represent the unknown relationships we aim to explore in this study. Two anatomical models are proposed: Model A assumes that LSC can be improved increasing the hydraulic diameter (d_h), whereas Model B assumes that for the same cross-section of petiole, a similar LSC could be reached by increasing the conductive area with a smaller d_h. References that support the proposed relationships are: (1) Sancho-Knapik et al. (2021); (2) Mediavilla et al. (2008) and Kikuzawa et al. (2013); (3) Mencuccini et al. (2019); and (4) Blackman et al. (2024). [Color figure can be viewed at wileyonlinelibrary.com]

conductive tissue structures in petioles—that is, conductive area and hydraulic diameter of the conduits—and leaf area, for both xylem and phloem; (2) to verify whether deciduous and evergreen species exhibit the same LSC, and to check if deciduous and evergreen oaks follow a different strategy when producing and packaging their conduits to improve their efficiency; (3) in case that two different models are observed, to relate them with climatic variables (mainly aridity and cold) that could be explaining such differences; and (4) to assess if the hydraulic architecture resulting from the observed scaling correlates with the physiological demands of the leaf, that is, stomatal conductance and photosynthesis rate. We hypothesised that xylem and phloem petiole traits should scale proportionally with leaf area, which ultimately reflects the water demands (stomatal conductance) and export requirements (photosynthesis rate) of the leaf lamina.

2 | Material and Methods

2.1 | Plant Material

In this study, we sampled 22 oak species occurring in the living collection in CITA de Aragón (41°39′N, 0°52′W, 200 m a.s.l., Zaragoza, Spain). To get a greater diversity of species and to cover a wider range of leaf areas, we also sampled 11 additional species from Jardín Botánico de Iturrarán 43°15′′N 2°09.3′′W, 200 m a.s.l., Gipuzkoa, Spain) (Supporting Information: Table S1).

We sampled five mature leaves of south-exposed branches from 3 to 5 trees per species. They were sealed in plastic bags and carried to the laboratory. The mid-section of the petiole was cut and stored in 70% ethanol. Then, leaf area (LA) was measured using ImageJ software by scanning the leaf lamina.

2.2 | Anatomical Traits

Petiole sections were dehydrated in a graded ethanol series and subsequently embedded in Paraplast Plus embedding medium (Leica). The resulting paraffin blocks were cut in the microtome (HM 350S; MICROM) to obtain 15–20 µm cross-sections that were stained with saffranine (0.1% w/v), picric acid (0.5% v/v) and AstraBlue (0.1% w/v) after being deparaffinated with Isoparaffin H and rehydrated using a graded ethanol series (100%, 95%, 90% and 70%). Then, sections were observed and photographed under a light microscope (OPTIKA B-600TiFL; Optika Microscopes) (Figure 2). We measured the total petiole cross-sectional area (A_{pet}), as well as the xylem cross-section area ($_{Ax}$) and phloem cross-section area (A_p) (Figure 2a). Hereafter, we will use conductive area (A_c) to refer to the sum of the two vascular tissues and we will distinguish between xylem area ($_{Ax}$) and phloem area (A_p) when treated separately. Besides, we measured the total number of xylem vessel elements and phloem conduits and their mean diameter in three subsamples of the whole conductive area of each tissue per photograph (Figures 2b, 2c). In the phloem, our aim was to measure only sieve tubes; however, we cannot claim to have exclusively measured these ones since a visible sieve tube plate (with electron microscopy or their identification with callose staining) is necessary to properly identify a sieve tube. We discarded the first brick-shaped cell layers (i.e., the procambium). Medullary rays and cells with a visible nucleus and organelles were also neglected. Finally, very small cells (what we interpret as companion cells or oblique/bevel cuts) as well as the big rounded thick-wall cells in the distal part of the phloem (phloem fibres) were also avoided (Esau 1939) (Figure 2c). The rest of the cells were considered as potentially sieve tubes and as such, measured. Afterwards, we calculated the hydraulic diameter for xylem (d_{hx}) and phloem (d_{hp}) using the formula proposed by Sperry et al. (1994):

$$d_h = \frac{\sum_i d_i^5}{\sum_i d_i^4}$$

where d_i is the mean diameter of each conduit measured. Then, we also calculated the ratio of the conductive area normalised by the leaf area following the next formula:

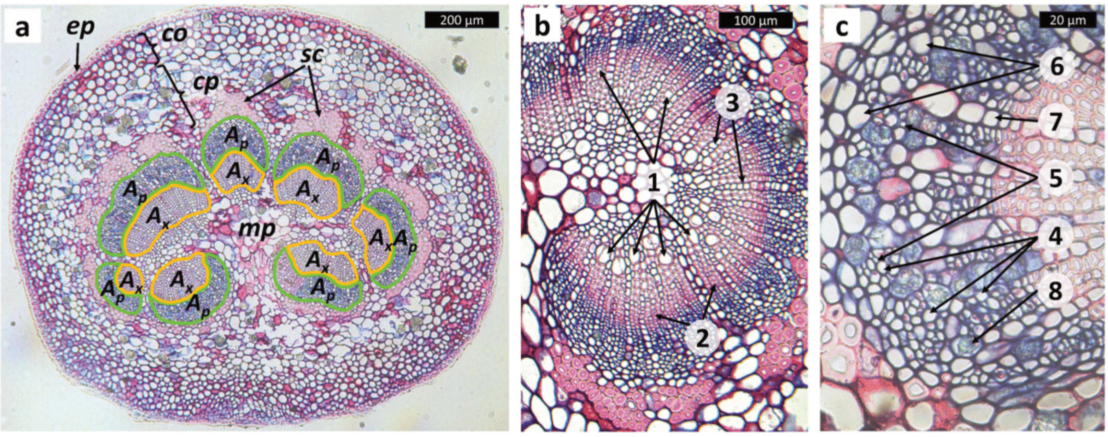

FIGURE 2 | Histological cross-section of *Quercus agrifolia* petiole. (a) General scheme of the whole petiole with the main tissues: epidermis (ep), collenchyma (co), cortical parenchyma (cp), sclerenchyma (sc), medullary parenchyma (mp) and the conductive tissues, measured in this study: xylem (A_x, highlighted in yellow) and phloem (A_p, highlighted in green). (b) Magnified view of xylem with its main cellular types: xylem vessels (1; measured in this study), tracheids (2) and parenchymatic medullary rays (3). (c) Detailed view of phloem with its main cellular types: potential sieve tubes (4; measured in this study), potential companion cells (5), phloem fibres (6), medullary rays (7) and parenchyma (8). [Color figure can be viewed at wileyonlinelibrary.com]

$$\text{For xylem: } XLA = \frac{A_x}{LA \times 10000};$$

$$\text{For phloem: } PLA = \frac{A_p}{LA \times 10000}$$

Where A_x is the total xylem area, A_p is the total phloem area and LA is leaf area. Leaf area was multiplied by 10 000 to obtain values close to one and to transform units to $\text{cm}^2\,\text{m}^{-2}$. All measurements were done using ImageJ software. Traits analysed are compiled in Table 1.

2.3 | Hydraulic Conductivity

We calculated the theoretical hydraulic conductivity of the whole petiole (K_h) as the sum of each conduit conductivity assuming that both types, xylem vessels (K_{hx}) and phloem cells (K_{hp}), follow the Hagen-Poiseuille law (Tyree and Zimmermann 2002; Hirose et al. 2005; Woodruff 2014):

$$K_h = \sum_i \frac{d_i^4 \pi \rho}{128\eta}$$

where ρ is the density of the fluid moving along the conduits at 25°C, assuming pure water for xylem ($997\,\text{kg}\,\text{m}^{-3}$) and a specific sap density dependent of sucrose concentration for phloem ($1068\,\text{kg}\,\text{m}^{-3}$) (Jensen, Savage, and Holbrook 2013), η is the viscosity of the fluid at 25°C, pure water for xylem ($8.9 \times 10^{-10}\,\text{MPa s}$) and 1.91 times that value for phloem sap ($1.7 \times 10^{-9}\,\text{MPa s}$) (Thompson 2006; Jensen, Savage, and Holbrook 2013); and d_i is the mean lumen diameter of each conduit.

Additionally, we calculated leaf-specific hydraulic conductivity of xylem (LSC):

$$\text{LSC} = \frac{K_{hx}}{LA}$$

where LA is leaf area. We also calculated the specific conductivity for xylem (K_{sx}):

$$K_{sx} = \frac{K_{hx}}{A_x}$$

where A_x is the area of xylem in the cross-section of the petiole.

2.4 | Climatic Variables

To get a mean representative value of different climatic variables for each species we followed the same procedure as in Martín-Sánchez et al. (2024). In short, we first downloaded GBIF individual presence points for the 33 species studied, we thinned the data to one presence point per square kilometre using SDMtune R package (Vignali et al. 2020) and we extracted the climatic variables from the WorldClim version 2.1 database (Fick and Hijmans 2017. WorldClim 2. https://www.worldclim.org). We additionally added an aridity index (AI) calculated as mean annual precipitation (MAP) divided by potential evapotranspiration (PET) (Mencuccini et al. 2019; Peguero-Pina et al. 2020). All individual values were summarised into a mean value for each species. To test our hypothesis whether hydraulic diameters are restrained by climatic factors, we chose variables related to cold and aridity: mean annual temperature (MAT), mean of daily minimum temperatures during the coldest quarter (T_{min}), MAP and the aridity index (AI).

2.5 | Leaf Gas Exchange

We obtained the mean photosynthesis rate (A_N) and stomatal conductance (g_s) of 26 species. For nine species, we measured

TABLE 1 | Appendix of traits measured in this study, their abbreviations and units.

Parameter	Abbreviation	Unit
Leaf area	LA	cm^2
Petiole area	A_{pet}	μm^2
Conductive area	A_c	μm^2
Xylem area	A_x	μm^2
Hydraulic diameter of xylem	d_{hx}	μm
Ratio xylem area/leaf area	XLA	Adimensional
Hydraulic conductivity of xylem	K_{hx}	$Kg\ m\ Mpa^{-1}\ s^{-1}$
Leaf-specific conductivity	LSC	$Kg\ m^{-1}\ Mpa^{-1}\ s^{-1}$
Specific conductivity of xylem	K_{sx}	$Kg\ m^{-1}\ Mpa^{-1}\ s^{-1}$
Phloem area	A_p	μm^2
Hydraulic diameter of phloem	d_{hp}	μm
Ratio phloem area/leaf area	PLA	Adimensional
Hydraulic conductivity of phloem	K_{hp}	$Kg\ m\ Mpa^{-1}\ s^{-1}$
Photosynthesis net assimilation per leaf	$A_{N,\ leaf}$	$\mu mol\ CO_2\ s^{-1}$
Stomatal conductance per leaf	$g_{s,\ leaf}$	$mmol\ H_2O\ s^{-1}$
Mean annual temperature	MAT	°C
Mean annual precipitation	MAP	mm
Mean of daily minimum temperatures during the coldest quarter	T_{min}	°C
Aridity Index	AI	Dimensionless

these parameters using an open gas exchange system (CIRAS-3; PP-Systems) fitted with an automatic universal leaf cuvette (PLC6-U; PP-Systems) in six current-year fully developed leaves per species from our living collection between 10:00 and 12:00 h solar time during summer 2023. All measurements were conducted under the following standard environmental conditions: CO_2 concentration surrounding the leaf (C_a) of 400 μmol mol^{-1}, leaf temperature of 25°C, vapour pressure deficit of 1.25 kPa and saturating photosynthetic photon flux density of 1500 μmol $m^{-2}\ s^{-1}$. We complemented our own measurements with data for 17 species compiled from other studies (Vaitkus and McLeod 1995; Nagel et al. 2002; Thadani, Berlyn, and Ashton 2009; Huang, Hu, and Zhang 2016; Llusia et al. 2016; Jafarnia et al. 2018; Alonso-Forn et al. 2020; Kar, Montague, and Villanueva-Morales 2021). Assuming a mean value of A_N and g_s for each species and taking into account the mean LA measured for each species, we calculated the theoretical mean photosynthesis rate and stomatal conductance at whole leaf level. ($A_{N,leaf}$ and $g_{s,leaf}$, respectively).

2.6 | Statistical Analyses

First, we tested the potential effect of the garden throughout several analyses of variance (ANOVAs) and linear regressions via mixed models. On the one hand, we performed ANOVAs for each single trait to see how much variance was explained by species and the garden (Supporting Information: Table S2). On the other hand, we did linear regressions via mixed models including the garden as a random factor in correlations between pairwise traits, besides a subsequent ANOVA to see how much variance is explained by the random factor. Variance explained by garden in the first ANOVA showed that, for every trait, species accounted for more variance than the garden and in most of the cases species accounted over 60% of variance. Besides, correlations remain significant even when garden is included as a random factor (Supporting Information: Table S3). Finally, when we tested differences in the leaf habit, we additionally accounted for garden and the interaction between leaf habit and garden in the ANOVAs. In most of the cases, the interaction resulted to be nonsignificant, except for three traits (Supporting Information: Table S4). Even among those exceptions leaf habit and/or residuals accounted for more variance than the garden. In view of these results, we conclude that correlations are not strongly skewed by an effect of the garden.

Cross-correlations were performed between the different anatomical, hydraulic and physiological traits, assuming a log-log correlation. Alternatively, linear cross-correlations were performed between xylem and phloem hydraulic diameters and climatic variables. Post hoc analyses of every regression fit were performed using DHARMa R package to test normality, homoscedasticity and outliers (Hartig 2022). Additionally, we used SMATR R package to check if the scaling relationships were isometric or allometric (Warton et al. 2012). This calculates the slope for the bivariate linear relationship between two variables (after being log_{10}-transformed) following the standardised major axis regression. If the 95% confidence interval of the slope includes the value of 1, isometry cannot be rejected, whereas allometry can be assumed when this confidence interval does not include such value. For anatomical traits and conductivities, we performed slope tests including leaf habit as factor. Finally, for physiological traits, all species were considered together.

3 | Results

The range of variation covered in this study in terms of leaf area goes from 1.9 cm^2 (*Quercus monimotricha*) up to 151 cm^2 (*Quercus macrocarpa*). If species are compared by their leaf habit, significant differences ($p < 0.001$) can be found between deciduous (DEC; 68.7 ± 46 cm^2) and evergreen (EVE; 16.6 ± 15 cm^2) (Figure 3a). Raw measurements of all the measured diameters are represented as a violin plot to notice the range of variation either between leaf habits or between conductive tissues (Figure 3b). Hydraulic diameter (d_h) is always significantly wider—either for xylem (d_{hx}) or phloem (d_{hp})—in deciduous than in evergreen species although the range of variation in xylem vessels diameter (25 ± 7.64 µm in deciduous; 15.8 ± 5.6 µm in evergreen) is higher than in phloem cells diameter (8.19 ± 1.77 µm in deciduous; 6.28 ± 1.42 µm in evergreen). When the conductive area (A_c) is examined (Figure 3c), deciduous species also display larger xylem and phloem cross-sectional areas ($A_x = 297 \pm 180 \times 10^3$ $µm^2$, $A_p = 224 \pm 147 \times 10^3$ $µm^2$) compared to evergreen species ($A_x = 175 \pm 121 \times 10^3$ $µm^2$, $A_p = 128 \pm 116 \times 10^3$ $µm^2$) (Figure 3c). Nonetheless, the ratio between cross-sectional areas of the vascular elements in the petiole and LA—that is, XLA and PLA—reveals significantly lower values in deciduous species (XLA = 4628 ± 1371, PLA = 3885 ± 2320) compared with evergreen species (XLA = 14207 ± 9038, PLA = 9242 ± 3923) (Figure 3d), which means that evergreen oaks have a higher conductive area per leaf area. Calculated xylem hydraulic conductivity (K_{hx}) and xylem specific conductivity (K_{sx}) also present highly significant ($p < 0.001$) differences between deciduous and evergreen species. By contrast, K_{hp} does not show significant differences when leaf habit is considered ($p = 0.902$) (Table 2).

Although the cross-sectional petiole (A_{pet}) area shows a positively significant relationship with LA ($p < 0.001$), the dispersion of the data is quite high, especially in deciduous species (DEC: $R^2 = 0.486$, EVE $R^2 = 0.752$) (Figure 4a). Conductive area (A_c) presents a strong correlation with LA for both leaf habits ($p < 0.001$, DEC: $R^2 = 0.816$, EVE $R^2 = 0.816$) (Figure 4b, Table 3).

3.1 | Xylem and Phloem Anatomy

We analyse how the conductive area of both tissues, xylem and phloem, as well as the hydraulic diameter of conduits scale with leaf area. In all cases, traits scale positively and significantly ($p < 0.001$) with LA (Figure 5, Table 3). The larger a leaf is, the larger is the investment in conductive area (Figure 5a,d) and wider conduits (Figure 5b,e). However, the relationships are not linear but logarithmic, so that for small leaves, a slight increment in leaf area implies a big increase in both, A_c and d_h, especially in evergreen species. Xylem and phloem also present the same behaviour when their ratios are analysed, that is, how much A_x and A_p a petiole develops divided by leaf area (Figure 5c,f). XLA and PLA scale negatively and significantly ($p < 0.001$) with LA either for deciduous or evergreen species (Figure 5c,f). Evergreen species show a huge heterogeneity in their ratio values for both xylem and phloem for the smallest values of LA. In other words, there is a group of small-leaved evergreen species that invests more in A_x for a specific LA in comparison to large-leaved evergreen leaves. XLA and PLA values above a LA of c.a. 50 cm^2 become asymptotical. The scaling relationships are allometric in all cases (Table 3).

When both conductive areas are correlated, a strong linear relationship ($p < 0.001$) can be appreciated (Supporting Information: Figure S1; $R^2 = 0.716$ for DEC, $R^2 = 0.811$ for EVE). When leaf habit is taken into account, the scaling for deciduous species can be considered isometric (Supporting Information: Figure S1). By contrast, evergreen species present an allometric scaling between A_x and A_p, with more xylem produced than phloem (Supporting Information: Figure S1).

