Universidad de Alcalá
COMISIÓN DE ESTUDIOS OFICIALES
DE POSGRADO Y DOCTORADO

Diligencia para hacer constar que donde dice: "En el día de hoy 25/03/18" debe decir "En el día de hoy 25/05/18"

ACTA DE EVALUACIÓN DE LA TESIS DOCTORAL

Año académico 2017/18

DOCTORANDO: **FERNÁNDEZ PÉREZ, LAURA**
D.N.I./PASAPORTE: ******0926N**

PROGRAMA DE DOCTORADO: **D413-ECOLOGÍA. CONSERVACIÓN Y RESTAURACIÓN DE ECOSISTEMAS**
DPTO. COORDINADOR DEL PROGRAMA: **CIENCIAS DE LA VIDA**
TITULACIÓN DE DOCTOR EN: **DOCTOR/A POR LA UNIVERSIDAD DE ALCALÁ**

En el día de hoy 25/03/18, reunido el tribunal de evaluación nombrado por la Comisión de Estudios Oficiales de Posgrado y Doctorado de la Universidad y constituido por los miembros que suscriben la presente Acta, el aspirante defendió su Tesis Doctoral, elaborada bajo la dirección de **MIGUEL ÁNGEL DE ZAVALA GIRONÉS // PEDRO VILLAR SALVADOR.**

Sobre el siguiente tema: *RESPUESTAS FUNCIONALES DE LAS CONÍFERAS AL FRÍO Y A LA SEQUÍA: UNA APROXIMACIÓN MULTIESCALAR*

Finalizada la defensa y discusión de la tesis, el tribunal acordó otorgar la CALIFICACIÓN GLOBAL[1] de (**no apto, aprobado, notable** y **sobresaliente**): SOBRESALIENTE

Alcalá de Henares, 25 de mayo de 2018

EL PRESIDENTE
Fdo.: Marta PARDOS

EL SECRETARIO
Fdo.: PALOMA RUIZ BENITO

EL VOCAL
Fdo.: Pedro A. Tíscar Oliver

UNIVERSIDAD DE ALCALÁ, PATRIMONIO DE LA HUMANIDAD

Con fecha 11 de junio de 2018 la Comisión Delegada de la Comisión de Estudios Oficiales de Posgrado, a la vista de los votos emitidos de manera anónima por el tribunal que ha juzgado la tesis, resuelve:

☐ Conceder la Mención de "Cum Laude"
☒ No conceder la Mención de "Cum Laude"

La Secretaria de la Comisión Delegada

FIRMA DEL ALUMNO,

Fdo.: ....

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[1] La calificación podrá ser **"no apto" "aprobado" "notable"** y **"sobresaliente".** El tribunal podrá otorgar la mención de "cum laude" si la calificación global es de sobresaliente y se emite en tal sentido el voto secreto positivo por unanimidad.

Universidad
de Alcalá
COMISIÓN DE ESTUDIOS OFICIALES
DE POSGRADO Y DOCTORADO

Diligencia para hacer constar que donde dice: "el día 25 de marzo de 2018" debe decir: "el día 25 de mayo de 2018"

En aplicación del art. 14.7 del RD. 99/2011 y el art. 14 del Reglamento de Elaboración, Autorización y Defensa de la Tesis Doctoral, la Comisión Delegada de la Comisión de Estudios Oficiales de Posgrado y Doctorado, en sesión pública de fecha 11 de junio, procedió al escrutinio de los votos emitidos por los miembros del tribunal de la tesis defendida por *FERNÁNDEZ PÉREZ, LAURA*, el día 25 de marzo de 2018, titulada *RESPUESTAS FUNCIONALES DE LAS CONÍFERAS AL FRÍO Y A LA SEQUÍA: UNA APROXIMACIÓN MULTIESCALAR*, para determinar si a la misma se le concede la mención "cum laude", arrojando como resultado, 3 votos en contra.

Por lo tanto, la Comisión de Estudios Oficiales de Posgrado **resuelve no otorgar la Mención de "cum laude"** a dicha Tesis.

Alcalá de Henares, 12 de junio de 2018
EL VICERRECTOR DE INVESTIGACIÓN Y TRANSFERENCIA

F. Javier de la Mata

F. Javier de la Mata de la Mata

Copia por e-mail a:
Doctorando: FERNÁNDEZ PÉREZ, LAURA
Secretario del Tribunal: PALOMA RUIZ BENITO
Director/a de Tesis: MIGUEL ÁNGEL DE ZAVALA GIRONÉS //PEDRO VILLAR SALVADOR

Universidad
de Alcalá

ESCUELA DE DOCTORADO
Servicio de Estudios Oficiales de Posgrado

DILIGENCIA DE DEPÓSITO DE TESIS.

Comprobado que el expediente académico de D./Dª Laura Fernández Pérez reúne los requisitos exigidos para la presentación de la Tesis, de acuerdo a la normativa vigente, y habiendo presentado la misma en formato: ☒ soporte electrónico ☐ impreso en papel, para el depósito de la misma, en el Servicio de Estudios Oficiales de Posgrado, con el nº de páginas: 178 se procede, con fecha de hoy a registrar el depósito de la tesis.

Alcalá de Henares a 15 de marzo de 2018

Purificación Reviejo Arandia

Fdo. El Funcionario

Universidad de Alcalá

RESPUESTAS FUNCIONALES DE LAS CONÍFERAS AL FRÍO Y A LA SEQUÍA: UNA APROXIMACIÓN MULTIESCALAR

Laura Fernández Pérez

Tesis Doctoral

PROGRAMA DE DOCTORDO EN ECOLOGÍA, CONSERVACIÓN Y RESTAURACIÓN DE ECOSISTEMAS

2018

Foto portada. Piet Mondrian “El árbol rojo”, 1910.

Universidad
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D. Miguel Ángel de Zavala Gironés, Catedrático del Departamento de Ciencias de la Vida de la Universidad de Alcalá y Director de esta Tesis Doctoral,

hace constar:

Que el trabajo descrito en la presente memoria, titulado **"Respuestas funcionales de las coníferas al frío y a la sequía: una aproximación multiescalar"**, ha sido realizado dentro del Programa de Doctorado "Ecología, Conservación y Restauración de Ecosistemas" (D413), reuniendo los requisitos necesarios para su aprobación como Tesis Doctoral por acuerdo de la Comisión Académica de dicho programa de doctorado.

Alcalá de Henares, 14 de Marzo de 2018.

Universidad de Alcalá
DEPARTAMENTO DE CIENCIAS DE LA VIDA

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D. Pedro Villar Salvador, Profesor Titular de Universidad del Departamento de Ciencias de la Vida de la Universidad de Alcalá y Codirector de esta Tesis Doctoral,

hace constar:

Que el trabajo descrito en la presente memoria, titulado **"Respuestas funcionales de las coníferas al frío y a la sequía: una aproximación multiescalar"**, ha sido realizado dentro del Programa de Doctorado "Ecología, Conservación y Restauración de Ecosistemas" (D413), reuniendo los requisitos necesarios para su aprobación como Tesis Doctoral por acuerdo de la Comisión Académica de dicho programa de doctorado.