3.2 | Petiole Hydraulic Conductivity

Calculated hydraulic conductivity of xylem (K_{hx}), that is, the theoretical capacity of the whole petiole to supply water to the leaf, results to be positively and significantly ($p < 0.001$, DEC: $R^2 = 0.724$, EVE $R^2 = 0.560$) correlated with LA (Figure 6a). Deciduous species with the largest leaves present up to ten-fold higher values of K_{hx} than the evergreen ones with the lowest values (Supporting Information: Figure S2a). For both leaf habits an allometric relationship between K_{hx} and LA is supported (Table 3).

The specific conductivity of xylem (K_{sx}) also presents a high significance ($p < 0.001$) in both groups in relation with LA, although correlations are much weaker in comparison to K_{hx}, especially for evergreen species (DEC: $R^2 = 0.415$, EVE: $R^2 = 0.281$) (Figure 6b, Table 3). Deciduous species present significant higher values of K_{sx} than evergreen ones (Supporting Information: Figure S2b). In this case, isometry cannot be rejected for either deciduous or evergreen species (Table 3). Leaf-specific conductivity (LSC) is significantly higher in deciduous species than evergreen ones ($p < 0.001$) (Supporting Information: Figure S2c).

Calculated phloem hydraulic conductivity (K_{hp}) is in all cases much lower than for xylem with weaker or nonsignificant correlations with LA (DEC: $R^2 = 0.276$, $p = 0.06$; EVE: $R^2 = 0.036$, $p = 0.55$) (Table 3, plot not shown). It is over 100 times lower than the K_{hx} on average for deciduous species (data not shown). Differences among evergreen species are less remarkable, with a K_{hx} c.a. 20 times higher than K_{hp} on average and some specific individuals with a similar conductivity for both conductive tissues (data not shown).

When XLA is compared with the K_{sx} (Figure 7a), it can be noticed how deciduous species, whose leaves are larger, hardly present variation in their XLA values. By contrast they display a wide range of values in their K_s. Conversely, evergreen species show a wide range of variation in their XLA values without an apparent increase in their K_{sx}, with the exception of *Q. costaricensis*, which is, in turn, among the species with the largest leaves within evergreen oaks.

Similarly, in the comparison between XLA with the respective d_{hx} (Figure 7b), it can be seen how individuals tend to contribute mainly to one axis depending on their leaf habit. This is, deciduous species basically present much higher range of variation in d_{hx} than in XLA. By contrast, evergreen oaks present a larger variation in XLA than in d_{hx}. Both increments, either in d_{hx} or in XLA leads to an improvement in the LSC, although species that increase their d_{hx}, represented by deciduous oaks, improve their LSC more than evergreen oaks that increase their xylem area.

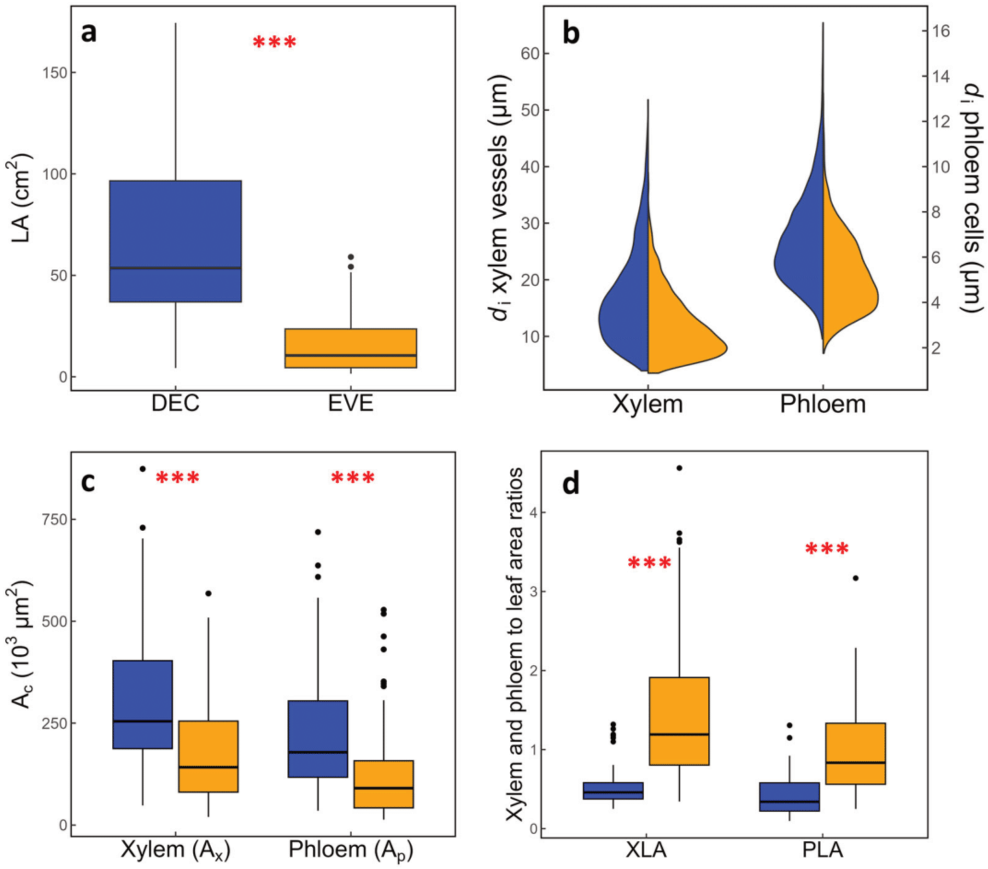

FIGURE 3 | Distribution of the main traits measured: (a) Boxplot of the leaf area (LA), (b) Violin plot of the diameters (d_i) of the conduits (xylem vessels and phloem cells), (c) Boxplot total conductive area (A_c) in the petiole, and (d) Xylem and phloem to leaf area ratios, that is, conductive area divided by LA. Red asterisks show significant ($p < 0.001$) differences between leaf habits (blue, deciduous; orange, evergreen). Note the double Y scale in panel (b). [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 | Percentage of variance explained by leaf habit (deciduous and evergreen) according to the ANOVA performed for each trait measured individually. Leaf traits notation as in Table 1. Significance level is showed with asterisks (***$p < 0.001$, **$p = 0.001$-0.01, *$p = 0.01$–0.05, n.s.> 0.05).

Trait	Leaf habit		Residuals
LA	37.43	***	62.57
A_x	13.97	***	86.03
d_{hx}	32.51	***	67.49
$Ratio_x$	32.95	***	67.05
K_{hx}	18.22	***	81.78
K_{sx}	28.61	***	71.39
A_p	11.72	***	88.28
d_{hp}	25.17	***	74.83
$Ratio_p$	29.23	***	70.77
K_{hp}	0.88	n.s.	99.12

3.3 | Climatic Correlations

Mean annual precipitation reveals a significant relationship with d_{hx} for evergreen species ($p = 0.02$) but no significance is found in deciduous ($p = 0.07$). Taking into account the potential evapotranspiration, that is, comparing the aridity index (AI) with d_{hx} improves the relationships. Aridity index shows positive correlation with d_{hx} for both deciduous ($p = 0.03$; $R^2 = 0.249$) and evergreen species ($p = 0.02$; $R^2 = 0.274$) (Supporting Information: Figure S3). The smallest hydraulic diameters are displayed in the most xeric species. Regarding temperature, MAT does not seem to be significantly related to d_{hx} either for deciduous ($p = 0.24$) or evergreen species ($p = 0.91$). Conversely, T_{min} was only compared for evergreen species since deciduous oaks lack leaves during winter. The correlation did not present significance ($p = 0.37$; Supporting Information: Figure S4).

3.4 | Relationships Between Vascular Traits, Stomatal Conductance and Photosynthesis Net Rate

When the main attributes of the xylem in the petiole are correlated with the stomatal conductance at leaf level ($g_{s,leaf}$), significant relationships can be appreciated in all cases ($p < 0.001$) (Figure 8). There is a significant increase in $g_{s,leaf}$ as A_x becomes larger with an allometric relationship ($R^2 = 0.512$) (Figure 8a, Table 4). For a given value of A_x, deciduous species tend to present higher values of $g_{s,leaf}$ than evergreen ones. Stomatal conductance also increases

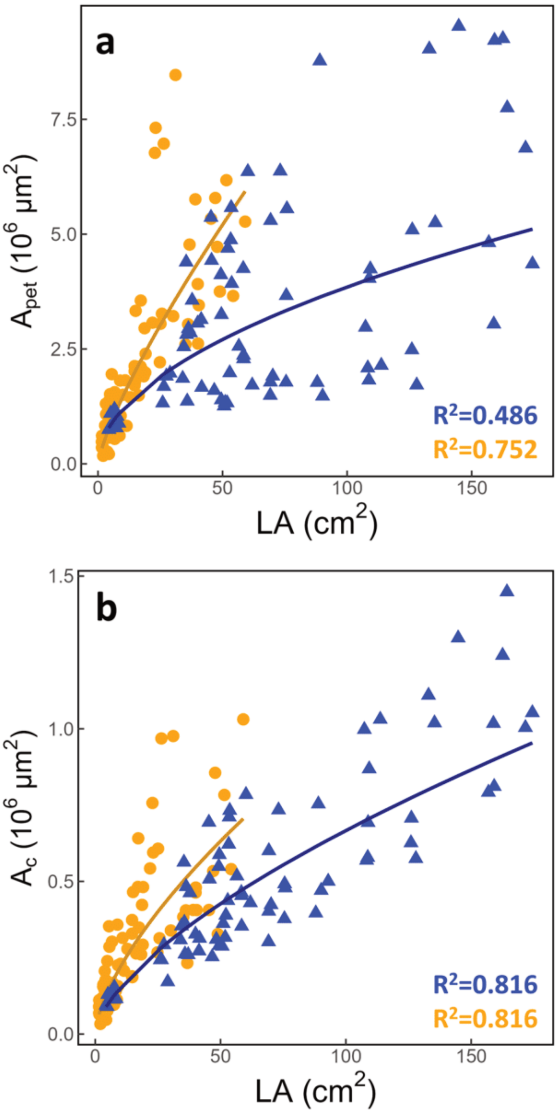

FIGURE 4 | Scaling relationships of (a) leaf area (LA) with cross-sectional petiole area (A_{pet}) and (b) LA with conductive area (A_c, the sum of xylem and phloem areas) for deciduous (blue triangles) and evergreen (orange dots) species. Each point represents one individual measure. Coloured continuous lines represent the best fit for each leaf habit separately. All regressions are highly significant ($p < 0.001$). [Color figure can be viewed at wileyonlinelibrary.com]

allometrically as d_{hx} becomes wider, but with a steeper slope ($R^2 = 0.586$) (Figure 8b, Table 4). Likewise, deciduous species usually present higher values of $g_{s,leaf}$ for the same d_{hx} than evergreen ones. Once again, K_{hx} also scaled allometrically (Figure 8c, Table 4) and deciduous species have higher values than evergreen species on average.

When the same xylem traits are correlated with the photosynthesis net rate, exactly the same trends arise (Table 4). The $A_{N,leaf}$ appears to be related to A_x (Figure 8d, $p = 0.001$, $R^2 = 0.591$), d_{hx} (Figure 8e, $p < 0.001$, $R^2 = 0.671$) and K_{hx} (Figure 8f, $p < 0.001$, $R^2 = 0.786$).

Photosynthesis net rate at leaf level ($A_{N,leaf}$) is significantly correlated with phloem anatomical traits ($p < 0.001$) (Figure 9, Table 4). $A_{N,leaf}$ increases with larger A_p ($R^2 = 0.561$) (Figure 9a) and wider d_{hp} ($R^2 = 0.509$) (Figure 9b) being on both cases an allometric relationship. Deciduous species tend to present higher photosynthesis net rates at leaf level than evergreen ones. Finally, the relationship between $A_{N,leaf}$ and K_{hp} is barely significant ($p = 0.043$) with a very week correlation ($R^2 = 0.130$) (plot not shown).

4 | Discussion

4.1 | Anatomical Traits Scale With Leaf Area

In the *Quercus* species studied, we found associations between the anatomical traits of the petioles and leaf area. First, the cross-sectional area of the petiole displays a rather scattered association with LA (yet significant). In general, there is an allometric relationship for deciduous species, whereas evergreen oaks are better adjusted to an isometric scaling. As we first hypothesised, both hydraulic diameter and conductive area scale with LA, either as a whole (A_c) or separating between xylem (A_x) and phloem (A_p). This means that the larger the leaf, the greater the ability for bulk transport of water and carbohydrates. Increasing the conductivity can be achieved either by increasing the number of conduits, by producing wider conduits or by a combination of both strategies. It should be noted that widening the conduits results in a fourth-power increment in the efficiency, whereas a higher number of conduits only increases the conductivity linearly, according to Hagen-Poiseuille law.

The weaker scaling in conductive areas and hydraulic diameters in larger leaves may reflect the constraints that can compromise the functionality of the conducting tissues (Figure 5). The trade-off between support and conduction functions of petioles could be explaining the constraint to produce linearly larger conductive areas in larger leaves. The hydraulic diameter cannot scale infinitely either. In the xylem, wider conduits are more susceptible to cavitation by both drought and freezing. For phloem, leaves with wider sieve tube elements would require a sugar production commensurate with the size of such conduits to generate an adequate turgor pressure gradient to transport the phloem sap. Otherwise, allocation of sugars would be hindered. Sieve elements differences can be found depending on the organ, age and life-form. (Ewers and Fisher 1991; Woodruff 2014; Prislan et al. 2019; Kopanina et al. 2022; Losada, He, and Holbrook 2022). Despite these differences, interspecific variation of sieve element diameter is lower than for xylem vessel diameter, which agrees with our results. Thus, the limitations imposed to phloem seem to be more restrictive than those imposed to xylem. This makes sense if we consider that maintaining the proper function of phloem is more critical than xylem for several reasons. First, phloem sap flows during the whole day, night included, in contrast to xylem flow, which reaches the highest values when stomata are opened during the day. Second, phloem must maintain a constant turgor to achieve a steady flow since either an excessive viscosity or a loss of turgor level will hinder the sap flow (Lang 1978).

The scaling relationship between xylem and phloem areas has been also explored in several studies (Table 5), which find an isometric scaling between A_x and A_p. Nonetheless, most of these studies only

TABLE 3 | Scaling exponents of each leaf habit (deciduous and evergreen) separately for standardised major axis (SMA) regressions. All variables were $\log_{10}$ transformed. The scaling relationship (isometry or allometry) was selected taking into account if the 95% confident interval of the slope includes the value 1 (isometry) or not (allometry). All correlations are significant ($p < 0.001$) but K_{hp} with LA. Leaf traits notation as in Table 1. Differences in the slope and elevation between deciduous (DEC) and evergreen (EVE) species are shown with asterisks (***$p < 0.001$ **$p < 0.01$, *$p < 0.05$, NS = No significant).

			Deciduous			Evergreen			DEC vs. EVE	
y	***x***	**Figure**	**Slope**	**Scaling relationship**	R^2	**Slope**	**Scaling relationship**	R^2	**Slope**	**Elevation**
A_{pet}	LA	Figure 3a	0.73	Allometry	0.486	0.9	Isometry	0.752	*	***
A_c	LA	Figure 3b	0.71	Allometry	0.816	0.79	Allometry	0.816	NS	***
A_x	LA	Figure 4a	0.74	Allometry	0.874	0.76	Allometry	0.651	NS	***
d_{hx}	LA	Figure 4b	0.33	Allometry	0.507	0.37	Allometry	0.563	NS	NS
$Ratio_x$	LA	Figure 4c	−0.4	Allometry	0.579	-0.59	Allometry	0.429	*	*
A_p	LA	Figure 5a	0.75	Allometry	0.599	0.87	Allometry	0.69	NS	***
d_{hp}	LA	Figure 5b	0.24	Allometry	0.2	0.24	Allometry	0.443	NS	*
$Ratio_p$	LA	Figure 5c	−0.63	Allometry	0.439	-0.56	Allometry	0.24	NS	NS
A_p	Ax	Figure 6	1.01	Isometry	0.716	1.15	Allometry	0.811	NS	NS
K_{hx}	LA	Figure 7a	1.57	Allometry	0.724	1.66	Allometry	0.56	NS	NS
K_{sx}	LA	Figure 7b	1	Isometry	0.415	1.18	Isometry	0.281	NS	NS
K_{hp}	LA	Plot not shown	1.13	Isometry	0.276	1.61	Isometry	0.036	NS	NS

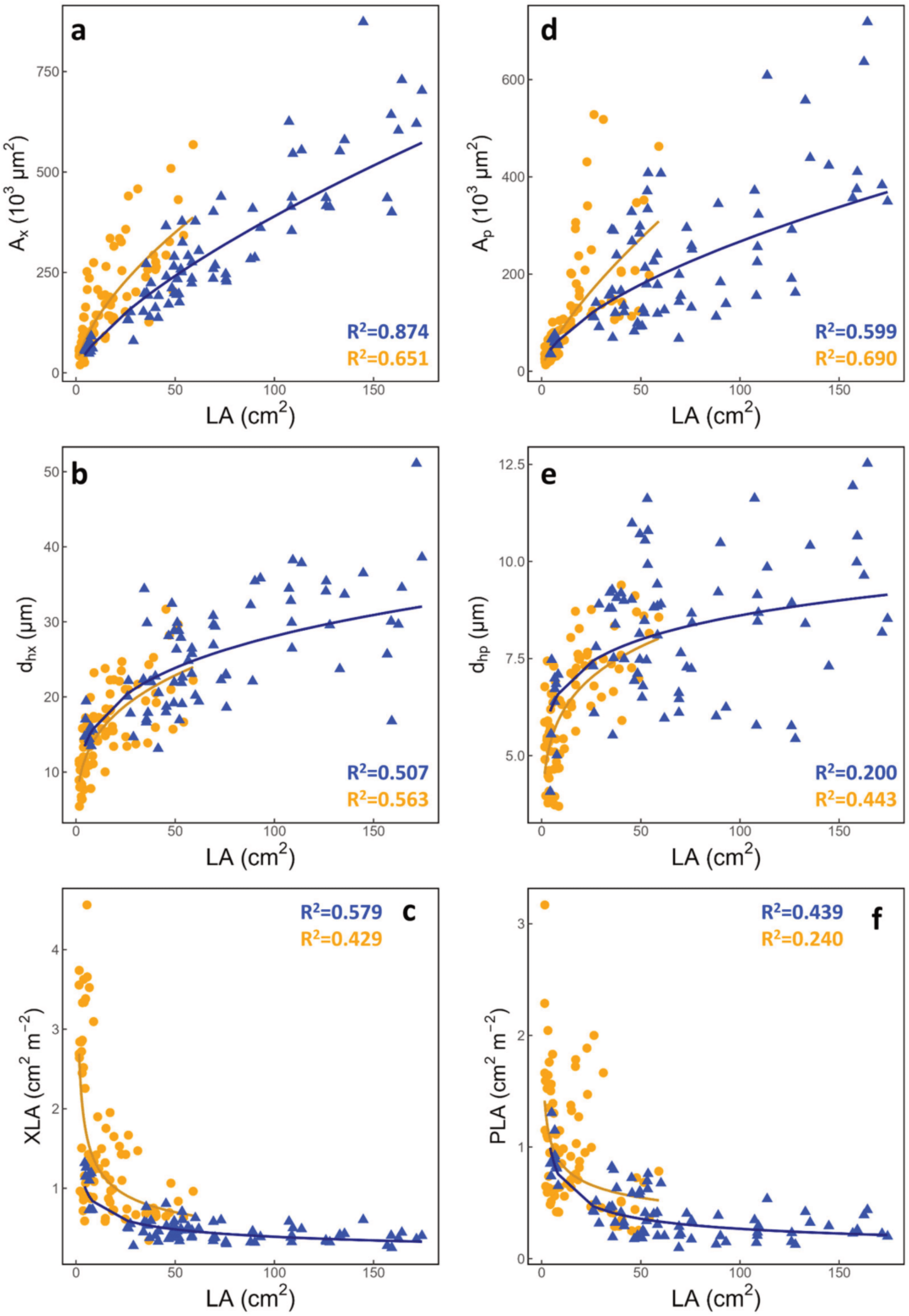

FIGURE 5 | Correlations related to xylem (a–c) and phloem (d–f) anatomy for deciduous (blue triangles) and evergreen (orange dots) species between leaf area (LA) and: (a) xylem cross-sectional area (A_x), (b) xylem hydraulic diameter (d_{hx}), (c) xylem cross-sectional area divided by LA (XLA), (d) phloem cross-sectional area (A_p), (e) phloem hydraulic diameter (d_{hp}) and (f) phloem cross-sectional area divided by LA (PLA). Each point represents one individual measure. Coloured continuous lines represent the best fit for each leaf habit separately. All regressions are highly significant ($p < 0.001$). [Color figure can be viewed at wileyonlinelibrary.com]

focus on single species. Our work clearly improves this by exploring the scaling relationship in a great number of species, closely related but different enough in leaf habit and climatic ranges. Our data support an isometric scaling in the case of deciduous species, but an allometric scaling between xylem and phloem in evergreen species, favoring more production of xylem than phloem area (Supporting Information: Figure S1), which reflects the higher values of XLA in evergreen species (see next section for further details). A scaling relationship between the conductive areas in any case should be expected, since both tissues are originated from the same

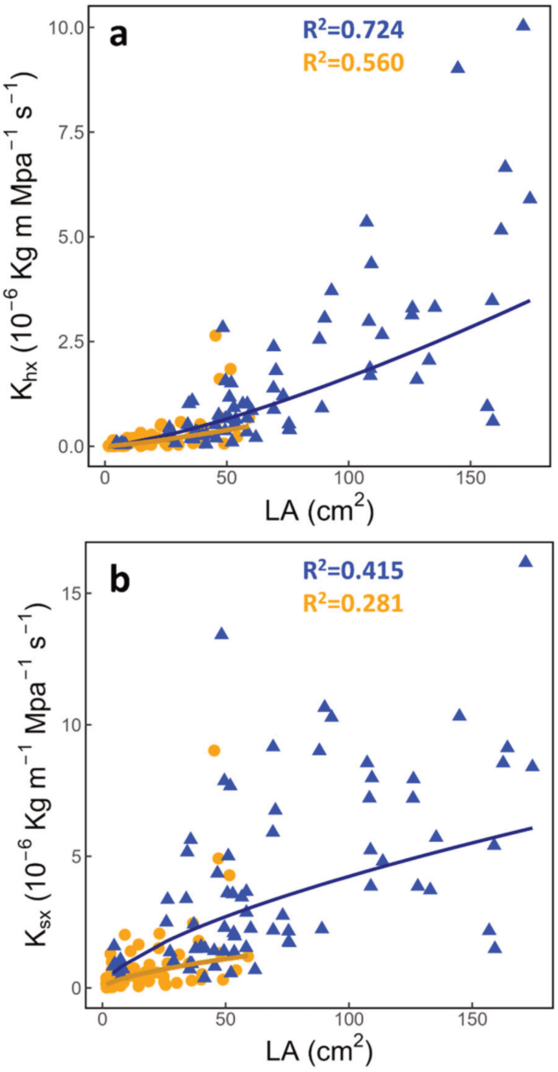

FIGURE 6 | Correlations related to (a) xylem hydraulic conductivity (K_{hx}) and (b) specific hydraulic conductivity (K_{sx}) for deciduous (blue triangles) and evergreen (orange dots) species. Each point represents one individual measure. Coloured continuous lines represent the best fit for each leaf habit separately. All regressions are highly significant ($p < 0.001$). [Color figure can be viewed at wileyonlinelibrary.com]

meristematic tissue, that is, the procambium. In addition, despite having very different function, xylem and phloem are interconnected. The main hydric relationship relies on xylem supplying water to load phloem companion cells and sieve tubes according to a lateral water potential gradient between both tissues. The flux of sugars depends on the product of water flux and sugar concentration. Since the sugar concentration declines with distance from the leaf, water flux must increase to keep the sap flux steady. In other words, there is an influx of water from the xylem throughout the transport phloem to compensate for the lower sugar concentrations. The balanced interaction between xylem and phloem is an essential requirement for long-distance transport (Dinant and Lemoine 2010; Sevanto 2014).

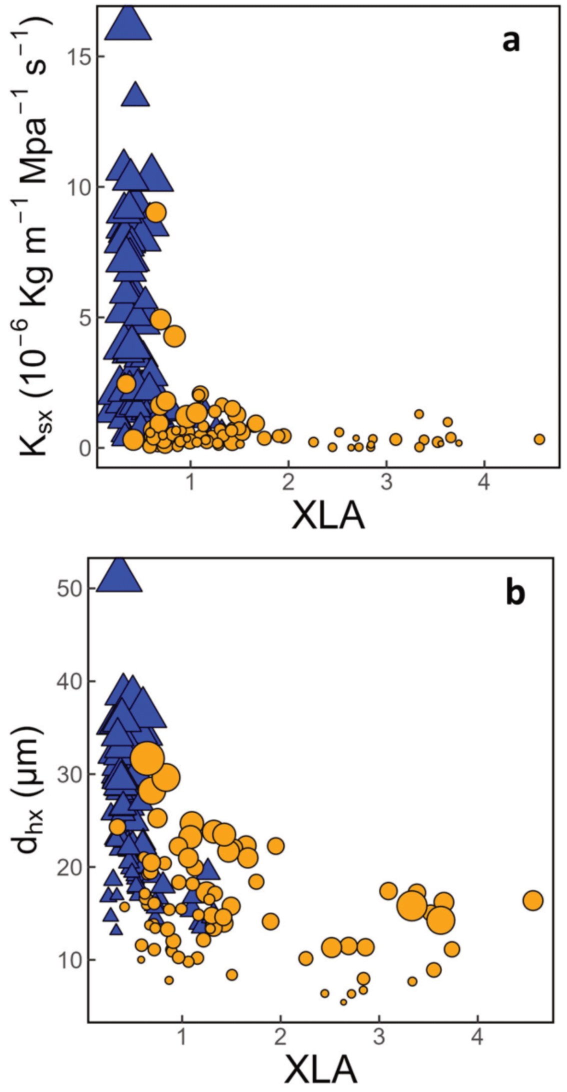

FIGURE 7 | Correlations between (a) XLA and the xylem specific conductivity (K_{sx}) showing the leaf area as the relative size of the symbols (larger symbols represent larger leaf areas); and (b) between the XLA and the xylem hydraulic diameter (d_{hx}) showing the leaf specific conductivity (LSC) as the relative size of the symbols (larger symbols show higher LSC values). Leaf habit is represented by deciduous in blue triangles and evergreen species in orange dots. Each point represents one individual measure. [Color figure can be viewed at wileyonlinelibrary.com]

Concerning the hydraulic conductivity after applying Hagen-Poiseuille law, we reported an improvement in xylem K_h with LA, due to the combination of both a larger A_x and wider d_h. This increment in xylem efficiency is still reflected even after removing the effect of developing more A_x due to larger leaf areas, that is, the K_{sx}. However, this is not the case for phloem, where the dispersion of the data is much higher (Table 3). Here, solely diameter of the phloem cells does not seem to predict the actual hydraulic conductivity of phloem by itself, probably due to a mix of cellular types in the measurements. In addition, some other factors related to the nature of sieve plates, such as the number of pores, diameter of such pores and even number of plates per sieve element, are likely to modulate the hydraulic conductivity of phloem.

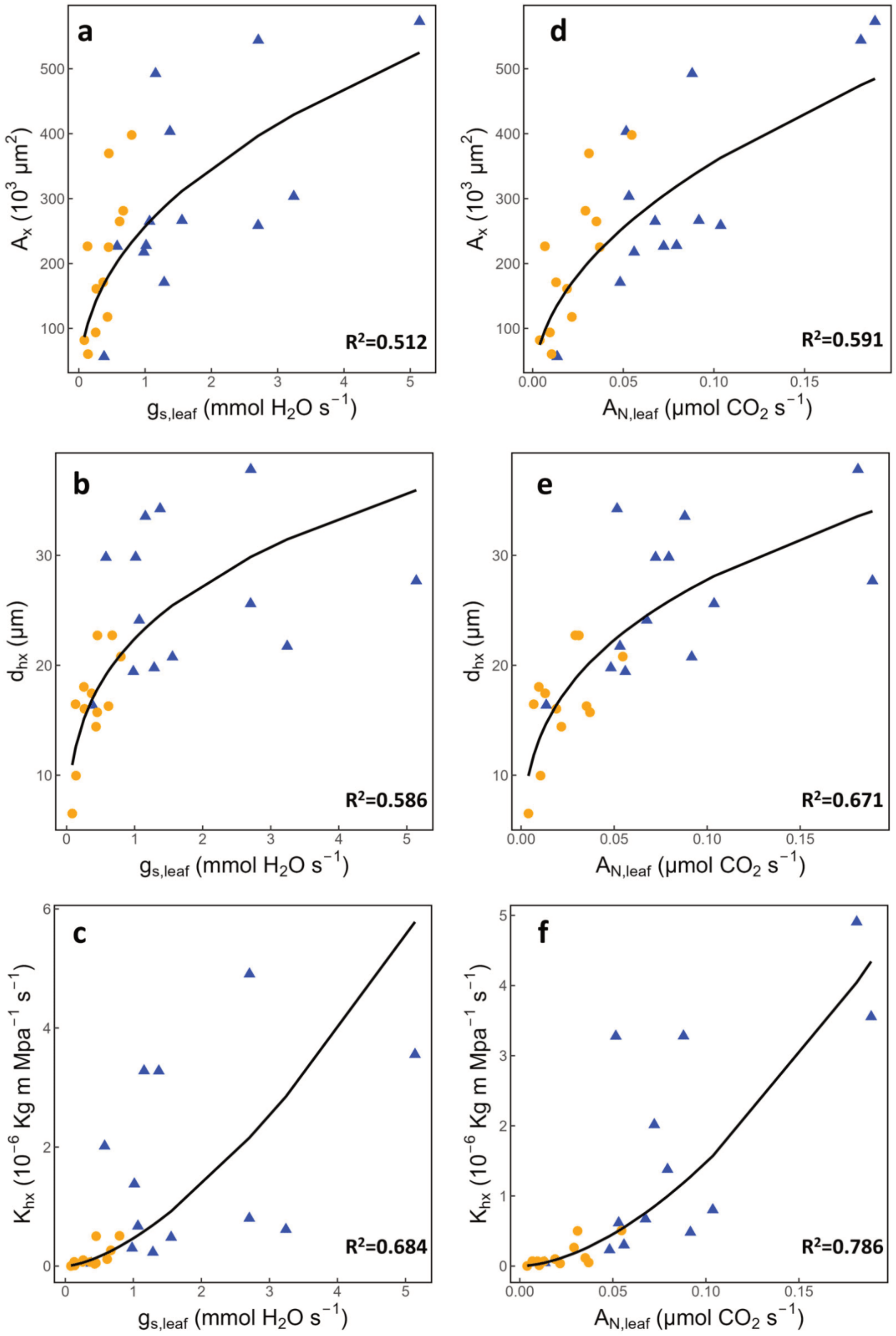

FIGURE 8 | Relationships of xylem traits (cross-sectional area (A_x), hydraulic diameter (d_{hx}) and calculated hydraulic conductivity (K_{hx})) with stomatal conductance ($g_{s,leaf}$) (a-c), and with photosynthesis net rate ($A_{N,leaf}$) (d-f). Blue triangles are deciduous, and orange dots are evergreen species. Each point represents the mean value of a species. The black continuous line is the correlation considering species altogether. All regressions are highly significant ($p < 0.001$). [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 4 | Scaling exponents of physiological traits for standardised major axis (SMA) regressions. All variables were $\log_{10}$ transformed. Leaf traits notation as in Table 1. Every correlation is significant ($p < 0.05$) and scale allometrically (slope significantly different of 1).

y	x	Figure	Slope	Scaling relationship	R^2
A_x	$g_{s,leaf}$	Figure 8a	0.61	Allometry	0.512
d_{hx}	$g_{s,leaf}$	Figure 8b	0.38	Allometry	0.586
K_{hx}	$g_{s,leaf}$	Figure 8c	1.86	Allometry	0.684
A_x	$A_{N,leaf}$	Figure 8d	0.63	Allometry	0.591
d_{hx}	$A_{N,leaf}$	Figure 8e	0.39	Allometry	0.671
K_{hx}	$A_{N,leaf}$	Figure 8f	1.91	Allometry	0.786
A_p	$A_{N,leaf}$	Figure 9a	0.73	Allometry	0.561
d_{hp}	$A_{N,leaf}$	Figure 9b	0.23	Allometry	0.509

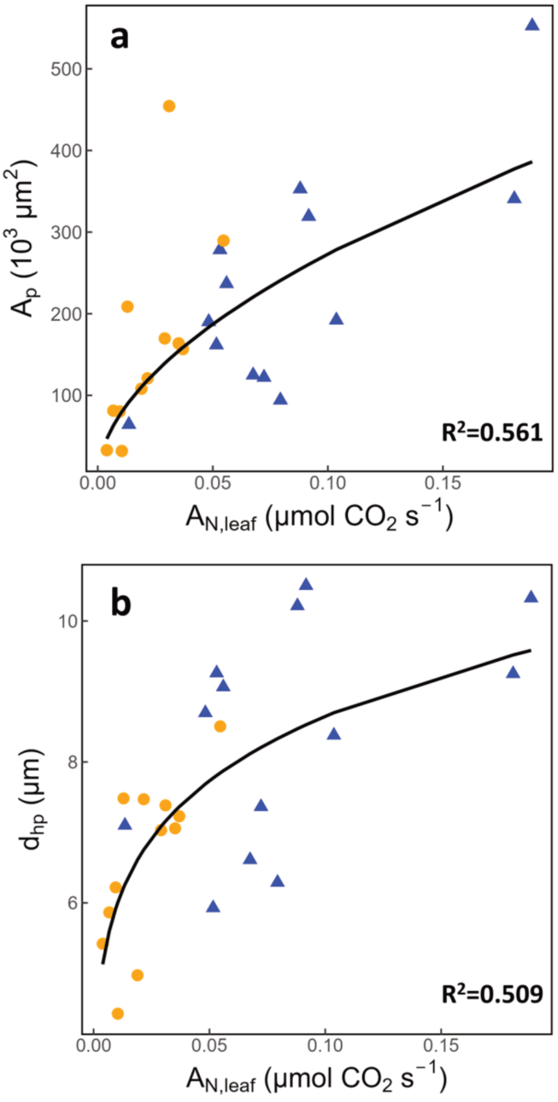

FIGURE 9 | Main relationships between photosynthesis net rate ($A_{N,leaf}$) and phloem anatomical traits ([a] cross-sectional area [A_p] and [b] hydraulic diameter [d_{hp}]). Deciduous as blue triangles and evergreen species as orange dots. Each point represents the mean value of a species. The black continuous line is the correlation considering species altogether. Both regressions are highly significant ($p < 0.001$). [Color figure can be viewed at wileyonlinelibrary.com]

4.2 | Deciduous and Evergreen Oaks Follow Different Strategies Producing and Packaging Their Conduits

The main differences between deciduous and evergreen oak species arise when we compare the conductive area standardised by LA (i.e., the XLA) with the K_{sx} and d_{hx} (Figure 7). In this scenario, the range of variation in both K_{sx} and d_{hx} mainly corresponds with deciduous species, whereas the range of variation in XLA mostly corresponds with evergreen species. The larger LA of deciduous oaks requires a higher water supply which is, in turn, reflected by a higher photosynthetic rate and stomatal conductance compared to evergreen species. Thus, deciduous species display up to an order of magnitude higher K_{sx} values than evergreen oaks. An increase in K_{sx} can be achieved either by reducing the xylem area or by widening the xylem vessels for the same size and number of vessels, which ultimately increases K_{hx}. Since the xylem area increases with LA, this increase in K_{sx} in deciduous species can only be modulated by an increase in the diameter of the xylem vessels. By contrast, evergreen oaks hardly present range of variation in their K_{sx} values, except for *Q. costaricensis*, the evergreen oak with the widest vessels in this study.