Alcalá de Henares, 14 de Marzo de 2018.

Universidad
de Alcalá

DEPARTAMENTO DE CIENCIAS DE LA VIDA
UNIDAD DOCENTE DE ECOLOGÍA

RESPUESTAS FUNCIONALES DE LAS CONÍFERAS AL FRÍO Y A LA SEQUÍA: UNA APROXIMACIÓN MULTIESCALAR.

Memoria presentada para optar por el grado de
Doctora por la Universidad de Alcalá

Programa de Doctorado:
“Ecología, Conservación y Restauración de Ecosistemas” (D413)

Laura Fernández Pérez

Director: Miguel Ángel de Zavala Gironés
Co-director: Pedro Villar Salvador

Alcalá de Henares, Marzo de 2018.

UNIVERSIDAD DE ALCALÁ, PATROMINIO DE LA HUMANIDAD

Esta Tesis Doctoral se realizó en el Programa de "Doctorado en Ecología, Conservación y Restauración de Ecosistemas" (D413), coordinado por el Departamento de Ciencias de la Vida de la Universidad de Alcalá, y al que también contribuyen las universidades Complutense, Politécnica y Rey Juan Carlos de la Comunidad de Madrid.

Durante el tiempo de realización de esta tesis Doctoral he disfrutado de una beca del Consejo Nacional de Ciencia y Tecnología del gobierno de México (291826), la cual agradezco enormemente. Sin este financiamiento no hubiese podido realizar este sueño.

Dedicada a la mujer más importante de
mi vida, Alejandra.

AGRADECIMIENTOS

Antes de empezar con la larga lista de personas que han formado parte de esta etapa de mi vida, me gustaría que los próximos doctorandos que consulten mi tesis (y en especial los que quieran hacer un doctorado en el extranjero) sean conscientes de lo que representa hacer un doctorado. Para mi ha sido un tanto hipócrita leer en los agradecimientos de las tesis que todo fue maravillosamente, que fue una experiencia enriquecedora y que después de mucho esfuerzo lo han conseguido. Para mi, éstos cuatro años han sido los peores mi vida, tanto académica como personalmente. Sólo los migrantes entendemos lo difícil que significa irse de la tierra natal. Dejarlo todo por un sueño, en el que, desafortunadamente no siempre te vas a sentir triunfador, si no todo lo contrario. Me he perdido eventos maravillosos como los nacimientos y las bodas de mis mejores amigos (esto va para Pilar y Fiolita), y los no tan maravillosos, como dar el último adiós a personas que ocuparon un lugar muy importante en mi vida. Acostumbrarse a nuevas expresiones, a la comida (en especial siendo la comida mexicana la mejor del mundo!), y enfrentarse con el racismo, que al ser hispanomexicana es difícil de entender que hasta en tu propio país sean crueles sólo por hablar distinto, vaya ironía. Con todo esto lidiamos los migrantes. Si ya de por si es difícil adaptarse a una nueva cultura, por encima le sumas el enorme desafío que representa hacer un doctorado y la responsabilidad social que tiene mantener un beca. Por regla general se espera mucho de los doctorandos, como si el doctorado significara que todo lo has de saber. Terror a no llegar a los estándares previstos, temor a fracasar, no saber escribir, no entender, no sentirse en el lugar correcto, saber que los hijos y/o estudiantes de 15-20 años de tus profesores lo hace cien veces mejor que tú. Esos sentimientos me inundaron estos cuatro larguísimos años. Le recomiendo a los interesados en hacer un doctorado que dediquen su vida a algo que les haga mucho más feliz. Viajar, aprender un oficio, vivir hombre…VIVIR! Espero que estas líneas no desalienten a algunos soñadores, pero hay que ser realistas y decir la verdad, que es parte de la formación de todo científico.

Tengo que reconocer que nunca me había costado tanto conseguir algo. Quizás por todas las malas rachas que he tenido durante la realización de la tesis. Además tengo asumido y más que interiorizado que nunca he sido una estudiante ejemplar. Sin embargo, en la vida de cada estudiante hay maestros que dejan huella. Ellos nos guían y se convierten durante un tiempo de nuestra vida en figuras de referencia para nosotros, que sin su apoyo estamos perdidos. Mi gran maestro ha sido Pedro Villar Salvador. En todos estos años has sido increíblemente paciente conmigo. Muchas gracias por todos esos días que invertiste en mi, tus enseñanzas "pizarrescas", por hacerme un huequito en tu apretada agenda para esclarecer dudas y ver resultados "sexys" donde yo ni sabía que existían. "Educar a una persona no es hacerle aprender algo que no sabía, sino hacer de él alguien que no existía". Gracias por no desistir. También quiero agradecer a Miguel Ángel de Zavala por darle la oportunidad de hacer la tesis a una chica que no conocía de nada, por mostrarme una nueva visión modélica de los bosques, y por todas esas ridiculeces como "mi variable aleatoria", que no entendía para que, pero al final han tenido su objetivo.

Realizar esta tesis doctoral ha sido especialmente difícil para mi debido a que durante estos años perdí a muchas personas. Uno de ellos fue mi padre, Pablo Fernández Oubiña, que a pesar de su ausencia a lo largo de mi vida, estaré siempre agradecida por estos saludables genes, consecuencia de históricas migraciones entre Europa y América. Espero que donde se encuentre tu alma, estés orgulloso de mi. Mi abuelita, Mamá Tere, la auténtica matriarca mexicana, fuerte y con muchos huevos, capaz de reventarle la bolsa del mercado a cualquiera que se metiera con su nieta. Mi María bonita, me encantaba escucharte hablar. Para ti también va mi tesis. Mi tía Elba Abuela Becerril, otra gran matriarca. De ti aprendí el sarcasmo y a reírme de la vida. Eras una de las más queridas. Mi tío Manolo López que perdió la batalla contra el cáncer. Mi segundo padre. A todos ellos les doy las gracias por estar presentes a lo largo de mi vida y enseñarme a ser una mejor persona. Aunque sé que nunca podrán leer éstas líneas, su ausencia durante este proceso me ha dolido en el alma. Los llevaré en mi corazón por siempre hasta que nos volvamos a encontrar.

Sin embargo, para aminorar los duros golpes que da la vida, siempre contamos con gente extraordinaria en nuestro andar. Quiero darle las gracias a la mujer más maravillosa, mi madre, Alejandra Pérez Becerril. Sin ti no hubiera llegado ni a la esquina. Eres una mujer increíble a la que admiro y amo profundamente. Has sido mi mejor maestra: me has enseñado a luchar por lo que es correcto, independencia, y sobre todo, a enfrentarme a mis miedos. Durante este proceso (y muchos anteriores a este!), has acudido a mi llamado. La respuesta positiva era "Tu puedes hija, venga! No exageres! Ya casi, espérate que te enciendo una velita". En cambio la negativa era: "pues manda todo a la fregada y regrésate para acá, que aquí tienes a tu madre para lo que haga falta". Al final de cuentas, dejaste que decidiera por mi misma y por fin terminé. Se que unas cuantas líneas no pueden expresar todo lo que siento por ti, pero que sepas que te agradezco infinitamente lo que has hecho siempre. Te amo con todo mi corazón!

Durante mis años por España conocí a personas maravillosas de lo cual me siento muy afortunada. Una de tantas es Daniel Pinel Bermejo, que ha sido otra persona crucial en todo este proceso. Tuvieron que pasar muchísimas cosas para que los astros se alinearan y por fin encontrarte. Tengo tanto que agradecerte: tu tiempo, tu simpatía, tu música, tu singular risa, tu dulzura excepcional, tu protección, tu amor y en especial, lo paciente que has sido conmigo sobre todo en el último empujón. Gracias por enseñarme que la vida puede ser más simple y relajada. Muchas gracias a ti siempre ☺! Lo que te quiero! Muchas gracias a toda tu maravillosa familia Isaac, Juan Antonio, Irene, César, Corina, Pilar, Pepe, Beatriz, David, Denis, Alberto, Sandra, Paco y todos los sobrinos, en especial Marquitos y Adrián. Con especial agradecimiento le dedico unas líneas a mis maravillosos suegros Rosa Bermejo y Juan Pinel, las personas más cariñosas, dadivosas y protectoras que conozco. Muchas gracias por abrirme las puertas de su casa y hacerme un huequito en su mesa, por tratarme como una más de la familia, por todas esas comidas vegetarianas, las risas, los niños, los paseos, por todo.

Lo bueno de ser migrante es que aprendí desde muy pequeña a hacer amigos a donde quiera que fuera. Bien dice Emily Dickinson "Todo mi patrimonio son mis amigos", que son parte fundamental en mi andar. Muchas gracias a mi mejor amiga, Ethel Martínez-Aguilera, la chica más fuerte que he conocido. Cuando te he necesitado has estado ahí para escucharme. No eres hermana de sangre, pero eres mi hermana de corazón. Siempre que estoy en algún aprieto pienso: "¿Que haría Ethel?" Interminables batallas con el pequeño Mafaldo y con un mundo injusto y hostil. Sin embargo, siempre sales victoriosa de cualquier problema y además, con una hermosa sonrisa. Eres un gran libro lleno de aventuras e historias que contar y espero estar siempre en algún lugar de cada capítulo de tu vida.

Los vecinos se convierten en amigos, y a su vez, en la familia que uno añora. Les agradezco mucho a Esther Nuñez, Iván Sanz y sus respectivas familias. Muchas gracias por abrirme la puerta de su casa y de su corazón! Si no hubiera sido por esa noche de póker, la historia hubiera sido muy diferente ;). Mis otros amigos Jorge y Vane y sus respectivas familias. Muchas gracias por las verduras frescas, por las borracheras y por su sincera amistad. En mi caótica y corta estancia por Villanueva de la Torre hice otros grandes amigos: Miguel Martín-Loeches, Elena Perea, Francisco Rodríguez, Rita Pardo y Carlos Palacios. Y por último, en mi reciente mudanza he conocido a una pareja increíble, Mónica Bravezo e Iñaki Martínez. Las cenas, paseos, persecuciones con las fieras y discusiones sobre problemas "profundos" de la vida me han enseñado tanto. También le quiero agradecer mucho a mi linda concuna, Laura Rivera, y a sus dos retoños, Sara y Joel por todas sus alegrías! Muchas gracias a cada uno de ustedes!

La distancia no separa a los verdaderos amigos. Desde muy lejos vinieron a visitarme, me escucharon, nos reímos, "turisteamos" y compartimos momentos que atesoraré siempre en mi memoria. Muchas gracias Fabiola Gutiérrez-Medina, Jhibran Ferral-Piña, Laura Ferrero García, Claudia Flores, Julio Vargas e Irene Ortiz. Su compañía hicieron esto mucho más feliz. Mis amigos de la Secu 95. Su apoyo llegó en el momento correcto. Estaba pasando por una etapa horrenda y apunto de dejarlo todo, pero sus mensajes hicieron que riera como desde hacía 20 años no lo hacía. No tienen idea de lo feliz que me hizo leerlos. Gracias por acordarse de mi!

Mi más sincero agradecimiento a mi buen amigo, Denis Conrado Da Cruz. Mi estancia por la universidad no hubiera sido la "mesma" sin ti. Sabes el significado de la palabra luchar en toda su extensión. Todo lo que te ha costado llegar hasta donde estás, te ha convertido en una persona increíble, llena de simpatía y alegría. En especial tengo que agradecerte enormemente por la elaboración de los mapas que en esta tesis se presentan, todos y cada uno de ellos. El principio de la tesis no hubiera sido tan divertida si no hubiera conocido a tan singulares chicas: Deisi Shulz y Michelle Ajala que tendrán una amiga por donde quiera que me encuentre. Muchas gracias por su ayuda en los experimentos, por compartir viajes y muchas alegrías. No cabe duda que Brasil produce gente maravillosa!!

Las generaciones anteriores a nosotros siempre están llenas de sabiduría y de buenos consejos. Mi abuela María Oubiña es una de ellas. Alguien a quien también tengo que agradecerle mis multiculturales genes. Es todo un orgullo ser tu nieta! Fuerte como un roble gallego, amable, dulce y servicial. Ojalá que si llego a tu edad, tenga la misma energía y fuerza que tienes tú. Nunca dejas de mostrar amor por tu familia y por tu aldea. A mi amiga y nueva abuelita, Concepción Cruz Salanueva y a toda su linda familia. Eres todo un ángel. Las patatitas, la cerveza y el oído en el que desahogarse han ablandado hasta los días más duros. Mi vida es mejor contigo a mi lado! Deseo de todo corazón que la vida siga siendo igual de maravillosa contigo!

En la vida de todo doctorando, debe haber un terapeuta capaz de aguantar berrinches y lágrimas. Dedico unas líneas a mi buena amiga Rita Alarcón. Me has mantenido a raya estos cuatro años. No se que hubiera hecho sin todo tu apoyo. Todos tus consejos los atesoro en mi memoria y los llevo a cabo siempre que puedo…y quiero! Gracias de todo corazón!

La música ha sido una de mis mejores compañeras a lo largo de todo camino recorrido. Compartir las maravillosas melodías en acetato de Led Zeppelin, y un buen whiskey con mi buen amigo Jorge Fernández disiparon el estrés en numerosos momentos. Muchas gracias por todas esas tardes de explicaciones físicas, de nubes y de rock.