Subsequently, we compared d_{hx} with XLA, but this time transforming K_{sx} into a more physiologically meaningful variable, that is LSC, which links the capacity of xylem to transport water with the leaf water demands (Mencuccini et al. 2019). In this correlation (Figure 7b), deciduous oaks always present low XLA values, close to or lower than one, but they display a wide range of variation in their d_{hx}. Conversely, evergreen oaks exhibit a wider variation in their XLA values but narrower d_{hx} values. In other words, deciduous species tend to produce wider conduits to improve their xylem hydraulic conductivity for a given leaf area, whereas evergreen species choose to increase their A_x for the same leaf area over the d_h.

This dichotomous strategy between deciduous (Figure 1, Model A) and evergreen (Figure 1, Model B) oaks could be directly related with both, their leaf life spans and the climatic niches they occupy. First, deciduous leaves only have to keep functional for a few months (typically 6–9 months). Thus, they can take a riskier but, simultaneously, a more effective—showed by high LSC values—and a cheaper strategy (Ni et al. 2022). On the other hand, evergreen species, whose leaves must remain productive for longer periods, tend to follow a safer strategy at the expense of a more costly investment (Hacke, Sperry, and

TABLE 5 | References of studies exploring the scaling relationship between xylem and phloem conductive areas, specifying the species and organs studied as well as the slope and the nature of the scaling found, either isometry or allometry.

Reference	Species	Organ	Slope	Scaling relationship
Jyske and Hölttä (2015)	*Picea abies*	Stem	0.93	Isometry
Carvalho et al. (2017a)	*Populus × canescens*	Leaf, petiole	0.96	Isometry
Carvalho et al. (2017b)	*Ginkgo biloba*	Leaf	0.91	Isometry
Kiorapostolou and Petit (2019)	*Fraxinus ornus*	Stem	0.96	Isometry
Ray and Jones (2018)	*Pelargonium* (11 spp.)	Petiole	0.87	Isometry
Losada, He, and Holbrook (2022)	*Austrobaileya scandens*	Leaf, petiole and twig	1.25	?

Pittermann 2000). Nonetheless, this investment in larger A_x also increases the LSC in those species with high values of XLA, partly counterbalancing their lower K_{hx} values and reaching efficiencies close to deciduous species. Besides, this safer strategy could be the main contributor to the allometry found in xylem for evergreen species in comparison to the isometry that most studies find and is also present in our deciduous species.

Second, deciduous oaks considered in this study are mainly represented by species occupying temperate forests. These habitats rarely present stressful conditions (drought and/or cold) during the lifespan of the leaves (Peguero-Pina et al. 2016). Hence, it is reasonable to think that deciduous oaks could afford more efficient vessels at the expense of more vulnerability. Accordingly, most of the evergreen oak species (with the exception of some tropical ones; for example, *Q. costaricensis*) must cope with at least one stressful period during the year (typically a drought period), and even two in the case of Mediterranean species (summer drought and winter cold) (Martín-Sánchez et al. 2022). Therefore, it is justifiable to consider that these species choose a conservative strategy for building their conductive tissues. Furthermore, deciduous oaks occupying extra-temperate habitats with stressful periods such as the Mediterranean Basin (e.g., *Q. faginea* and *Q. ithaburensis*) or winter-dry temperate climates in Mexico (e.g., *Q. crassipes*) present the smallest values of both leaf area and hydraulic diameter among deciduous oaks, suggesting the reduction of xylem vessels in environmental restrictive habitats. Indeed, aridity index shows correlation with d_{hx} for both deciduous and evergreen species. The more xeric the climate is, the narrower the xylem vessels are. This relationship between drought and vessel size has been widely reported by numerous authors in stems, branches and leaves, resulting in a trade-off between efficiency and safety (Hajek et al. 2014; Pivovaroff et al. 2016; Schreiber et al. 2016; Barotto et al. 2018). It has also been recently found in petioles by comparing XLA and resistance to cavitation in several *Eucalyptus* species (Blackman et al. 2024). Likewise, this compensation of improving the hydraulic conductivity by increasing the conductive area over the diameters of the conduits has been also reported in stems of several species (Nardini, Pedà, and Rocca 2012) but, to our knowledge, it has never been attributed to leaf habit in any case.

We demonstrate the presence of two models for producing and packaging the conduits, and we also prove the relationship between aridity and hydraulic diameter. However, we did not find significant correlation between cold, here represented by the WorldClim2 variable "mean of daily minimum temperatures during the coldest quarter", and d_{hx} in evergreen oaks. Nonetheless, cavitation induced by winter cold is caused by freeze-thaw cycles, a climatic variable for which global-scale data are not available. The lack of significance is mainly due to two species: *Q. semecarpifolia* and *Q. engleriana*. These evergreen species present a wide range of distribution in Asia, in habitats that present a complex orography, resulting in very different climatic conditions. A detailed study in their natural habitats along altitudinal and climatic gradients, measuring the daily temperatures, might reveal a reduction in d_{hx} in those sites where trees have to withstand more frequent freeze-thaw cycles. Other species, such as *Q. chrysolepis* and *Q. monimotricha*, for instance, the two species with the narrowest vessels, can be found in very high-altitude habitats, where they are exposed to recurrent frosts during the coldest months. Thus, according to the leaf economic spectrum, these species would not recover the investment in case such expensive leaves died earlier due to a hydraulic failure. By contrast, evergreen species with the widest vessels (e.g., *Q. costaricensis*, *Q. virginiana*) occupy tropical or subtropical habitats with the absence of strong and frequent frosts.

4.3 | Anatomy of Petioles Accommodates Physiological Demands

Our data supports a strong correlation between the petiole anatomical traits of both, xylem and phloem, and the estimated g_s and A_N at leaf-level. The strongest relationships are found between xylem traits and $A_{N,leaf}$, albeit xylem-$g_{s,leaf}$ correlations shows similar statistical power. Even though Figures 8 and 9 represent deciduous and evergreen species in different colours, the aim is not to see differences in leaf habit but explore the anatomical architecture in response to the physiological demands of the leaf lamina. This link function-structure has been proposed to be mediated throughout several physiological processes, such as water potential, hydraulic conductance, turgor pressure or sugar concentration (Holtta et al. 2010). These factors would influence the ontogeny and development of the cells in a tissue (Cosgrove 1993).

Relationships between xylem area in the petiole and leaf transpiration were proposed more than one hundred years ago by Salisbury (1913). However, this author suggested that the nature of the conduits—that is, number and size—should receive more attention. Here, we explore not only such relationship between A_x and $g_{s,leaf}$, but also the size of the conduits and the calculated K_{hx}, which all resulted to be highly correlated with $g_{s,leaf}$. Brocious and Hacke (2016), presented a study among different *Populus* hybrids they found similar trends to our findings for A_x and K_{hx} in relation to g_s, suggesting that 'lamina size is constrained by the transport capacity of the vascular tissue in the petiole'. Concerning the scaling relationship, our results show an allometry in all cases. In this regard, Zhong et al. (2020) also found allometric scaling in 53 woody species between xylem area in the midrib and the number of stomata in the leaf lamina, but they reported an isometric scaling of leaf area and total stomatal area. Nonetheless, it must not be disregarded that stomatal conductance is only showing the capacity of stomata to release water to atmosphere, but the transpiration rate is the variable actually measuring water losses in leaves by taking into account the vapour pressure deficit (VPD). In this context, part of the scatter observed in the association between petiole xylem traits and $g_{s,leaf}$ might be attributed to adaptations to different VPD levels during the growing season.

A higher photosynthetic rate is related to a larger xylem hydraulic conductance because of a greater water usage (Brodribb and Feild 2000; Holtta et al. 2010). The largest leaves among our species correspond with deciduous species, and they present a higher $A_{N,leaf}$ compared to evergreen species. However, when A_N—expressed in m^2—is compared, no significant differences linked to leaf habit are found (Peguero-Pina et al. 2017). In this case, considering the total photosynthesis rate at leaf level is more logical than standardising it for a given area because a petiole must have an anatomical structure able to export the sucrose produced by the leaf. Sucrose is the most abundant photosynthate transported by sieve elements, but viscosity of a sucrose solution increases exponentially with increasing concentration (Morison 2002). Furthermore, the viscosity of the sap is one of the main factors that limit phloem transport, since the more viscous the solution, the lower the flow rate (Lang 1978; Sevanto 2014). To deal with this disadvantage, plants can choose between two strategies. On the one hand, sink organs could lower their sugar concentration, increasing the source-sink concentration gradient. On the other hand, they could develop wider sieve tubes, since an increment in the radius of a sieve tube should improve the hydraulic conductivity to the fourth power. This latter strategy seems to be more feasible for the plant (Hölttä, Mencuccini, and Nikinmaa 2009; Sevanto 2014). Thus, the largest leaves in oak species, which are in turn the ones which produce more sucrose, would need wider sieve tubes to avoid a depleted flow rate caused by an excessive viscosity.

5 | Conclusion

The anatomy of the petiole adapts to the demands of the leaf, with xylem and phloem scaling to meet both transpiration and photosynthesis needs. Both tissues adjust in a coordinated manner, balancing the conductive area and cell diameters, reflecting species' habitat adaptations. For instance, species in arid environments have narrower conduits compared to those in cooler, wetter habitats.

Deciduous and evergreen oaks use different strategies to optimise hydraulic conductivity. Deciduous oaks produce wider vessels for the same leaf area, enhancing water flow but at a higher risk. In contrast, evergreen oaks adopt a safer strategy by increasing the conductive area rather than vessel diameter, ensuring safety over efficiency.

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Data Availability Statement

The data that supports the findings of this study are available in https://doi.org/10.5281/zenodo.13735281.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

SUPLEMENTARY MATERIAL

Table S1. List of oak species studied, the section they belong to, the botanical garden they have been sampled and their leaf habit.

Species	**Section**	**Garden**	**Leaf Habit**
Quercus acutissima	*Cerris*	CITA	Deciduous
Quercus agrifolia	*Lobatae*	CITA	Evergreen
Quercus alba	*Quercus*	Iturrarán	Deciduous
Quercus berberidifolia	*Quercus*	Iturrarán	Evergreen
Quercus brantii	*Cerris*	CITA	Deciduous
Quercus chrysolepis	*Protobalanus*	CITA	Evergreen
Quercus coccifera	*Ilex*	CITA	Evergreen
Quercus costaricensis	*Lobatae*	Iturrarán	Evergreen
Quercus crassipes	*Lobatae*	Iturrarán	Deciduous
Quercus engleriana	*Ilex*	Iturrarán	Evergreen
Quercus faginea	*Quercus*	CITA	Deciduous
Quercus falcata	*Lobatae*	Iturrarán	Deciduous
Quercus fusiformis	*Virentes*	Iturrarán	Evergreen
Quercus hemisphaerica	*Lobatae*	CITA	Evergreen
Quercus ilex	*Ilex*	Iturrarán	Evergreen
Quercus ithaburensis	*Cerris*	CITA	Deciduous
Quercus leucotrichophora	*Ilex*	CITA	Evergreen
Quercus longispica	*Ilex*	Iturrarán	Evergreen
Quercus macrocarpa	*Quercus*	CITA	Deciduous
Quercus microphylla	*Quercus*	Iturrarán	Evergreen
Quercus monimotricha	*Ilex*	Iturrarán	Evergreen
Quercus montana	*Quercus*	Iturrarán	Deciduous
Quercus muehlenbergii	*Quercus*	CITA	Deciduous
Quercus myrsinifolia	*Cyclobalanopsis*	CITA	Evergreen
Quercus pyrenaica	*Quercus*	CITA	Deciduous
Quercus robur	*Quercus*	CITA	Deciduous
Quercus rubra	*Lobatae*	CITA	Deciduous
Quercus semecarpifolia	*Ilex*	CITA	Evergreen
Quercus stellata	*Quercus*	CITA	Deciduous
Quercus suber	*Cerris*	CITA	Evergreen
Quercus velutina	*Lobatae*	Iturrarán	Deciduous
Quercus virginiana	*Virentes*	CITA	Evergreen
Quercus yunnanensis	*Quercus*	Iturrarán	Deciduous

Table S2. Summary of the ANOVA performed for each trait individually. Percentage of variance explained by species, garden and residuals. Significance level is showed with asterisks (*** < 0.001, ** = 0.001-0.01, * = 0.01-0.05).

Trait	Species		Garden		Residuals
LA	89.25	***	3.26	***	7.48
A_{pet}	79.43	***	8.51	***	12.05
A_{c}	73	***	9.27	***	17.73
A_{x}	77.74	***	3.27	***	18.99
d_{hx}	82.29	***	2.08	***	15.62
XLA	79.97	***	12.39	***	7.63
K_{hx}	64.49	***	0.15		35.36
K_{sx}	75.61	***	0.24		24.14
LSC	61.51	***	1.45	*	37.04
A_{p}	63.25	***	17.99	***	18.77
d_{hp}	57.8	***	23.73	***	18.47
PLA	76.32	***	0.38		23.3
K_{hp}	57.35	***	19.1	***	23.55

Table S3. Summary of the linear regressions via mixed model including the garden as a random factor. Transformation applied to data; variance explained by the random factor (garden) and significance of the correlations. Significance level is showed with asterisks (*** < 0.001, ** = 0.001-0.01, * = 0.01-0.05). Note that mixed models resulted to have the same significative correlations as simple linear regressions.

y	x	Variance explained by Garden	P-value
A_{pet}	LA	0.04	***
A_c	LA	0.01	***
A_x	LA	0	***
d_{hx}	LA	0	***
XLA	LA	0	***
A_p	LA	0.1	***
d_{hp}	LA	0.01	***
PLA	LA	0.1	***
A_p	A_x	0.08	***
K_{hx}	LA	0.12	***
K_{sx}	LA	0.11	***

Table S4. Summary of the variance explained in the ANOVA performed for each trait individually taking into account the garden, leaf habit and the interaction between both (Garden*LeafHabit). Significance level is showed with asterisks (*** < 0.001, ** = 0.001-0.01, * = 0.01-0.05). The sum of the variances may not be 100 because of rounding.

Trait	**Garden**		**Leaf Habit**		**Garden*Leaf Habit**		**Residuals**
LA	3.26	**	34.9	***	0.005		61.84
A_{pet}	8.51	***	4.39	**	0.1		87
A_{c}	9.27	***	10.88	***	0.05		79.8
A_{x}	3.27	*	12.23	***	0.04		84.46
d_{hx}	2.09	*	30.73	***	2.19	*	64.98
XLA	12.4	***	27.58	***	4.69	***	55.33
A_{p}	17.99	***	7.79	***	0.57		73.65
d_{hp}	23.73	***	18.97	***	5.15	***	52.15
PLA	0.38		28.9	***	1.25		69.47
K_{hx}	0.15		18.15	***	0.002		81.7
K_{sx}	0.24		30.2	***	0.82		68.74
K_{hp}	19.1	***	2.61	*	1.04		77.25

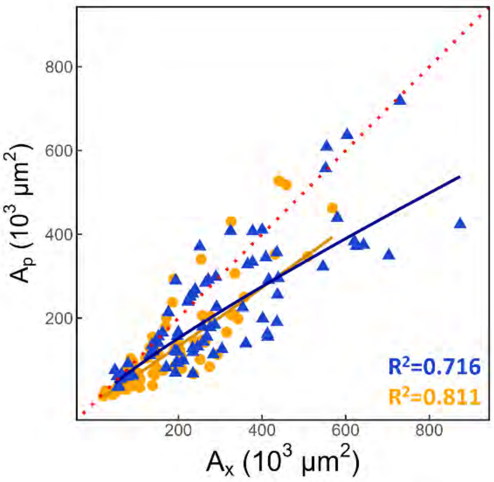

Fig. S1. Scaling relationship between xylem (A_x) and phloem (A_p) conductive areas for deciduous (blue triangles) and evergreen (orange dots) species. Each point represents one individual measure. Colored continuous lines represent the best fit for each leaf habit separately. The dotted red line shows the theoretical isometric scaling between the two conductive areas. Both regressions are highly significant ($P < 0.001$).

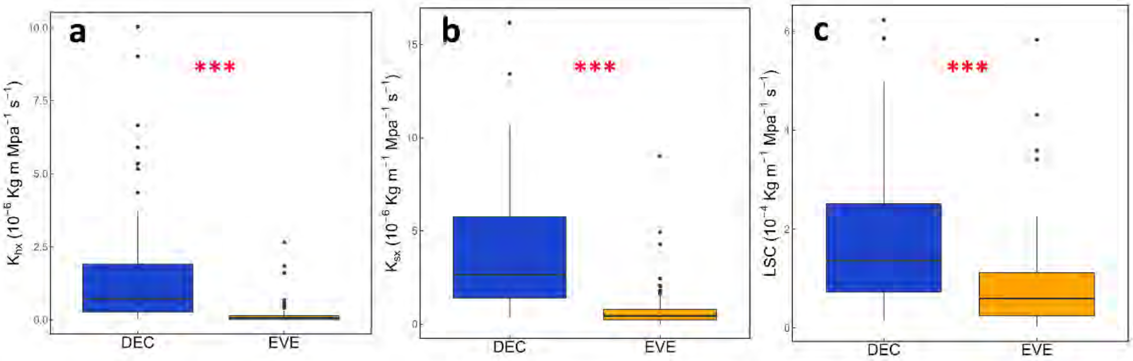

Fig. S2. Boxplots of the different xylem conductivity-related measurements for deciduous (blue) and evergreen (orange) species: a) Hydraulic conductivity (K_{hx}), b) xylem specific conductivity (K_{sx}) and c) leaf-specific conductivity (LSC). Red asterisks show significant ($P < 0.001$) differences between species leaf habit.

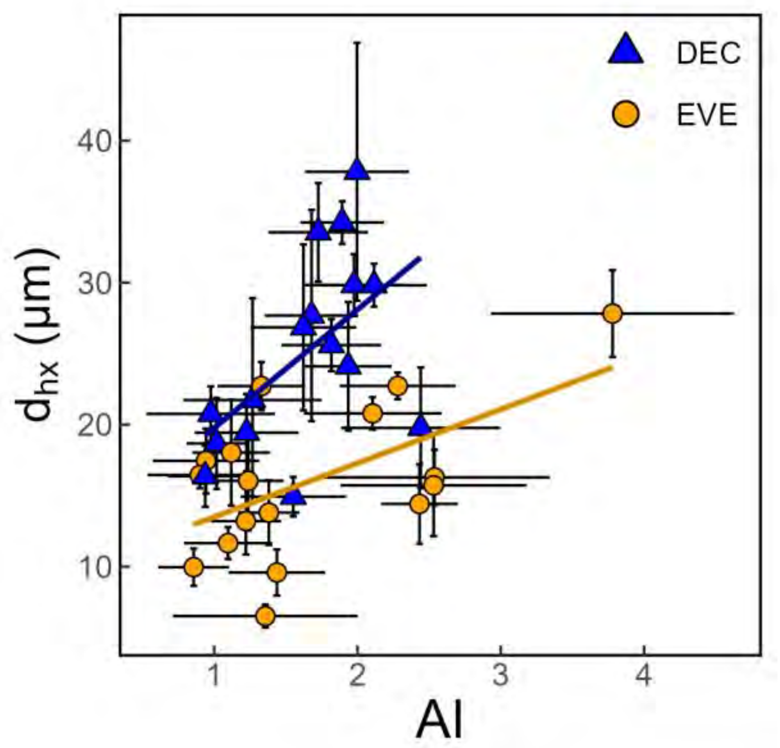

Fig. S3. Correlation between the Aridity Index (AI) and hydraulic diameter of xylem (d_{hx}) for deciduous (blue triangles) and evergreen (orange circles) species. Each point represents the mean value (± SD) of a species. Low values of AI represent xericity and high values humidity. The relationship is significant for both deciduous ($P = 0.028$; $R^2 = 0.249$) and evergreen species ($P = 0.018$; $R^2 = 0.274$).

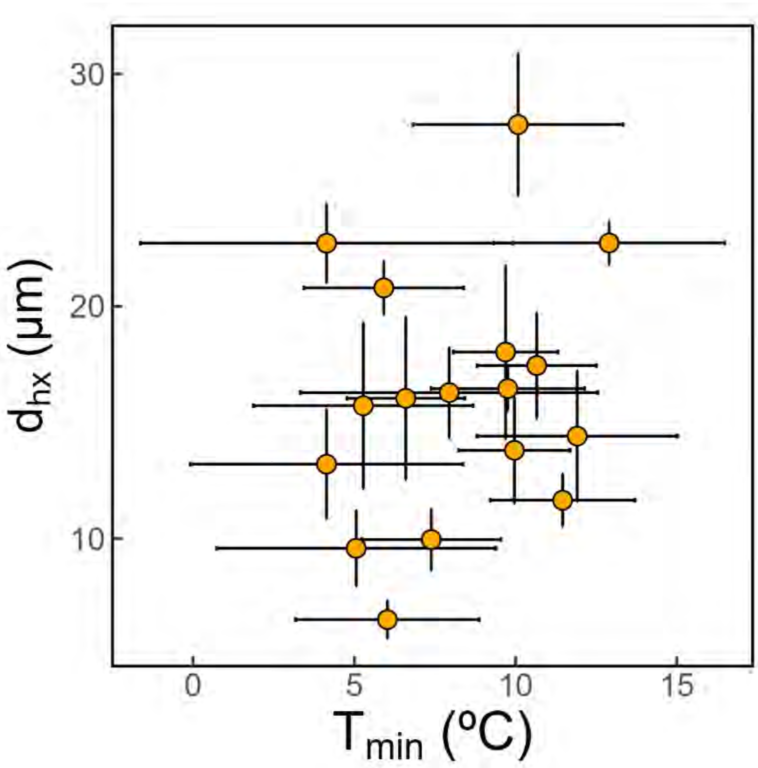

Fig. S4. Correlation between the mean of daily minimum temperatures during the coldest quarter (T_{min}) and hydraulic diameter of xylem (d_{hx}) for evergreen species. Each point represents the mean value (± SD) of a species. There is not significant relationship between the variables (P = 0.368).

Capítulo V

Trade-offs among leaf toughness, constitutive chemical defense, and growth rates in oaks are influenced by the level of leaf mass per area

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RESEARCH PAPER

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Trade-offs among leaf toughness, constitutive chemical defense, and growth rates in oaks are influenced by the level of leaf mass per area

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Abstract

Key message Among the variety of leaf defensive strategies to counteract herbivory attacks, the oak species analyzed in this study maximize investment in no more than one, with high-LMA oaks developing very tough leaves and low-LMA oaks favoring between high concentrations of condensed tannins or high growth rates.

Context Plants develop a variety of defense strategies to counteract herbivory attacks, from physical and chemical defenses to tolerance strategies. Tradeoffs between strategies have been widely assessed from a resource allocation perspective, but there is a need to consider eventual interactions among them.

Aim We evaluate the among-species tradeoff between three main constitutive plant defense traits, while considering the leaf mass per area ratio (LMA) as a proxy of leaf construction investment on area basis.

Methods Leaf toughness measured as work of fracture, condensed tannins, and relative growth rate (RGR) were analyzed in a set of 19 *Quercus* L. species with contrasting LMA.

Results Most species had low values either in two or in the three traits analyzed. Moreover, the highest values of work of fracture appeared in the species with high LMA; the highest values of condensed tannins were found in the species with the lowest LMA; and high values of RGR were measured in species with intermediate or lower values of LMA.

Conclusion Oaks showed a trade-off among leaf defensive strategies influenced by LMA. Oaks with high LMA developed very tough leaves while oaks with low LMA presented lower values of toughness but favored between one of the two other strategies.

Keywords Condensed tannins, Defensive traits, Relative growth rate, *Quercus*, Work of fracture

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1 Introduction

Plant herbivory, referred to the consumption of all or a part of a plant, is a key ecological process affecting primary production, vegetation shape, and composition, which is likely to intensify in temperate regions due to global warming (Schowalter 2006; Coley and Barone 1996; Schemske et al. 2009; Currano et al. 2010; Birkemoe et al. 2016; Nakamura et al. 2021). To counteract herbivory attacks, plants have developed a variety of defense mechanisms (Rasmann et al. 2014; Dostálek et al. 2016). Direct defenses are the first kind and comprise physical and chemical defenses that increase plant resistance and reduce plant palatability (Schoonhoven et al. 2005). The second category, referred to as indirect defenses, is the attraction with volatile organic compounds of the herbivores' natural enemies (Dicke and Baldwin 2010; Lehrman et al. 2013). The third type of defense mechanism is the tolerance of plants to herbivory, which measures how plants are able to overcome herbivory damage (Strauss and Agrawal 1999; Schoonhoven et al. 2005).

Focusing on the leaf, the most common physical defenses analyzed in herbivory studies are those associated with its mechanical resistance, specifically, the leaf toughness (Pearse and Hipp 2009; 2012; Sanson et al. 2001; Onoda et al. 2011). Leaf toughness is defined as the resistance of the leaf to crack propagation and is a measure of the energy required to fracture the leaf. The calculation of the toughness is associated with the measurement of the work of fracture (WF) which needs a simultaneous measure of the punch strength—maximum force at which the leaf breaks—and the punch displacement (Vincent 1992; Wright and Vincent 1996). Leaves with higher toughness are those with higher values of work of fracture, and therefore, leaves with an improved mechanical resistance to predation (Vincent 1991). Work of fracture has also been correlated with leaf mass per area ratio (LMA) (e.g., Alonso-Forn et al. 2023). Due to this association, LMA has sometimes been used as an estimator of leaf toughness. However, leaves can show higher WF values than expected at a given LMA (Alonso-Forn et al. 2023). Thus, there is an inability of LMA to account adequately for the wide variation in WF indicating that there are variations in the mechanical properties that do not contribute to an increase in the accumulated mass per surface area (Alonso-Forn et al. 2023).

Leaf chemical protection includes secondary compounds known by their antiherbivore actions (Seigler 1998; Lokvam et al. 2004; 2006; 2007). The most common and widespread secondary metabolites analyzed in herbivory studies are the tannins, including the condensed tannins also known as proanthocyanidins (Kursar et al. 2009; Pearse and Hipp 2009; Barbehenn and Constabel 2011; Eichenberg et al. 2015; Solla et al. 2016; Galmán et al. 2019; Fyllas et al. 2022). Condensed tannins cause oxidative damage within the epithelial cells of an insect's midgut by acting as toxins and feeding inhibitors (Barbehenn and Constabel 2011), although the level of such effects may depend on how the digestive system of the herbivore species interact with the plant-specific tannins (Barbehenn and Constabel 2011; Salminen and Karonen 2011; Büchel et al. 2016). Condensed tannins have been shown to most affect chewing insects damage in a set of oak species (Pearse and Hipp 2009) or have driven a latitudinal gradient in leaf herbivory when studying *Quercus robur* (Moreira et al. 2018). Condensed tannins have also been reported as a tolerance, rather than a chemistry resistance factor in aspens, indicating that condensed tannins may benefit plants under extreme herbivory through the improvement of the nutrient cycling (Madritch and Lindroth 2015).

The relative growth rate (RGR), or the rate of accumulation of new dry mass per unit of existing dry mass, has been shown to be the best predictor of tolerance to herbivory (Gianoli and Salgado-Luarte 2017). Having higher levels of relative growth rate, not only reduce leaf predation on the more vulnerable, young, expanding leaves (Aide and Londoño 1989; Moles and Westoby 2000) but also has reported to increase the tolerance to leaf herbivory through a greater ability to recover lost material (Danckwerts 1993; Strauss and Agrawal 1999; Gianoli and Salgado-Luarte 2017). Relative growth rate is an indirect measurement of the rate of resource acquisition. Thus, the faster a species accumulates biomass, the more carbon is available to increase growth of roots, shoots, and photosynthetic material for greater access to resources, which in turn promotes further biomass accumulation. In this sense, Gianoli and Salgado-Luarte (2017) found a strong positive correlation between RGR and tolerance to herbivory in a set of tree species from a temperate rainforest showing, effectively, that a higher RGR entails a higher tolerance.

Tradeoffs between the different defensive strategies have been widely assessed in herbivory studies to evaluate the investments from the perspective of resource allocation (Rohner and Ward 1997; Adams et al. 2009; Cárdenas et al. 2014). It has been proposed that resistance and tolerance are alternative plant strategies to deal with herbivore damage because if plant resistance mechanisms are relatively more costly or ineffective, herbivory might result in selection for tolerance because resistance is not a viable strategy (van der Meijden et al. 1988). In addition, it is also in agreement that maximizing multiple defensive strategies might not occur in the same individual as it is costly for a plant and allocating in defensive traits is assumed to reduce resource availability for other processes (Heil and Bostock 2002; Koricheva et al. 2004;

Ballhorn et al. 2008). In this regard, some previous studies showed that tree growth was negatively correlated with chemical defense, concluding that production of defensive compounds had a growth cost (Kosola et al. 2004; Stevens et al. 2007; Paul-Victor et al. 2010; Züst and Agrawal 2017; Cope et al. 2021; Perkovich and Ward 2021). Other studies found tradeoffs within resistance strategies, i.e., between physical and chemical defenses (e.g., Eichenberg et al. 2015; Wang et al. 2016). However, some other studies did not find any tradeoff or found weak correlations between defensive traits (Gianoli and Salgado-Luarte 2017; Moreira and Pearse 2017). So far, most studies have only analyzed two of the three most important strategies (e.g., growth vs. chemistry, or physical vs. chemical defense), thus there is a need to consider eventual interactions among the three defense strategies.

The aim of our study was to evaluate the among-species tradeoff between three of the most important factors involved in plant defense described above: leaf toughness (physical defense), condensed tannins (chemical defense), and relative growth rate (tolerance strategy), in a set of *Quercus* L. species growing in a common garden. The genus *Quercus* is an excellent system to perform this research, since it not only minimizes phylogenetic variation (in comparison to studies conducted across diverse species) but also exhibits significant trait variation among species (Pearse and Hipp 2009; Sancho-Knapik et al. 2021). We analyzed the interspecific tradeoff by studying the constitutive levels (i.e., basal levels expressed at leaf maturation) of defensive traits (Karban 2011) while considering the leaf mass per area ratio as a proxy of leaf construction investment on an area basis (Escudero et al. 2017). We hypothesized that, due to tradeoffs between the alternative strategies, each oak species would tend to maximize one single of the three strategies. We also hypothesized that the strategy maximized by the oak species would depend on the species' leaf mass area and therefore on the species' leaf construction cost on area basis.

2 Materials and methods

2.1 Study system, plant material, and field site

To test our hypotheses, we used the dataset of Alonso-Forn et al. (2023), which included oak species with contrasting leaf toughness and leaf mass area. In addition, plant material for condensed tannins and relative growth rate was sampled on 19 oak species during the same year and in the same specimens as those described in Alonso-Forn et al. (2023). Briefly, plant material was collected from a living collection of oak species, maintained in an experimental field under uniform nutrient supply and light conditions, and with no water restrictions, located at CITA de Aragón (41.728° N, 0.821° W, 218 m a.s.l., Zaragoza, Spain). The common garden features Mediterranean climatic conditions with a long-term mean annual temperature of 15.4 °C and total annual precipitation of 298 mm. The study site was in a river terrace, with clayey silt soil of alluvial origin. Oak trees were ca. 20 years old; they were drop irrigated twice per week from spring to autumn. Plant material was collected from south-exposed branches of one individual per species during the early morning, stored in sealed plastic bags and carried to the laboratory in portable coolers. For condensed tannins we collected a set of 9 current year, fully developed, mature leaves per species. For growth analysis, we sampled two sets of 10 developing shoots (including leaves and the stem) per species spaced in time (see below for details).

2.2 Leaf toughness

Leaf toughness was directly obtained from the values of work of fracture (WF, J m^{-1}) that appear in the dataset of Alonso-Forn et al. (2023) which are openly available in TRY Plant Trait Database at http://doi.org/10.17871/TRY.86. This previous study performed punch and die tests, which consisted on punching a hole through the leaf lamina and generating force–displacement curves. These mechanical tests were taken at room temperature on 10 full-hydrated leaves per species, with measurements avoiding major veins. The analysis of the force–displacement curves allowed to obtain the work of fracture as the area below the curve between the initial contact of the punch with the leaf and the highest load value registered.

2.3 Condensed tannins

Condensed tannin concentrations (CT, mg g^{-1} dry weight) of oak leaves were determined colorimetrically following the butanol – HCl assay (Porter et al. 1986; Pearse and Hipp 2009; Grabber et al. 2013). Briefly, 500 mL of leaf extract were incubated with 3 mL of acidified butanol (BuOH-HCL) and 100 mL of ferric reagent at 100 °C for 60 min. The mixture was cooled to room temperature and the absorbance of the mixture was read at 550 nm.

The dataset generated and the code used for the analyses during the current study are available in the citaREA repository (Sancho-Knapik et al. 2023).

2.4 Relative growth rate

Relative growth rate (RGR, d^{-1}) was measured for each species by making two harvests separated by a time-interval (Paul-Victor et al. 2010). For each species, the first harvest took place 7 days after bud bursting (t_1); the second harvest took place 28 days after bud bursting (t_2). Figure 1 shows the time-interval period for each species as well as the temperature throughout the growing

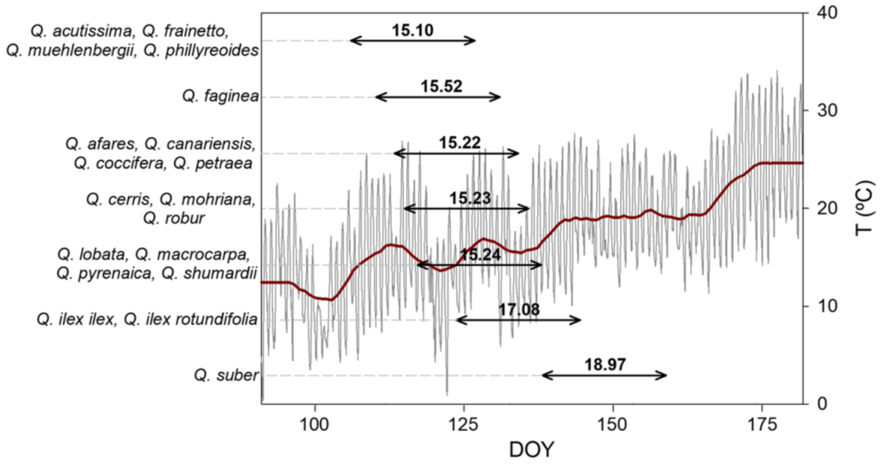

Fig. 1 Temperature (T) throughout the growing season (grey line) and smothering of the data (red line). Arrows indicate the 21-day-period for the RGR calculation of each species. Values above arrows are the mean temperatures of each period

season and the growth mean temperature for each species (climatic data are from the nearest weather station, 1 km away).

As mentioned above, 10 shoots (including leaves and the stem) per species were collected from the southern exposure of the tree for each harvest. Then, shoots were oven dried for 3 d at 70 °C and weighed using an analytical balance (Sartorius AG Model BP221S; Sartorius AG, Goettingen, Germany) obtaining the dry mass (M) of each shoot. RGR was then calculated as (Paul-Victor et al. 2010):

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

where M_1 and M_2 are the mean dry mass values of the 10 shoots recollected for each species during the first and the second harvest, respectively. Individual shoot dry mass values for each species are available in the citaREA repository (Sancho-Knapik et al. 2023).