En los lugares de trabajo científico el común denominador es lidiar con el ego, la soberbia y la avaricia científica. Sin embargo, el Departamento de Ecología es la gran excepción. Mis compañeros de despacho son grandes personas dispuestas a ayudar en cualquier momento. Muchas gracias Joaquín Calatayud, Joaquín Romero, Paloma Ruiz-Benito, Laura Marqués, Loreto Martínez, Verónica Cruz, Navila Monteagudo, Andrea Brihuega, Elena Velado, Zoe Roher, Enrique Andivia, Antonio Guerrero, Pablo Quiles y Daniel Gómez. En especial le dedico unas líneas a dos personas que han sido cruciales para poder llevar a cabo esta tesis. La primera es Jaime Madrigal-González, porque sin el, definitivamente esto lo hubiera dejado desde el primer año. Recuerdo que cuando llegué me dijiste: “yo en tu lugar no haría tesis”. ¡Qué razón tenías queridísimo Jaime, ja! Sin embargo, me has enseñado muchísimo durante estos cuatro años. Espero que la vida te regrese todo lo que das por tus compañeros, te compense con una excelente plaza y que seas muy feliz con tus pequeños retoños. La segunda es Judith Palacios. Tantos viajes te han llenado de experiencias, conocimientos y empatía, cualidades muy difíciles de encontrar en el ámbito personal y científico. Siempre con un buen consejo y una palabra amiga. Infinitas gracias por todo tu apoyo, por las tardes de café y chocolate, y por seguir motivándome a terminar la tesis.

Lo único bueno que obtuve en mi estancia por Zaragoza fue los buenos amigos que hice: Carlota, Pedro, Pau, Ona, Miguel, Graciela y Elena Granda. Gracias por darme un hogar temporal y por tantas enseñanzas de cómo vivir sin depender del mercado.

Agradezco a todo el personal del edificio de Ciencias: la dulce Anita Guerrero, siempre servicial y amable; Lorenzo Pérez siempre con un buen consejo y unas llaves de repuesto; las chicas de limpieza (en especial, Juani y Manoli), los conserjes, el personal del comedor y de la biblioteca. Su amabilidad y humildad se debería replicar en todo el personal. Siempre con una sonrisa, un amable saludo y una charla.

Y por último quiero dedicar unas cuantas lineas a tres seres que, a pesar de que no entienden de que va esto, comprenden lo que he sentido en estos arduos años. Quiero agradecer a mis compañeros de vida Maya y Lennon. Mi única constante. Si no hubiese sido por ellos, quien sabe donde estaría ahora. Ellos me han dado tres cosas fundamentales: estabilidad emocional, amor incondicional y pura felicidad. Maya ha estado conmigo en toda mi carrera profesional: desde que estaba terminando la universidad hasta ahora, 12 largos años! De ser una cachorrita latosa y destructora hasta convertirse en la tierna abuelita que enamora a cualquiera. Lennon y yo compartimos hasta el cumpleaños. Lo vi nacer, crecer y convertirse en el compañero ideal: protector como todo buen macho alfa pero dulce y cariñoso como ninguno. Hemos librado dolorosas cirugías, cambios radicales de vida, de clima, cruzamos el planeta juntos. Definitivamente no sería la misma persona sin mis amados y fieles compañeros. Muchas gracias por darme su vida, por acompañarme a donde quiera que vaya. Recientemente ha llegado el dulce Tosh, otro gran compañero. A pesar de que su pasado asoma dolor y tristezas, el sigue moviendo la cola como si nunca le hubiera pasado nada. Gracias por confiar en mi desde el primer momento. A ellos tres, gracias, las más sinceras.

"Si no puedes volar, corre, si no puedes correr, camina, si no puedes caminar, gatea. Sin importar lo que hagas, sigue avanzado hacia adelante".

Martin Luther King Jr.

TABLA DE CONTENIDO

CAPÍTULO 1

INTRODUCCIÓN GENERAL DE LA TESIS

Foto: *Pinus sylvestris*, Pinar de Navafría. Por Laura Fernández-Pérez

La vida de los seres vivos sólo es posible dentro de un rango de condiciones ambientales y de disponibilidad de recursos. Cada organismo presenta un valor óptimo de cada factor ambiental en el que sus funciones básicas se maximizan. Cualquier desviación con respecto a estos niveles óptimos reducen su desempeño funcional, fenómeno que se denomina estrés (Larcher, 2003). Un factor ambiental, por tanto, se considera "estresante" cuando está en unos niveles que producen estrés a un organismo (Levitt, 1980; Lambers et al, 2008). Así, la reducción del agua retenida en el suelo con respecto a la capacidad de campo causa estrés hídrico en las plantas, del mismo modo que las temperaturas del aire por debajo del punto de congelación del agua son un factor de estrés para la inmensa mayoría de organismos. Cuando un organismo sufre estrés se producen alteraciones en todos sus niveles de organización, desde los moleculares hasta los órganos. Estas alteraciones pueden ser reversibles, pero si el estrés es muy intenso y prolongado pueden ser permanentes y conducir a la muerte del organismo (Levitt, 1980; Lichtenthaler y Rinderle, 1988; Larcher, 2003).

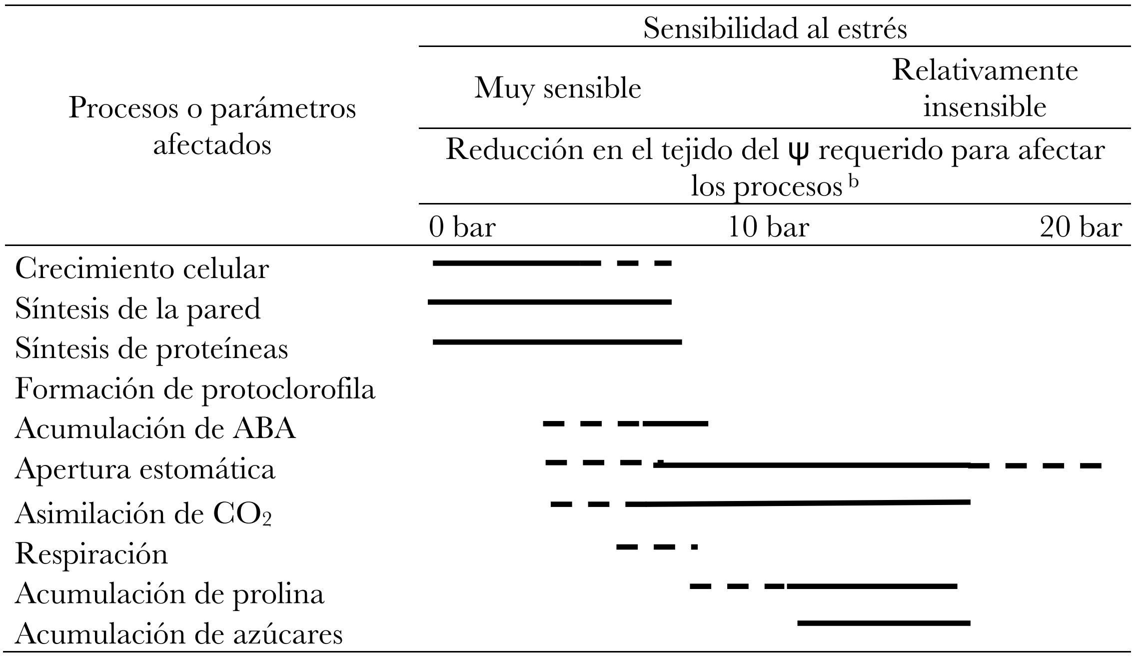
La reducción en la disponibilidad de agua y las temperaturas extremas, especialmente las bajas han condicionado la evolución de las plantas y su distribución en la Tierra (Beck et al, 2007; Prentice et al, 1992).