2.5 Statistical analysis

For the evaluation of the results, species were classified according to the value of leaf mass per area (LMA, g m^{-2}) reported in Alonso-Forn et al. (2023). Oak species with values of LMA higher than 151 g m^{-2} were considered species with high LMA and, therefore, species with a high construction cost on an area basis. Species with values of LMA between 150 and 101 g m^{-2} were considered species with intermediate values of LMA, and oak species with values of LMA lower than 100 g m^{-2} were considered species with low values of LMA and, therefore, species with a low construction cost on an area basis.

Relationships between work of fracture, condensed tannins and RGR were first related two by two. Then, values of the three defensive traits were normalized between 0 and 1 and were plotted in a three-dimension graph. To assess the probability to find a combination of two or more defensive traits, we divided the species in three categories, according to their position within the range of values (0 = none of the variables in the upper half of the range; 1 = one variable in the upper half of the range; 2 = two or more variables in the upper half of the range). Significance differences in the abundance of species among defense strategies were tested with a general linear model assuming a Poisson distribution using the basal function "glm" in R version 4.2.1 (R Core Team 2022). Finally, a principal components analysis (PCA) with two components was carried out to summarize the multivariate relationships among defensive traits and *Quercus* species.

3 Results

The three plant defense strategies showed a wide range of variation within our set of *Quercus* species studied. Leaf toughness, measured as work of fracture, ranged from the 0.07 J m^{-1} found in *Q. lobata* to the 1.08 J m^{-1} showed by *Q. ilex* subsp. *rotundifolia* (Fig. 2). Condensed tannins ranged from 9.6 mg g^{-1} of dry weight measured in *Q. petraea* to 109 mg g^{-1} d.w. found in *Q. lobata* (Fig. 2). RGR ranged from 0.037 day^{-1} found for *Q. lobata* to 0.160 day^{-1} measured in *Q. muehlenbergii* (Fig. 2). When arranging the oak species from highest to lowest leaf mass area (LMA), the highest values of work of fracture were measured in the species with the highest LMA, whereas the highest values of condensed tannins were found in the species with the lowest LMA (Fig. 2). By contrast, high values of RGR were measured in species with intermediate or lower values of LMA (Fig. 2). It

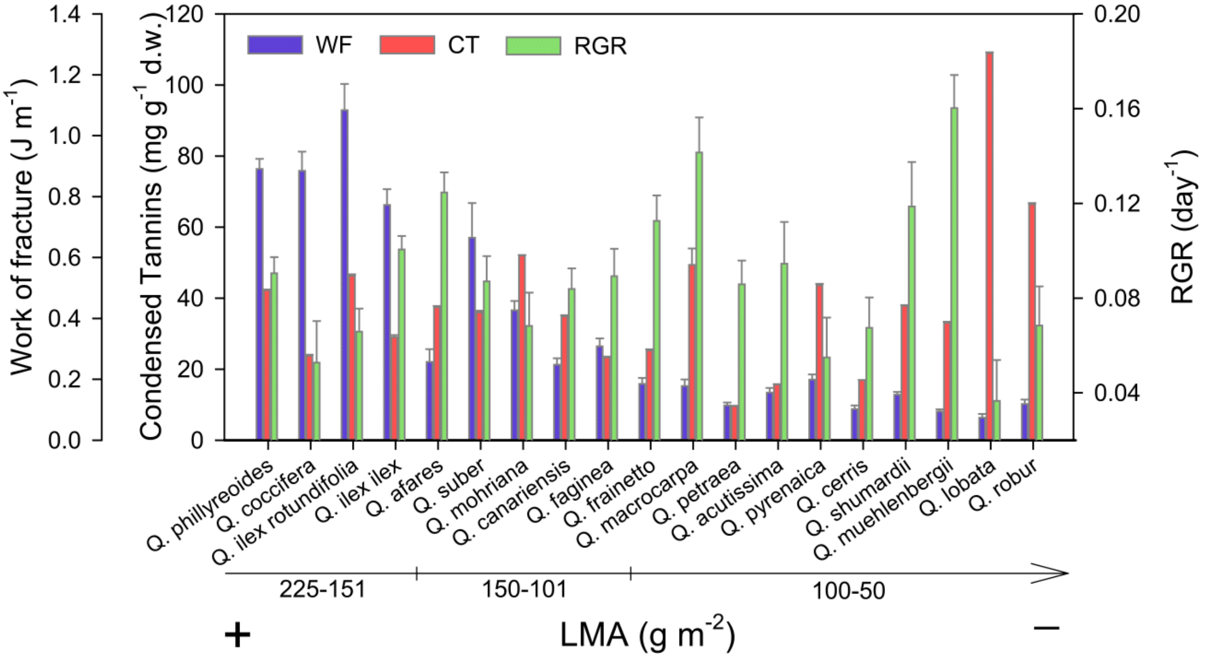

Fig. 2 Mean values and standard errors of work of fracture (WF), condensed tannins (CT) and relative growth rate (RGR) for the 19 oaks studied. Species are arranged from highest to lowest leaf mass area (LMA) and classified in three groups: 225 to 151, 150 to 101, and 100 to 50 g m^{-2}

should be noted that, among the species with the lower values of LMA, there was not any oak species that maximized both condensed tannins and RGR (Fig. 2).

When the defensive traits were related two by two (Fig. 3a, b, c) or in the three-dimensional graph (Fig. 3d), we found that most species had relatively low values (i.e., normalized values below 0.5) either in two or in the three traits analyzed. In the categorization of species according to their defense strategies, we found 7 species with a limited defense specialization, i.e., none of the mechanisms in the upper half of the range, 11 species with one of the mechanisms in the upper half of the range, and only 1 species with two mechanisms in the upper half of the range (*Q. ilex subsp. ilex*) (Fig. 3d). The basal function "glm" to assess differences between categories showed no significant differences in the number of species with none or one defense mechanism within the upper range ($p=0.3499$) but a marginally significant lower ($p=0.0687$) number of species with a combination of two or more defense mechanisms within the upper range.

The principal component analysis (PCA) among the three defensive traits showed that each trait axe was separated equidistant from the other two by an angle of ca. 120° (Fig. 4). Oak species were distributed along the three-trait vectors, with no species clearly located between any two-trait vectors. Species with high LMA were distributed along the upper range of the work of fracture vector, whereas species with low LMA were aligned either with the condensed tannins vector or the RGR vector (Fig. 4). Species with intermediate values of LMA were mainly located in the center on the principal component axes (Fig. 4).

4 Discussion

The evaluation of the three constitutive defensive strategies (leaf toughness, condensed tannins and RGR) among the oak species of this study revealed that none of the species have maximum values in more than one defensive trait. That is, species tend to maximize only one of the defensive strategies considered, confirming our first hypothesis. For example, while *Q. ilex* subsp. *rotundifolia* maximizes leaf toughness, *Q. lobata* and *Q. muehlenbergii* maximize condensed tannins and RGR, respectively (Fig. 2). The existence of an interspecific tradeoff among defensive traits agrees with previous studies in oaks (Mediavilla et al. 2018; Abdala-Roberts et al. 2018; Perkovich and Ward 2021) and other species (Dostálek et al. 2016; Eichenberg et al. 2015), where it is indicated that the co-expression of multiple defense strategies may be too expensive for a particular species, since investing in defensive traits is thought to reduce the resource availability for other processes, such as reproduction (Heil and Bostock 2002; Koricheva et al. 2004). By contrast, some species such as *Q. pyrenaica* or *Q. cerris* presented low values for the three defensive traits analyzed, when compared with the highest

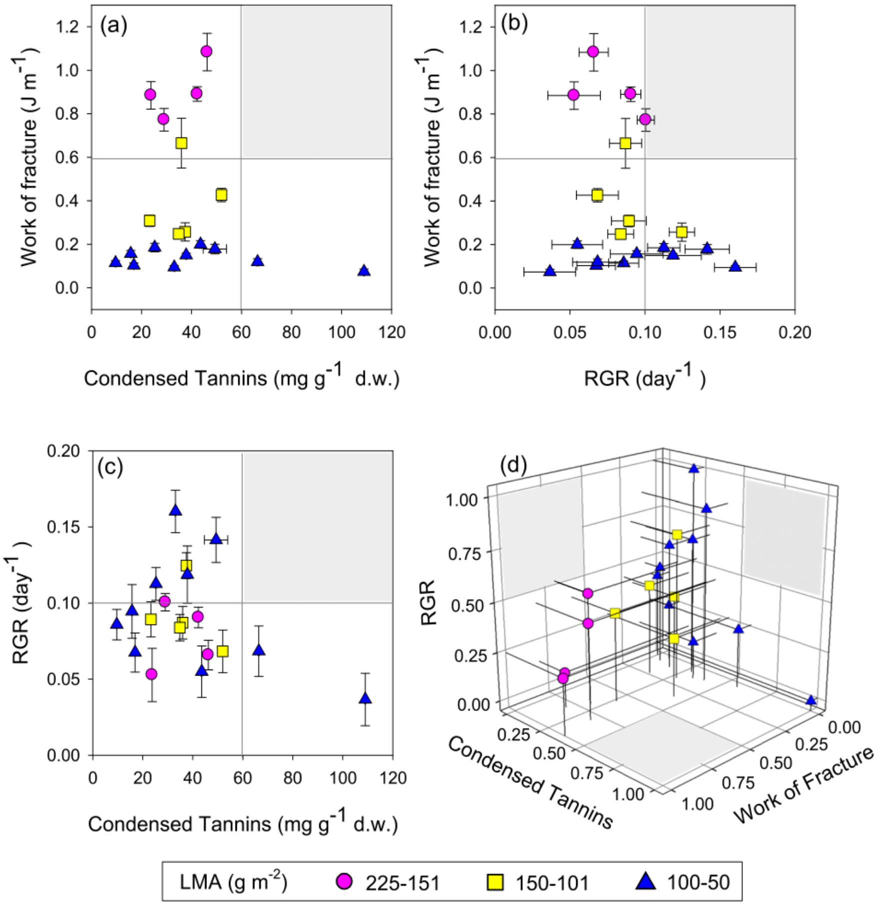

Fig. 3 Two-dimensional relationships between work of fracture, condensed tannins, and relative growth rate (RGR) for the 19 oaks studied ((**a**), (**b**), (**c**)). Three-dimensional relationship between defensive traits using normalized values ((**d**)). Shadow squares represent the areas with normalized values above 0.5. Species are classified in three levels of leaf mass area (LMA). Data are mean values ± SE

absolute values of their congeners (Fig. 2). In the case of *Q. pyrenaica*, its leaves have more trichomes than the leaves of all other species of the study (personal observations), being considered leaves densely pubescent, attribute that can act as a defensive barrier against herbivory (Kitamura et al. 2007) and thus compensating the lower constitutive values found in the traits analyzed in this study. In particular, *Q. cerris* seemed to be the most vulnerable oak to herbivory from a constitutive point of view. Perhaps this species allocates resources to other constitutive strategies such as indirect defenses (e.g., attraction of the herbivores' natural enemies with volatile compounds), or relies mostly on induced defense mechanisms, showing low constitutive values (Agrawal and Karban 1999; Lehrman et al. 2013; Moctezuma et al. 2014; Perkovich and Ward 2021). Nevertheless, our results should be interpreted with caution as, due to the restrictions imposed by the common garden, we did not contemplate the intraspecific variation in the plant defensive traits that might not be negligible (Hahn and Maron 2016).

Despite the lack of several individuals per species to analyze intraspecific variation, the LMA values used in this study to classify the species in three categories were good representative of the species values when compared with data from other studies (e.g., Niinemets 2001; Cornelissen et al. 2003; Wright et al. 2004; Kleyer et al. 2008), minimizing the phenotypic response to local environmental conditions. Taking into account these LMA categories, our results showed that developing very tough leaves was the main defensive strategy in oaks with high LMA, whereas making leaves with high constitutive concentrations of condensed tannins (chemical defense) or having high growth rates as a tolerance strategy were associated with low LMA values. Therefore, we confirm our second hypothesis: the strategy maximized by oak species would depend on the species' leaf mass area. More specifically, our results suggest that the elevated

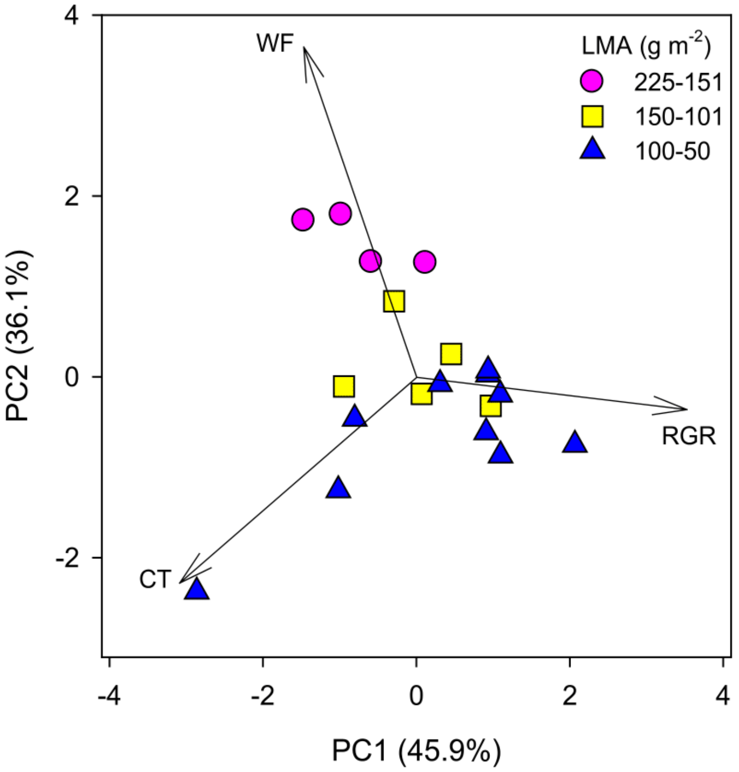

Fig. 4 Principal component analysis (PCA) of defensive traits in the 19 oaks studied. Species are classified in three levels of leaf mass area (LMA). Each dot corresponds to a single species. WF, work of fracture; CT, condensed tannins; RGR, relative growth rate

construction costs associated with high LMA limits the investment in alternative strategies, such as chemical defense or growth.

The higher toughness found in oaks with high LMA (> 151 g m^{-2}) reflects an improvement of a larger structural resistance that was related in previous studies to increased leaf density associated with a higher vein density, thicker cell walls, thicker bundle sheath extensions, and thicker epidermis outer walls (Peguero-Pina et al. 2017; Sancho-Knapik et al. 2021; Alonso-Forn et al. 2023). In addition, high values of LMA and structural resistance have been associated with a longer leaf lifespan (Reich et al. 1991; Wright and Westoby 2002; Onoda et al. 2011; Alonso-Forn et al. 2020). Effectively, the oaks from this group have the longest leaf lifespans of the studied species, from 12 up to 26 months in the case of *Q. ilex* (Alonso-Forn et al. 2020). It has been argued that leaves with higher structural resistance had longer leaf life spans to compensate their costly investment in construction, although the underlying mechanisms are controversial (Turner 1994).

Within the group with low LMA (< 100 g m^{-2}) and in opposition to the previous group, species constructed leaves with the lowest values of toughness and therefore the lowest mechanical resistance, which agreed with their shorter leaf life span (5 to 8 months, Alonso-Forn et al. 2020). These species, with a low investment in mechanical resistance and reduced construction costs per area, would favor one of the two other strategies, chemical defense or tolerance (Fig. 4). Regarding the chemical defense, the contribution of condensed tannins to LMA seems to be very small, as the two species with the highest concentration of tannins are those with the lowest LMA (Fig. 2). Concerning the tolerance strategy, the species with low LMA tent to have higher leaf area ratio (LAR, plant area/plant dry weight, m^2 g^{-1}) (Cornelissen et al. 1996, 1998), which results in higher values of RGR (Antúnez et al. 2001). Another way to increase RGR is to increase the net assimilation rate (NAR) as RGR can also be calculated as LAR x NAR (Antúnez et al. 2001). Growing climatic conditions, especially temperature, can also affect RGR in *Quercus*, although the explanatory power of this factor can be low when compared to other environmental factors such as tree age or tree density (Villar et al. 2017). In our experiment, RGR of most species was obtained for an interval period with a mean temperature ca. 15.2 °C (Fig. 1), i.e., growth temperature of most species was similar, minimizing the possible effect of temperature on RGR. Only three of the species (the Mediterranean evergreens) grew with a higher interval growth temperature (17–19 °C; Fig. 1) due to their late phenology. In any case, despite this higher temperature, RGR of these three species was not remarkable (Fig. 2). By contrast, *Q. muehlenbergii*, a cold-temperate oak, showed the highest RGR with one of the lowest interval temperatures (15.1 °C).

Additionally, we found that those oaks with high condensed tannins tended to have low RGR (e.g., *Q. lobata*, Fig. 2), and conversely, those oaks with high RGR had low to intermediate values of condensed tannins (e.g., *Q. muehlenbergii*, Fig. 2). This is in agreement with previous works reporting a tradeoff between constitutive chemical defense and tolerance (i.e., growth rate) within this group (Kosola et al. 2004; Stevens et al. 2007; Perkovich and Ward 2021). Nevertheless, as pointed out by Perkovich and Ward (2021), species with low constitutive values of chemical defense may develop a strong induced response, which would be compatible with high potential growth rates. Indeed, Perkovich and Ward (2021) found a negative association between growth rates and constitutive chemical defense, partially explained by the phylogeny, but did not find any tradeoff with induced chemical defense. In this regard, we encourage further assessment of the tradeoffs between defense traits, considering both constitutive and induced responses, and the eventual role of phylogenetic constraints, such as those imposed by elevated LMA (e.g., in the species from section ilex, which tend to show high values of LMA; Sancho-Knapik et al. 2021).