1.1 Efecto del estrés hídrico sobre la fisiología de las planta

La escasez de precipitaciones y la alta demanda evaporativa del aire debido elevadas temperaturas y baja humedad relativa (Larcher, 2000) son la principal causa de estrés hídrico en las plantas. Sin embargo, el enfriamiento y la congelación del agua del suelo además del aumento en la salinidad del suelo también generan estrés hídrico (Kozlowski, 1971).

El estrés hídrico afecta negativamente la mayor parte de las funciones vitales de las plantas. En la Tabla 1 se muestra la sensibilidad al estrés hídrico de algunos procesos fisiológicos en las plantas. A nivel celular, la turgencia de las células es muy sensible al estrés hídrico. Reducciones en la misma se traduce en la disminución del volumen de la célula y, en consecuencia, en el tamaño de los tejidos y órganos. La pérdida súbita de agua de las células debido a la deshidratación de los tejidos puede dañar las membranas celulares, que se despegan de la pared, por lo que las células dejan de crecer. Además, la síntesis de proteínas también disminuye con el estrés hídrico, y las proteínas se desnaturalizan al estar desprovistas de la capa de hidratación. El estrés hídrico provoca el cierre estomático que disminuye la evaporación del agua por las hojas, pero como contrapartida la asimilación de CO_2 y la respiración disminuyen (Kozlowski et al, 1991).

Tabla 1. Sensibilidad general al estrés hídrico de una serie de procesos fisiológicos de las plantas. [a]La longitud de las líneas horizontales representa el rango de los niveles de estrés en el cual el proceso se ve afectado. Las porciones discontinuas de las líneas significan deducciones basadas en datos. [b] Con un ψ de plantas regadas bajo demandas medias de evaporación como un punto de referencia. (Reproducido a partir de Hsiao, 1973).



Procesos o parámetros afectados	Sensibilidad al estrés		
	Muy sensible		Relativamente insensible
	Reducción en el tejido del ψ requerido para afectar los procesos [b]		
	0 bar	10 bar	20 bar
Crecimiento celular	——— – –		
Síntesis de la pared	————		
Síntesis de proteíneas	————		
Formación de protoclorofila			
Acumulación de ABA	– – –——		
Apertura estomática	– – –	———————	– – – –
Asimilación de CO_2	– –	———————	
Respiración	– – -		
Acumulación de prolina		– – –————	
Acumulación de azúcares		————	

El estrés hídrico también reduce el flujo de agua en la planta, un proceso clave para su balance de carbono y economía hídrica. Esta reducción se debe al cierre de los estomas pero también puede ocurrir por la formación de embolias en el sistema vascular de la planta. La circulación del agua en las plantas ocurre desde la raíz a las hojas a través del xilema. En las plantas con crecimiento secundario por el cambium vascular, el xilema es la madera. En el caso de las coníferas (el grupo de plantas objeto de esta Tesis doctoral), el xilema es un tejido compuesto principalmente por traqueidas y en menor abundancia por células parenquimáticas, que forman los radios medulares. Las traqueidas son de forma alargada (Fig. 1) y están muertas. Transportan el agua y los solutos absorbidos por las raíces hasta las superficies de evaporación. (Evert, 2006). Las paredes de las traqueidas están provistas de punteaduras, que son zonas adelgazadas de la pared celular por donde circula el agua entre traqueidas (Fig. 1) (Evert, 2006).

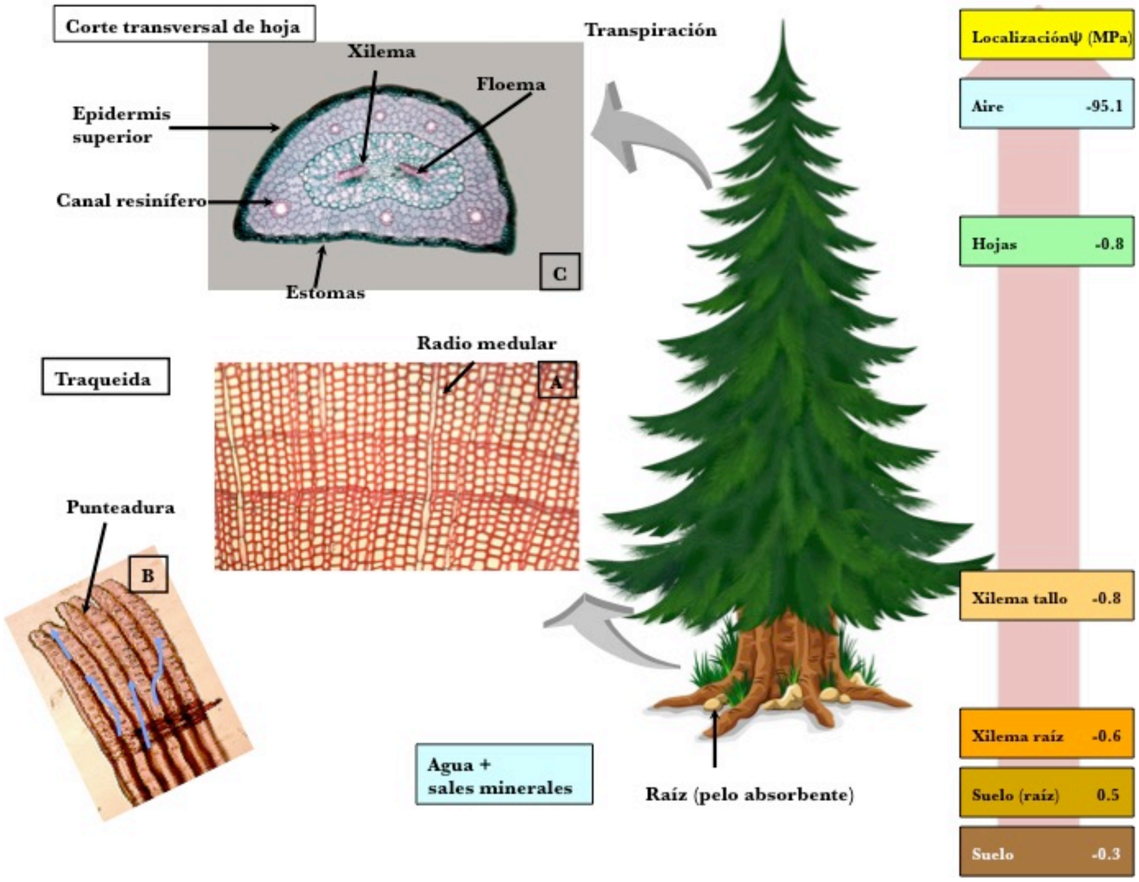


Fig 1. Esquema de la circulación del agua desde el suelo hasta las hojas. En el panel de la derecha se muestran las diferencias de potencial hídrico a lo largo del continuo suelo-planta-atmósfera. A) Corte transversal de tallo de *Pinus sylvestris* (200x), B) Traqueidas (*Juniperus oxycedrus*, 400x (Imágenes de Villar-Salvador, 2000), C) Corte transversal de células *Pinus sylvestris* (Imagen modificada de http://duplexjuanmiguel.blogspot.com.es/2015/02/el-pino-silvestre.html).

El agua asciende por la columna de traqueidas debido las diferencias del potencial hídrico (ψ_w) que se establecen entre la atmósfera, las distintas partes de la planta y el suelo; el agua fluye, por tanto, desde las zonas con mayor potencial hídrico (suelo y raíces) hacia las de menor potencial (hojas) (Fig. 1) (Kozlowski et al, 1991; Lambers et al, 2008). La velocidad con la que fluye el agua a lo largo del xilema dependerán de la intensidad de dicho gradiente potencial hídrico, la apertura y número de los estomas (Fig 1; Kramer y Kozlowski, 1979) y las propiedades de las traqueidas, especialmente de su longitud y diámetro, y el grosor de las paredes de las células (Sperry et al, 2006). La construcción de elementos traqueales de mayor diámetro tiene dos consecuencias funcionales. En primer lugar se incrementa muy significativamente la capacidad de transportar agua. Un incremento del doble del diámetro de las traqueidas incrementa 16 veces la capacidad de transporte de agua (Zimmermann, 1983; Tyree y Ewers, 1991). Por otro lado, se reducen los costes de construcción al producirse menos pared celular por unidad de volumen de la madera.

El estrés hídrico induce la formación de embolias en los conductos del xilema. Ocurre cuando la disminución del ψ_w provoca que el aire contenido en las células vecinas de las traqueidas es aspirado a través de las punteaduras de las traqueidas (Figura 2). Este aire aspirado se expanden en el lumen de la traqueida porque la columna de agua está sometida a una tensión causada por el gradiente de potencial hídrico que se genera a lo largo de la planta (Zimmermann, 1983; Sperry y Tyree, 1990). Al expandirse la burbuja se tapona la traqueida, interrumpiendo el flujo del agua.

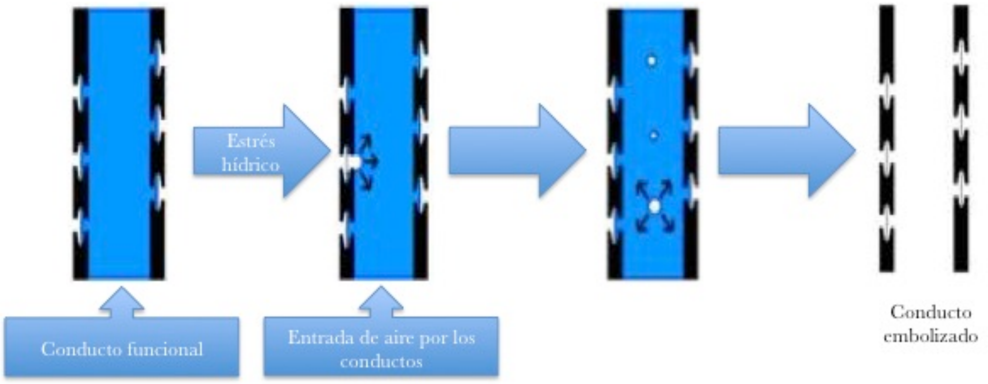


Fig. 2. Esquema de la formación de embolias causada por estrés hídrico. (Modificado de Sperry, 1993)

1.2 Efecto del estrés causado por las heladas sobre la fisiología de las plantas

La congelación del agua en los tejidos de las plantas por las heladas puede causar daños por los propios cristales de hielo (Pearce, 2001) y/o por deshidratación (Beck et al, 2004). A nivel celular, las heladas causan la pérdida de la integridad funcional de las membranas celulares. Esto sucede cuando el agua intercelular (la del apoplasto) se congela y provoca que el agua intracelular (la del simplasto) salga a los espacios intercelulares. Ello produce un cambio brusco en el volumen del protoplasma que puede dañar la membrana celular. Si el daño es muy intenso, puede desembocar en la pérdida de la integridad de la célula (Prentice et al, 1992; Sakai y Larcher, 1987; Wesley-Smith et al, 2015). En este sentido, este daño causado por las heladas es parecido a algunos daños causados por el estrés hídrico

(Levitt, 1980). Las heladas también reducen la capacidad de fotosíntesis debido a distorsiones en los tilacoides que reducen la superficie funcional de los cloroplastos (Larcher, 2005). Además, ciertos procesos metabólicos se detienen y se acumulan radicales libres que son altamente oxidantes. (Larcher, 2005).

Las heladas también pueden reducir la circulación de agua por la planta mediante la formación de embolias. Las embolias inducidas por las heladas ocurren cuando se descongela el agua del xilema (por ejemplo, por la mañana al incidir el sol en la planta después de las heladas nocturnas) y el gas contenido en el agua líquida forma burbujas. Éstas se expanden y se unen entre sí bloqueando los conductos del xilema (Fig. 3; Sperry and Sullivan, 1992; Feild and Brodribb, 2001; Mayr et al, 2003; Pittermann and Sperry, 2006; McCulloh et al, 2011; Charrier et al, 2014). Por tanto, el mecanismo de formación de las embolias por las heladas es diferente al mecanismo inducido por estrés hídrico y (Zimmermann, 1983; Sakai y Larcher 1987). Sin embargo, ambos mecanismos de embolización tienen algo en común y es que la propagación e intensidad de la embolia aumenta con la reducción del ψ_w del xilema (Davis et al. 1999; Mayr et al, 2003). Si la planta presenta un potencial hídrico muy bajo en el momento de la helada, la formación de embolias será mayor. Por tanto, en inviernos muy secos con heladas intensas es esperable que las embolias sean más intensas que en inviernos húmedos (Peguero-Pina et al, 2011).

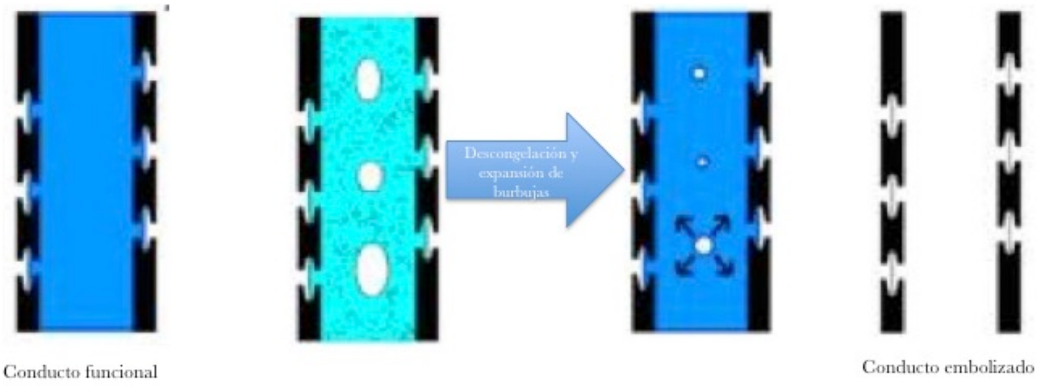


Fig. 3 Esquema de la formación de embolias causada por heladas (Imágen tomada de Sperry, 1993).