5 Conclusion

Oaks within this study showed a trade-off among three leaf defensive strategies: leaf toughness, condensed tannins, and RGR that were associated to different levels of LMA. Oak species with high LMA developed very tough leaves that was associated with a larger structural resistance and longer leaf lifespans. By contrast, oaks with low LMA presented the lowest values of leaf toughness but favored between one of the two other strategies, higher constitutive concentrations of condensed tannins or having high growth rates.

Code availability

Not applicable.

Authors' contributions

Conceptualization: Domingo Sancho Knapik, Eustaquio Gil-Pelegrín, Juan Pedro Ferrio, José Javier Peguero-Pina; methodology: Rubén Martín-Sánchez, David Alonso-Forn, Eustaquio Gil-Pelegrín; formal analysis and investigation: Domingo Sancho Knapik; writing—original draft preparation: Domingo Sancho Knapik; writing—review and editing: Rubén Martín-Sánchez, José Javier Peguero-Pina, Juan Pedro Ferrio, Eustaquio Gil-Pelegrín; funding acquisition: Juan Pedro Ferrio, Domingo Sancho Knapik; supervision: Eustaquio Gil-Pelegrín. The authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated during and/or analyzed during the current study are available in the citaREA repository, https://doi.org/10532/6533.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors gave their informed consent to this publication and its content.

Competing interests

The authors declare that they have no competing interests.

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Publisher's Note

Capítulo VI

Discusión general y Conclusiones

6.1. Discusión general

Partiendo del objetivo general de esta tesis, se ha conseguido aportar más información acerca de cómo afectan los diferentes estreses ambientales, especialmente los abióticos, al género *Quercus*. Así, quedan reflejadas numerosas adaptaciones y explicados varios procesos para enfrentarse a dichos estreses a todos los niveles: (i) en cuanto al número de especies, se abordan desde estudios monoespecíficos (**Capítulo II**), hasta estudios multiespecíficos (**Capítulos III, IV** y **V**), con especial detalle al **Capítulo III**, que abarca un tercio de la biodiversidad del género; (ii) se explican desde adaptaciones morfoanatómicas (**Capítulos II, III, IV** y **V**), hasta algunos procesos fisiológicos (**Capítulos II** y **IV**); (iii) desde la escala "macro", estudiando caracteres morfológicos foliares (**Capítulo III**), hasta la escala "micro", analizando la anatomía de los tejidos (conductores, soporte, protección...) en diferentes partes de la planta (lámina, pecíolo, tallo) (**Capítulos II, IV** y **V**) y, (iv) aunque sobre todo se tienen en cuenta factores abióticos (clima) (**Capítulos II, III** y **IV**), también se consideran —en menor medida— el papel de factores bióticos (**Capítulo V**). En definitiva, esta tesis aporta contribuciones a explicar la gran variabilidad, diversidad y plasticidad de las especies del género *Quercus* que les permiten colonizar hábitats tan diferentes a lo largo de todo el hemisferio norte.

Comenzando por el estudio más simple contemplado en la presente tesis (**Capítulo II**), esto es, considerando una sola especie (*Q. ilex*) y un solo clima (Mediterráneo), se pueden observar claros patrones de respuesta a las restricciones ambientales. Como se vio en el **Capítulo I**, el clima Mediterráneo destaca por la existencia de dos periodos separados de estrés que limitan el periodo vegetativo: el verano, a través de la aridez, y el invierno, a través de las bajas temperaturas. Estas condiciones tan particulares, que no se dan en ningún otro zonobioma arbóreo del mundo, conllevan una flora altamente especializada a dichas condiciones. Así, Breckle (2002) propone como propio de este zonobioma los bosques y arboledas perennifolias y esclerófilas, con *Q. ilex* como máximo representante en las zonas circummediterráneas *sensu stricto*. De hecho, la distribución de la encina solapa casi a la perfección con el clima mediterráneo en el Viejo Mundo, perdiendo esta especie importancia en el paisaje hacia el extremo oriental de su área de distribución, donde es sustituida por otras quercíneas como *Q. aucheri*. El taxón *Q. ilex* es un taxón tan complejo en lo morfológico, ecológico y climático, que ha llevado ha llevado a considerarlo como la suma de dos subespecies: *Q. ilex* subespecie *ballota* (Desf.) Samp. (sin. *Q. rotundifolia* Lam.) y *Q. ilex* subespecie *ilex* L. (Schwarz, 1993, Flora Europea, Segunda Edición; Amaral-Franco, 1990, Flora Ibérica), o de dos especies (Schwarz, 1964, Flora

Europea, Primera Edición). No obstante, con independencia de su estatus taxonómico, que no es el objeto de estudio de esta tesis, el **capítulo II** visita la existencia de dos morfotipos con claras y evidentes diferencias ecofisiológicas.

Este capítulo analiza, a partir de la información actualmente publicada, los principales estreses que genera el clima mediterráneo: el estrés hídrico, ya sea edáfico o atmosférico durante el verano; y el estrés térmico, tanto por altas como por bajas temperaturas. Comenzando por el estrés hídrico, y considerando el taxón *Q. ilex* en su conjunto, este demuestra tener una alta resistencia a la cavitación inducida por sequía, equivalente a la de otras especies de *Quercus* que ocupan zonas eminentemente áridas. No obstante, un análisis más detallado señala la existencia de un comportamiento bimodal en cuanto a esta respuesta. Así, los datos procedentes de poblaciones ibéricas o norteafricanas, asumiendo con gran probabilidad que se trate de la subespecie *Q. ilex ballota* (en adelante morfotipo Ballota) presentan valores significativamente más elevados de resistencia a la cavitación, es decir, un P_{50} más negativo (P_{50} = -5.5 MPa), que aquellas procedentes de poblaciones franceses o italianas, asumiendo su condición de *Q. ilex ilex* (en adelante morfotipo Ilex) (P_{50} = -3.9 MPa). Sin embargo, más casuística es necesaria para establecer la geografía de estas diferencias y sobre todo la genética de las mismas a través de las peculiaridades del xilema. Estas diferencias en P_{50} entre ambos morfotipos de encina pueden tener consecuencias críticas en su resistencia a la sequía, especialmente en lo relativo a su "margen de seguridad" (*safety margin*). De hecho, la distribución occidental del morfotipo Ballota coincide con las zonas de mayor aridez en toda el área de distribución de *Q. ilex*, ocupando zonas genuinamente semiáridas según el índice de Emberger en amplias zonas de la Península Ibérica y Marruecos. Por el contrario, el morfotipo Ilex ocupa condiciones subhúmedas-húmedas en la inmensa mayoría de su área de distribución, principalmente ligadas a zonas costeras, desde el sureste de Francia hasta Turquía. No obstante, el estudio de este parámetro ha revelado la existencia de una posible plasticidad en el valor de P_{50} en esta especie (Peguero-Pina *et al*., 2014). Un análisis más detallado de esta plasticidad debería ser considerado en sí mismo un posible objetivo a estudiar con mayor profundidad para esta especie.

Las altas temperatura son un factor estresante que afecta a varios procesos y estructuras celulares, como: (i) la estabilidad de las membranas (Bischof *et al*., 1995), (ii) la función de determinadas enzimas (p. ej., la rubisco) (Bernacchi *et al*., 2002), o (iii) la integridad del PSII (Arnold *et al*., 2021), entre otras. La temperatura letal calculada por nosotros para el morfotipo Ballota resultó ser de 50.2 ºC, temperatura muy similar a la publicada en otras especies vegetales estudiadas (Daniell *et al*., 1969; Ranney y Peet, 1994; Krause *et al*., 2013). Al alcanzarse esta temperatura se producen los daños más drásticos en la ultraestructura e integridad celular de la hoja, que pueden ser fácilmente detectados midiendo los valores de la fluorescencia variable de clorofilas (F_V/F_M). Dicho umbral térmico está, de momento, lejos de las temperaturas máximas normalmente alcanzadas incluso en el extremo más cálido del área de distribución de la especie. Por lo tanto, las altas temperaturas, en sí mismas, no parecen ser un factor de daño tan importante como el estrés hídrico. No obstante, otros procesos que no son críticos para la supervivencia del individuo o los órganos —como son la eficiencia de la fotosíntesis— sí que podrían verse afectados al exponerse a temperaturas elevadas por encima de las óptimas.

Una alta temperatura del aire combinada con una baja humedad relativa resulta en una elevada demanda evaporativa por parte de la atmósfera, asociada a valores de VPD altos. Varios estudios han puesto en evidencia que la encina es una especie sensible al valor de VPD, respondiendo con un cierre estomático a valores de 2-3 kPa, inferiores incluso a los reportados para otras quercíneas caducifolias con las que habita en simpatría (Mediavilla y Escudero, 2003; Sancho-Knapik *et al*., 2022). Además, valores altos de VPD suelen coincidir con valores bajos de Ψ_s durante el verano mediterráneo, lo cual provoca un efecto sinérgico que hace que la encina cierre aun antes sus estomas (Sancho-Knapik *et al*., 2022). Las consecuencias de mantener los estomas cerrados, bajo condiciones de alta luz, conlleva la llegada de un exceso de radiación a la hoja que no es derivada a la asimilación de carbono. Ante la simultaneidad de ambas circunstancias, la encina ha demostrado que es capaz de poner en marcha diferentes mecanismos de fotoprotección. Las bajas temperaturas invernales también exigirán de mecanismos de fotoprotección durante los frecuentemente luminosos días invernales. En este caso la inactivación metabólica asociada a las bajas temperaturas promueve un exceso de radiación que puede ser dañino al no poderse canalizar hacia procesos de asimilación de carbono.

Las bajas temperaturas invernales también provocan, a su vez, numerosos ciclos de hielo-deshielo a lo largo de toda la estación, los cuales pueden comprometer la integridad del xilema por los mecanismos expuestos en el **capítulo I, II**. El menor diámetro de los vasos en el morfotipo Ballota respecto del morfotipo Ilex (**Capítulo II**) podría garantizar una mayor resistencia a la cavitación asociada a estos ciclos de hielo-deshielo. No obstante, más estudios reportando el tamaño de los vasos en diferentes procedencias iberoafricanas son aún necesarios. De hecho, esto hace que el límite altitudinal y septentrional de *Q. ilex* esté más asociado al frío que a la sequía (Nardini *et al*., 2000). Sin embargo, esta afirmación tiene un sesgo asociado al hecho de que las poblaciones sobre las que se realizó este estudio están probablemente conformadas por morfotipo Ilex, donde se reportan el mayor diámetro de vasos. En las áreas donde esto se ha realizado, las temperaturas mínimas durante invierno son suaves, lo que le permite a la encina producir vasos más grandes con un menor riesgo de cavitación por ciclos de hielo-deshielo (Lo Gullo y Salleo, 1993), con los beneficios hidráulicos que ello conlleva. De hecho, se encuentran diferencias significativas en la temperatura letal por congelación entre ambas subespecies, siendo el morfotipo Ilex más vulnerable que el morfotipo Ballota. Este último morfotipo, por el contrario, vive en zonas expuestas a temperaturas menores y heladas más frecuentes en invierno, especialmente en áreas del Sistema Ibérico y el Atlas marroquí. Hay que tener en cuenta que esta temperatura letal por heladas, al igual que la temperatura letal por calor, está alejada de las temperaturas mínimas promedio alcanzadas en sus respectivas procedencias. No obstante, como ya se ha indicado anteriormente, el frío invernal obliga a la planta a activar mecanismos de fotoprotección para sobrellevar el efecto combinado de la alta radiación invernal y las bajas temperaturas (García-Plazaola *et al.*, 2017, Corcuera *et al.*, 2005). En cualquier caso, un mejor conocimiento de algunos aspectos básicos de la conformación del PSII en la encina, como la caracterización de la subunidad S del PSII (PsbS) serían aún necesarios. La reciente publicación del genoma completo de *Q. ilex ballota* (Rey *et al.*, 2023) facilitará este tipo de avances en el conocimiento de la encina.

Pasando a un modelo multiespecífico que contempla la gran mayoría de los climas ocupados por las quercíneas (con excepción de una representación significativa de especies de climas ecuatoriales), también se observan patrones de respuesta generalizados de las especies —síndrome foliares— como respuesta a las condiciones ambientales. Como se explica en el **capítulo III**, los climas de Köppen se agruparon en 6 macroclimas: árido, mediterráneo, tropical (zonobioma II de Walter), templado-cálido, templado-frío y ecuatorial (este último no considerado en el estudio). El espectro climático de los cinco macroclimas analizados segrega de manera bastante efectiva las diferentes condiciones climáticas de cada uno de los climas, con la excepción de los climas árido y mediterráneo, los cuales están completamente solapados, y una ligera superposición de ambos climas templados.

La morfología foliar, por su parte, presenta diferencias significativas en todos los caracteres analizados, especialmente en los cuantitativos. Esta morfología está explicada en mayor medida por el clima ocupado por las especies que por su proximidad filogenética, con la excepción de los valores de LMA. Esto no significa que éste no responda a variables climáticas, ya que ha demostrado ser bastante plástico como respuesta a la sequía, la exposición de luz, la herbivoría, etc. (Niinemets, 2001; Poorter *et al.*, 2009; Onoda *et al.*, 2011; Mediavilla *et al.*, 2018; Sancho-Knapik *et al.*, 2021), sino que probablemente se deba a la presencia de secciones bastante homogéneas en su LMA. Cuando se analizan todos los caracteres mediante el FAMD, se puede apreciar como la morfología de las quercíneas está reflejada por un gradiente continuo, ya que el análisis no segrega grupos completamente aislados. Incluso teniendo en cuenta los macroclimas de las especies, se puede ver un amplio solapamiento entre ellos. A pesar de dicho solapamiento, determinados caracteres morfológicos parecen estar asociados a macroclimas concretos, y, por ende, a determinadas variables climáticas. Esta interpretación del FAMD está apoyada estadísticamente, ya que la correlación entre los valores PC1-Morph y PC1-Clim presentan una relación significativa, incluso teniendo en cuenta la filogenia a través de una regresión usando el método PGLS. Esta relación parece estar indicando un eje de termicidad, ya que las especies tropicales ocupan un extremo y las templado-frías el opuesto, con una mezcla de los macroclimas restantes en el centro. Asimismo, la correlación entre los valores de PC2-Morph y PC2-Clim también arroja una relación significativa, lo que parece estar indicando un eje de xericidad, con algunas especies tropicales (de climas A de Köppen) y templado cálidas en el extremo lluvioso, mientras que las especies mediterráneas y áridas se ubicarían en el extremo seco.

En cualquier caso, la complejidad morfológica no puede ser fácilmente reducida a la explicada por solo dos dimensiones. Por cuanto la continuidad morfológica y climática entre las especies y la variabilidad intra e interespecífica impide proponer un solo síndrome foliar canónico por macroclima. La incorporación de más caracteres morfológicos, especialmente cuantitativos, sería deseable para futuros estudios. A pesar de esta dificultad, sí que se han podido reconocer algunas tendencias en los síndromes foliares, identificables con los macroclimas estudiados, y que podrían tener una explicación adaptativa. Así, las especies templado-frías, algunas templado cálidas y las submediterráneas están caracterizadas por ser hojas grandes, malacófilas, caducifolias, de márgenes desde profundamente lobuladas (submediterráneas) a ligeramente lobuladas y obovadas. Este síndrome estaría adaptado a (i) evitar las heladas invernales a través de la caducifolia, (ii) optimizar la captación de luz para fotosintetizar el corto periodo favorable del que disponen mediante el desarrollo de grandes hojas obovadas, (iii) reducir su coste de producción y optimizar su *payback* —siguiendo los postulados del LES—, mediante la producción de hojas malacófilas y, (iv) especialmente en el caso de la submediterráneas, a incrementar su conductividad hidráulica haciendo hojas profundamente lobuladas.

Las especies tropicales tienden a presentar hojas lanceoladas, alargadas, con ápices acuminados, de bordes enteros, serrados u ondulados, ligeramente esclerófilas, perennifolias (o brevideciduas) y glabras. La perennifolia estaría presente por los largos periodos vegetativos, siendo en la mayoría de los casos el año completo y, en aquellos con un breve periodo seco, volviéndose brevideciduas. La esclerofilia incrementa la resistencia mecánica de las hojas, que les serviría en estos climas como refuerzo estructural frente la climatología adversa (p. ej., precipitaciones extremas); y, además, como defensa a la intensa presión de los herbívoros en estos climas. La forma alargada, con ápices acuminados sería una adaptación para facilitar la evacuación del agua, con la presencia de un característico *drip-tip* (ápice caudado) en aquellas especies ocupando las zonas más lluviosas de todo el rango de distribución del género. El cambio de una pubescencia por una glaucescencia podría estar orientado, una vez más, a la ayuda de la evacuación del agua de la lámina foliar.

Las especies áridas y las genuinas mediterráneas se caracterizan por desarrollar hojas pequeñas, muy esclerófilas, mayoritariamente perennifolias, redondeadas, de bordes enteros y con tendencia a desarrollar pubescencia. La forma pequeña evitaría el sobrecalentamiento, aunque la forma redondeada, paradójicamente, dificultaría la disipación de calor. Se ha demostrado como la presencia de tricomas adaxiales aumenta la reflectancia de la hoja, disminuyendo la radiación recibida. La pubescencia abaxial ha sido interpretada como un rasgo xeromórfico, sin embargo, esta relación es ampliamente cuestionada (Aronne y DeMicco, 2001; Haworth y McElwain, 2008). Si que parece más claro el papel de los tricomas como elemento disuasorio contra herbívoros, al igual que la espinescencia que presentan algunas de estas especies (Karioti *et al*., 2011; Choong, 1996). La perennifolia les permitiría optimizar el periodo vegetativo sobre las caducifolias, al aprovechar periodos térmica e hídricamente favorables en primavera u otoño, cuando las caducifolias no tienen hojas. Por otro lado, la esclerofilia sería una inversión por parte de las hojas para asegurar su supervivencia ante las amenazas bióticas (**Capítulo V**) y abióticas, entre ellas la deshidratación (Oertli, 1986), permitiéndoles sobrevivir el tiempo necesario como para recuperar la inversión hecha en la producción de dichas hojas.