La vulnerabilidad a la formación de embolias producidas por las heladas se incrementa con el diámetro de los conductos del xilema (Sperry et al, 2006). Por tanto, la formación de embolias puede ser muy variable entre las especies en función del tamaño de los conductos de sus xilemas (Fig. 4) (Tognetti et al, 1998; Davis et al, 1999).

1.3. Respuestas del crecimiento y la morfología de los árboles a los factores de estrés.

Los árboles presentan tanto crecimiento longitudinal como radial. Éste último ocurre en aquellos órganos con cambium vascular, en concreto en troncos, ramas y raíces no finas. Las plantas leñosas de climas templados y fríos tienen un crecimiento radial rítmico que se traduce en la formación de un anillo por cada estación de crecimiento. Este anillo de crecimiento se forma por la actividad periódica del cambium vascular (Evert, 2006). Dentro del anillo de crecimiento de las coníferas, se distinguen la madera temprana (EW) y la madera tardía (LW) (Fig. 5) (Martinez-Meir et al, 2008). La EW se forma a principios de la temporada de crecimiento y tiene una menor densidad debido a que las células son más anchas y paredes más delgadas. La LW se produce al final de la temporada de crecimiento y tiene una mayor densidad porque las células son más estrechas y sus paredes son más gruesas (Larsson, 1994).

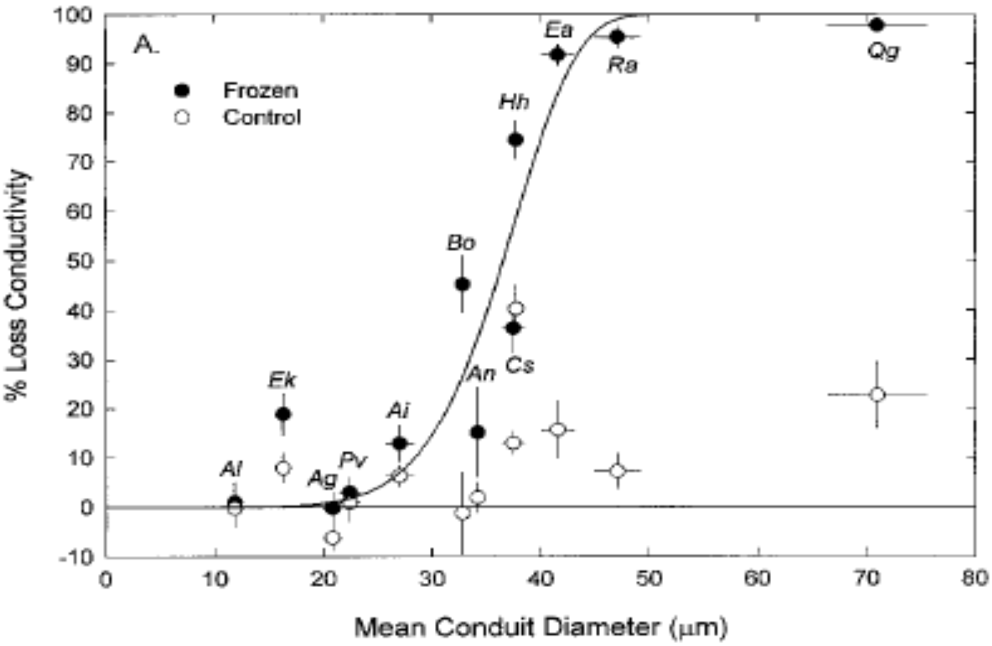


Fig. 4 Porcentaje de la pérdida de conductividad hidráulica por heladas de ≈ -15ºC (símbolos cerrados) y sin heladas (símbolos abiertos) en relación al tamaño de los conductos del xilema en diferentes especies leñosas. Al: *Abies lasiocarpa*; Ek: *Euonymus kiautschovicus* Loes; Ag: *Acer grandidentatum* Nutt.; Pv: *Prunus virginiana* L.; Ai: *Alnus incana* (L.) Moench; Bo: *Betula occidentalis* Hook.; An: *Acer negundo* L.; Cs: *Cornus sericea* L.; Hh: *Hedera helix* L.; Ra: *Rhus aromatica* Ait.; Ea: *Eleagnus angustifolia* L.; Qg: *Quercus gambelii* Nutt (Tomado de Davis et al, 1999).

La anchura de un anillo de crecimiento suele estar relacionada con la precipitación durante la estación de crecimiento en la que se formó el anillo. Las estaciones de crecimiento secas se asocian con anillos más estrechos (Fritts,1976; Säranpaa, 2003). También pueden existir diferencias en la anchura de EW y la LW en relación con las precipitaciones; la anchura de EW de un año concreto está más relacionada con las precipitaciones del año anterior, mientras que la anchura de LW suele estar más relacionada con las precipitaciones de la estación de crecimiento en la que se formó (Martín-Benito et al, 2013; Pompa-García y Camarero, 2015). Por tanto, los anillos de crecimiento integran la influencia de factores ambientales del año de crecimiento como el de los previos (Fritts, 1976).

Además de la anchura del anillo, la densidad de EW y la LW pueden también servir como indicadores del clima a largo plazo. La densidad de la madera es la relación entre su masa y volumen, y está relacionada con el soporte mecánico, la capacidad conductora y de almacenamiento de sustancias de la madera, y la resistencia al viento, la gravedad y la nieve (Hacke et al, 2001). Es común que las especies arbóreas con una mayor densidad de la madera (y por tanto baja conductividad hidráulica) muestren bajas tasas de crecimiento (Chave et al. 2009). Las especies con maderas blandas, por el contrario, tienen una mayor conductividad hidráulica y crecen más rápidamente (Hacke y Sperry, 2001; Bouche et al. 2014).