Mención especial merece el clima mediterráneo, ya que, de los estudiados, es el que induce mayor variabilidad morfológica. En el **capítulo III** se muestra como existe un gradiente morfológico desde el síndrome exhibido por la encina (**Capítulo II**), y compartido con las especies áridas, hasta el submediterráneo, compartido con las especies templado-frías (p. ej., *Q. pyrenaica* o *Q. cerris*). Estas especies pueden llegar a vivir en simpatría o en zonas climáticamente muy similares. El éxito de esta convivencia a pesar de tener morfologías foliares muy variadas radica en las diferentes estrategias fisiológicas y ecológicas de estas especies (Ramírez-Valiente *et al.*, 2020; Gil-Pelegrín *et al.*, 2017).

Continuando con el estudio multiespecífico, esta vez profundizando en parámetros anatómicos, llegamos a los resultados del **capítulo IV**. A pesar de que el estudio se realizó en un número de especies menor que el **capítulo III**, la selección de las mismas abarca una variabilidad climática y filogenética representativa de todo el género. En este capítulo queda patente que el pecíolo, como único punto de unión entre la lámina foliar y el resto de la planta, adapta la estructura de sus tejidos vasculares a las necesidades de la lámina. Aunque las tasas de transpiración y fotosíntesis por unidad de superficie pueden variar en gran medida entre especies, una hoja de mayor tamaño necesitará, en términos generales, un mayor suplemento de agua y producirá una mayor cantidad de azúcares, ambos por unidad de tiempo, que una de menor tamaño. Esto conlleva que el pecíolo desarrolle un xilema con una conductividad hidráulica suficiente para satisfacer dichas demandas, lo que puede lograrse mediante el aumento en el tamaño de los vasos, de la superficie vascular, o una combinación de ambas. La exportación de azúcares fotosintetizados requerirá, por su parte, un floema acorde a dicha producción, siguiendo la misma estrategia expuesta para el xilema.

Estos postulados son encontrados en el estudio, donde todos los parámetros analizados incrementan con el LA, sin embargo, no lo hacen de manera lineal, sino que siguen un escalado alométrico. Es decir, hay una restricción al incremento tanto al d_h como al área conductora en hojas de mayor tamaño. Incrementar la conductividad hidráulica a través del incremento en el d_h es mucho más efectiva que aumentar el área conductora, pero compromete la funcionalidad de los conductos (Wheeler *et al.*, 2005). Vasos del xilema más grandes son más susceptibles a cavitar por sequía o por frío, como se ha expuesto en el **capítulo I**. Por su parte, si los tubos cribosos fueran demasiado grandes (i) podrían tener dificultades para generar el gradiente de presión de turgencia necesario para mover la sabia elaborada por el floema y (ii) serían más susceptibles de ser atacados por áfidos.

Estas relaciones anatómicas reflejan —en última instancia— unas necesidades fisiológicas, que se traducen en un incremento en el suplemento de agua (K_h) por el xilema para abastecer las hojas de mayor tamaño, que tendrán —en promedio— una mayor conductancia estomática a nivel de hoja en comparación a una hoja de menor tamaño. Lo mismo ocurre con la fotosíntesis, donde una mayor área de floema y/o células floemáticas más grandes en el peciolo se correlacionan con tasas fotosintéticas, a nivel de hoja, mayores.

El otro resultado, quizás más interesante, de este estudio se halla cuando se analizan las diferencias en el hábito foliar. Partiendo de la base de que los estreses climáticos —principalmente frío y aridez— condicionan parámetros como son el LA, LMA (**Capítulo III**) y d_h (**Capítulo II**), el siguiente paso es analizar como repercuten en la LSC de caducifolios y perennifolios. En casi todos los parámetros analizados, las especies caducifolias presentan diferencias significativas con las perennifolias, probablemente debido a un LA promedio significativamente mayor en caducifolias comparado con perennifolias. De hecho, en el rango de áreas foliares donde se solapan, presentan escalados y comportamientos similares. No obstante, una vez se analiza la LSC, el factor del LA se elimina, ya que representa la eficiencia intrínseca de una hoja, es decir, cuanta agua es capaz de suministrar en relación con su área foliar (LSC = K_h /LA). A pesar de esto, las caducifolias siguen presentando LSC significativamente más altas que las perennifolias, lo cual se debe a la estrategia seguida para incrementar su K_h —y, por ende, su LSC— mediante el aumento del tamaño de los vasos. Aunque algunas perennifolias consiguen aumentar su LSC a través de la producción de vasos de mayor diámetro (p. ej., *Q. semecarpifolia*), estas especies coinciden con las perennifolias de LA más grandes. Las perennifolias de hoja pequeña, por el contrario, son capaces de incrementar sus LSC a través de la producción de más área de xilema, manteniendo diámetros de vasos pequeños, lo cual está indicando una clara estrategia conservadora al producir más vasos, pero menos vulnerables. Una vez más, de acuerdo con los postulados del LES, las hojas más longevas tienen que seguir estrategias más conservadoras para recuperar la mayor inversión que supone producir dichas hojas en comparación a hojas de vida más corta.

En lo que respecta a las estrategias defensivas frente a la presión biótica, en primer lugar, se observa como existe una amplia variación en la contribución relativa de los diferentes mecanismos de defensa considerados (WF, RGR y CT) según la especie. Sin embargo, dentro de esta variabilidad, las especies estudiadas tienden a maximizar una única estrategia defensiva en detrimento de las otras dos. Esto se explicaría por el coste que supone la inversión en estrategias contra la herbivoría, ya que los recursos destinados por parte de una planta a defenderse no pueden ser invertidos en otras funciones como son el crecimiento o la reproducción (Heil y Bostock, 2002; Koricheva *et al.*, 2004). Por otro lado, este *trade-off* entre los diferentes mecanismos estaría asociado con el gradiente de esclerofilia del género. Así, las especies más esclerófilas optarían por una estrategia basada en defensas físicas como es el WF (Alonso-Forn *et al.*, 2023). Las especies de LMA intermedios y bajos optarían, indistintamente, por defenderse mediante un mecanismo de tolerancia como es el RGR, o bien con defensas químicas como son los taninos condensados.

En ningún caso se encuentran especies que maximicen dos de las estrategias consideradas, aunque, por el contrario, sí que se encuentran especies con niveles relativamente bajos de los tres mecanismos de defensa. Los dos ejemplos más notables serían *Q. pyrenaica* y *Q. cerris*. En el caso de *Q. pyrenaica*, esta especie presenta una densa capa de tricomas tanto adaxial como abaxialmente, los cuales han sido descritos como una barrera física contra la herbivoría (Kitamura *et al.*, 2007). En cuanto a *Q. cerris*, parece ser la especie más vulnerable en este estudio. No obstante, su principal mecanismo de defensa podría estar basado en estrategias no contempladas en este estudio, por ejemplo, de naturaleza indirecta o evasiva.

Por último, en este estudio asumimos los postulados clásicos del LES acerca de que las hojas esclerófilas —de mayor LMA— son más caras, en términos de costes de construcción (gramos de glucosa por gramos de peso de seco). Si bien esta asunción generalmente se cumple (Wright *et al.*, 2004; Onoda *et al.*, 2017), la correlación podría deberse a que las hojas esclerófilas (y casi siempre perennifolias), son, generalmente, más pequeñas que las malacófilas caducifolias, una tendencia que también suele darse en las especies de *Quercus*, aunque con excepciones. De hecho, Merino (1987) no encuentra diferencias significativas al comparar los costes de producción en especies caducifolias y perennifolias del mismo tamaño. Por otro lado, Villar y Merino (2001) sugieren como las hojas de mayor tamaño alocarían más recursos a la producción de celulosa y hemicelulosa —como compuestos de refuerzo estructural—, que son, a su vez, los compuestos con menor coste de producción. No obstante, otros estudios han demostrado como las especies esclerófilas ganan dureza (p. ej., Fuerza máxima, WF, etc.,) a través de la inversión en compuestos baratos en términos de coste de producción, es decir, la celulosa o la hemicelulosa (Kitajima *et al.*, 2012; Alonso-Forn *et al.*, 2023). Por lo tanto, el debate sobre si las especies perennifolias son necesariamente más caras por unidad de superficie que las caducifolias está aún abierto.

6.2. Conclusiones

A continuación, se detallan las conclusiones extraídas de cada uno de los objetivos específicos expuestos en el **capítulo I**:

i) *Elaborar un sistema de estudio que incluya un clima con diferentes restricciones ambientales (clima mediterráneo) y una especie perennifolia de amplia distribución (Q. ilex) que tenga que soportar dichas restricciones. Evaluar y discutir en dicho sistema qué estreses son los que más comprometen la funcionalidad de la especie estudiada.*

1. La encina (*Q. ilex*) es una especie con multitud de adaptaciones para enfrentarse a las limitaciones impuestas por el clima mediterráneo. Como especie perennifolia, sigue estrategias extremadamente conservadoras para minimizar cualquier posible daño a sus hojas: su xilema presenta una alta resistencia a la cavitación, cierra estomas a valores de VPD bajos, es decir, sigue una estrategia ahorradora en el uso del agua y presenta numerosos mecanismos de fotoprotección.

2. Dentro de su gran conservadurismo en comparación a otras especies, *Q. ilex* presenta diferencias significativas entre sus dos morfotipos, estando el morfotipo Ballota ligado a condiciones más continentales y adversas que el morfotipo Ilex, el cual está ligado a zonas costeras con condiciones más suaves. Así, el morfotipo Ballota presenta vasos más pequeños, mayor resistencia a la cavitación y mayor tolerancia al frío que el morfotipo Ilex.

ii) *Estudiar en un diverso set de especies, que intenten representar la mayor cantidad posible de grupos filogenéticos y climas ocupados por Quercus, como la morfología foliar es modulada por las diferentes condiciones climáticas de los hábitats; así como discutir la ventajas adaptativas que representan dichas modificaciones.*

3. Las especies de *Quercus* se organizan, a nivel global, en grupos climáticos definidos que reflejan síndromes morfológicos foliares específicos. Incluso cuando crecen en las mismas condiciones climáticas, los robles desarrollan rasgos que sugieren adaptaciones ecológicas distintivas en sus lugares de origen.

4. Aunque no se puede rechazar una señal filogenética presente en los rasgos morfológicos analizados, especialmente en el caso del LMA, la morfología foliar responde de manera más efectiva al clima ocupado por las especies que a la filogenia.

iii) Analizar como la anatomía de los tejidos conductores del peciolo escalan proporcionalmente a las demandas fisiológicas de la hoja en un variado set de especies caducifolias y perennifolias de Quercus que ocupan diferentes climas.

5. Las especies de *Quercus* presentan dos estrategias diferentes a la hora de producir y empaquetar sus conductos vasculares que se identifican con los dos hábitos foliares. Así, las especies caducifolias que tienen hojas más grandes y necesitan una mayor K_h, optan por incrementar dicha conductividad mediante la producción de vasos muy grandes, con sus respectivas implicaciones sobre su vulnerabilidad. Por el contrario, las especies perennifolias, que suelen tener hojas más pequeñas, producen vasos más pequeños, y, por tanto, menos vulnerables. A pesar de tener una menor LSC que las caducifolias, las especies perennifolias son capaces de incrementar, hasta cierto punto, su LSC mediante el incremento en área de xilema.

6. El xilema y floema del pecíolo acomodan su anatomía a las necesidades fisiológicas de las hojas: transpiración y fotosíntesis, respectivamente. Existen claras relaciones de escalado entre el área conductora y el tamaño de los conductos con el LA, aunque dichas relaciones reflejan en todos los casos una alometría. Estas limitaciones al crecimiento lineal estarían explicadas por una serie de restricciones, principalmente de carácter ambiental.

iv) *Evaluar las diferentes estrategias posibles para hacer frente a la herbivoría en varias especies de Quercus abarcando todo el rango de esclerofilia del género.*

7. Las especies de *Quercus* muestran un *trade-off* entre los mecanismos defensivos estudiados, apostando principalmente por un único mecanismo y exhibiendo niveles de bajos a intermedios de los dos restantes.

8. Los mecanismos defensivos estudiados se asocian con el gradiente de LMA del género. Así, las especies más esclerófilas se defienden aumentando su WF, mientras que las especies malacófilas y de esclerofilia intermedia o bien presenten altos ratios de crecimiento relativo, o bien producen altas concentraciones de taninos condensados.

6.3. Conclusions

Below are the conclusions drawn from each specific objective presented in Chapter I:

i) *Develop a study system that incorporates a climate with various environmental constraints (Mediterranean climate) and an evergreen species with a wide distribution (Q. ilex) that must cope with these conditions. Evaluate and discuss within this framework which stresses most compromise the functionality of the studied species.*

1. The holm oak (*Q. ilex*) is a species with numerous adaptations to cope with the limitations imposed by the Mediterranean climate. As an evergreen species, it developpes highly conservative strategies to minimize potential leaf damage: the xylem shows high resistance to cavitation, stomata are closed at low VPD values, this is, a water-conserving strategy and has various photoprotection mechanisms.

2. Despite its conservative nature compared to other species, *Q. ilex* shows significant differences between the two morphotypes proposed. The Ballota morphotype is associated with harsher, more continental conditions, while the Ilex morphotype thrives in milder, coastal environments. Consequently, Ballota has smaller vessels, higher cavitation resistance, and greater cold tolerance than Ilex.

ii) *Study in a diverse set of species, represented by a wide range of phylogenetic groups and climates occupied by Quercus, how leaf morphology is modulated by different environmental conditions and discuss the adaptive advantages these modifications confer.*

3. Globally, *Quercus* species are grouped into distinct climate clusters that correspond to specific leaf morphological syndromes. Even when growing under the same climatic conditions, oaks display traits that relate to ecological adaptations in their native habitats.

4. While a phylogenetic signal cannot be neglected in the analyzed traits, particularly for LMA, leaf morphology is more strongly influenced by the climate occupied by the species than by the phylogenetic legacy.

iii) Analyze how the anatomy of the vascular tissues in the petiole scales proportionally to the physiological demands of the leaves in a varied set of deciduous and evergreen Quercus species occupying different climates.

5. *Quercus* species exhibit two distinct strategies for constructing and organizing their vascular conduits, corresponding to their leaf habits. Deciduous species, with larger leaves and higher hydraulic conductivity (K_h), increase this conductivity by producing large vessels, which in turn affect their vulnerability. Evergreen species, with generally smaller leaves, have smaller, less vulnerable vessels. Despite a lower leaf-specific conductivity (LSC) compared to deciduous species, evergreens can increase LSC to some extent through greater xylem area.

6. The xylem and phloem in the petiole adapt anatomically to meet the physiological requirements of the leaves: transpiration and photosynthesis, respectively. There are clear scaling relationships between both conductive area and conduit size with leaf area, though these relationships show allometry in all cases. These limitations to scaling are likely explained by a set of environmental constraints.

iv) Evaluate different strategies to cope with herbivory across several Quercus species that span the full range of sclerophylly within the genus.

7. Oak species exhibit a trade-off among the studied defensive mechanisms, typically investing in one primary strategy while maintaining low to intermediate levels of the other two.

8. The defensive mechanisms are associated with the LMA gradient present in the genus. Highly sclerophyllous species defend themselves by increasing their work to fracture (WF), while species with low to intermediate LMA either exhibit high relative growth rates or produce high concentrations of condensed tannins.

6.4. Referencias

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Apéndice

A continuación, se detallan las publicaciones derivadas de esta tesis, así como el área temática de la revista publicada para cada artículo, el índice de impacto y la contribución del doctorando:

Martín-Sánchez, R., Peguero-Pina, J. J., Alonso-Forn, D., Ferrio, J. P., Sancho-Knapik, D., & Gil-Pelegrín, E. (2022). Summer and winter can equally stress holm oak (*Quercus ilex* L.) in Mediterranean areas: a physiological view. ***Flora***, 290, 152058. https://doi.org/10.1016/j.flora.2022.152058

Factor de impacto: 1.9

Área temática: Plant Sciences

Contribución: El doctorando participó en la conceptualización del estudio, el análisis de los datos, la redacción y revisión del manuscrito.

Martín-Sánchez, R., Sancho-Knapik, D., Alonso-Forn, D., López-Ballesteros, A., Ferrio, J. P., Hipp, A. L., ... & Gil-Pelegrín, E. (2024). Oak leaf morphology may be more strongly shaped by climate than by phylogeny. ***Annals of Forest Science***, *81*(1), 14. https://doi.org/10.1186/s13595-024-01232-z

Factor de impacto: 2.5

Área temática: Forestry

Contribución: El doctorando participó en la conceptualización del estudio, el análisis de los datos, la redacción y revisión del manuscrito.

Martín-Sánchez, R., Sancho-Knapik, D., Ferrio, J. P., Alonso-Forn, D., Losada, J. M., Peguero-Pina, J. J., ... & Gil-Pelegrín, E. (2024). Xylem and phloem in petioles are coordinated with leaf gas exchange in oaks with contrasting anatomical strategies depending on leaf habit. ***Plant, Cell & Environment***. https://doi.org/10.1111/pce.15231

Factor de impacto: 6.1

Área temática: Plant Sciences

Contribución: El doctorando participó en la conceptualización del estudio, la toma y análisis de los datos, redacción y revisión del manuscrito.

Sancho-Knapik, D., **Martín-Sánchez, R.**, Alonso-Forn, D., Peguero-Pina, J. J., Ferrio, J. P., & Gil-Pelegrín, E. (2023). Trade-offs among leaf toughness, constitutive chemical defense, and growth rates in oaks are influenced by the level of leaf mass per area. ***Annals of Forest Science***, *80*(1), 39. https://doi.org/10.1186/s13595-023-01204-9

Factor de impacto: 2.5

Área temática: Forestry

Contribución: El doctorando participó en la toma y análisis de los datos, redacción y revisión del manuscrito.