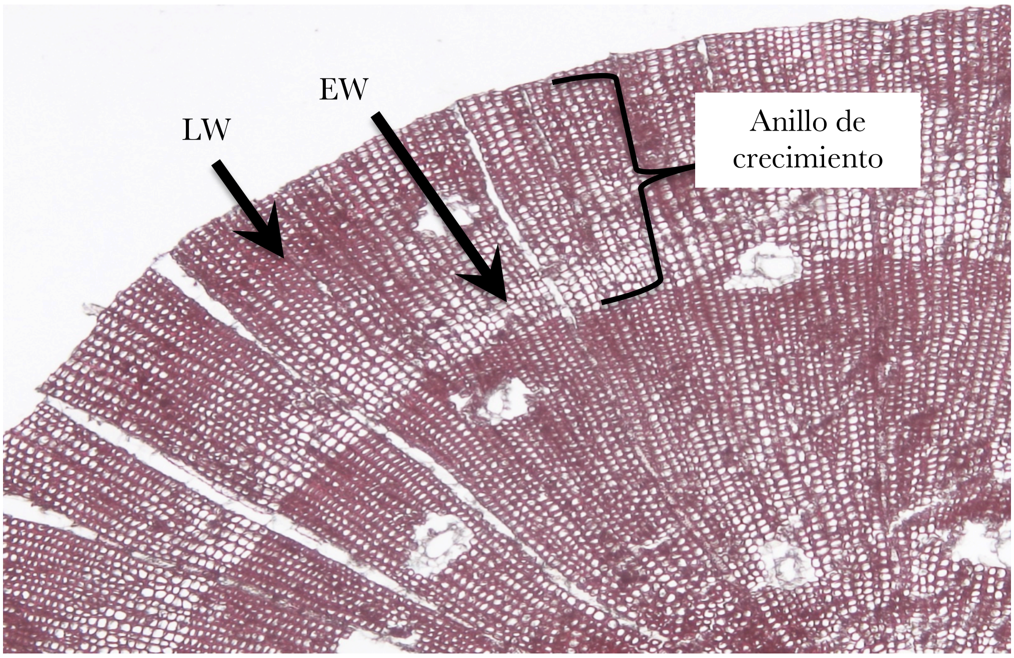


Fig. 5 Superficie de los anillos de crecimiento en un individuo de dos años de *Pinus pinaster* mostrando la madera temprana (EW) y tardía (LW) en el anillo formado el segundo año (Muestra anatómica y foto: Laura Fernández-Pérez).

Algunos estudios han sugerido que los valores mínimos y máximos de la densidad de la EW y LW pueden servir como indicadores en los cambios climáticos de la temporada de crecimiento. El valor máximo de la densidad (D_{max}) de LW es una medida de la densidad más alta de las paredes celulares más gruesas, formadas al final de la temporada de crecimiento (Jacoby y D'Arrigo, 1992). La D_{max} se se considera uno de los mejores parámetros para la reconstrucción del clima (Briffa et al, 2004; Bräuning y Mantwill, 2004; Luckman y Wilson, 2005). Se ha utilizado para reconstruir las variaciones de la temperatura estival y los períodos de sequía en el pasado (Rigling et al, 2002; Briffa et al, 2004; Bouriaud et al, 2005). La densidad mínima (D_{min}) de la EW está vinculada a condiciones climáticas prevalentes durante la primera parte de la temporada de crecimiento (Grabner et al, 2006; Dalla-Sala et al, 2009). La sequía provoca una reducción del tamaño de la luz en las células (Olano et al, 2012), por lo que D_{min} en los anillos de los árboles podría ser una variable importante para detectar períodos de sequía tempranos. Sin embargo, ha sido una variable poco utilizada para determinar las condiciones ambientales estivales. En resumen, la anchura y densidad de cada anillo de crecimiento puede variar enormemente entre especies (Muller-Landau 2004) y, dentro de las especies) de un año a otro en función de las condiciones ambientales como son la temperatura, las precipitaciones, la disponibilidad de agua en el suelo, la luz y la duración de la temporada de crecimiento (Fritts, 1976; Zobel y van Buijtenen, 1989; Kozlowski y Pallardy, 1997; Bouriaud et al, 2005; Martínez-Vilalta et al, 2009; Fajardo, 2016).

Los órganos de la planta guardan unas proporciones en sus tamaños denominadas relaciones alométricas. Estas relaciones, indican que existe una "coordinación" en la asignación de recursos entre las distintas partes de la planta para que permite mantener la estabilidad e integridad funcional del organismo. Las relaciones alométricas tienen unos límites de variación impuestos por constricciones físicas que determinan la estabilidad estructural y los estados de mínima energía termodinámica. Por ejemplo, un árbol no puede crecer indefinidamente en altura sin crecer en grosor porque perdería su estabilidad estructural o el follaje no recibiría suficiente agua para su funcionamiento normal. Dentro de los márgenes impuestos por dichas constricciones, las relaciones alométricas pueden variar en función del ambiente (respuestas plásticas), la ontogenia y la historia evolutiva de las especies (adaptaciones) (Weiner, 2004). Los cambios durante el desarrollo ontogénico reflejan los ajustes necesarios para mantener el equilibrio funcional y mecánico con la edad. Por ejemplo, los individuos jóvenes tienen en términos relativos más hojas que los individuos más viejos, los cuales presentan una mayor proporción de órganos de sostén y raíces (Climent et al, 2011).

La proporción de follaje en la planta, estimada por ejemplo como la proporción del volumen de la copa de un árbol, puede afectar a la economía energética e hídrica de la planta. En primer lugar, porque condiciona la proporción de tejidos autótrofos y heterótrofos que, a su vez, condicionará el peso relativo de la fotosíntesis y la respiración. Esta proporción determina la disponibilidad de carbohidratos y, por tanto, la capacidad de crecimiento en el futuro (Maherali et al, 2002). En segundo lugar, porque un mayor desarrollo relativo del follaje también suele implicar mayor transpiración. A su vez, un incremento en la proporción de los troncos, ramas gruesas y raíces, es decir, de órganos de sostén, transporte y almacenamiento, pueden incrementar la resistencia y resiliencia de la planta a factores de estrés, pero con el coste de una reducción de la capacidad de crecimiento (Maherali et al, 2002). Una utilidad aplicada de las relaciones alométricas de los árboles es que son una herramienta para estimar la biomasa aérea y radical y por tanto la cantidad de carbono almacenado en los bosques (Goodman et al, 2014).

El ambiente abiótico y biótico modifica las relaciones alométricas de las plantas. La capacidad de modificar una planta sus relaciones alométricas en respuestas al ambiente puede ser importante para su persistencia. Se conocen muy bien las respuestas de asignación de masa a nivel de juveniles (Villar-Salvador et al. 2015; Markesteijn y Poorter, 2009; Poorter et al, 2012) pero son mucho menos conocidas en individuos más viejos (Vizcaino-Palomar et al, 2016). En el norte de Europa, se ha observado un aumento en la masa aérea con respecto a la altura de algunas especies de pinos por el aumento de CO_2 y deposiciones de nitrógeno (Broadmeadow y Jackson, 2000). Sin embargo, en otros estudios no se han observado modificaciones en las relaciones alométricas de los árboles (Vanninen et al, 1996; Fréderic et al, 2009). Para ambientes mediterráneos, se desconoce si el patrón en las relaciones alométricas de los árboles se ha modificado por efecto del reciente cambio global.

1.4 Estrategias adaptativas de las plantas a factores de estrés

Las plantas poseen diversos mecanismos para mitigar o prevenir el desequilibrio entre el ambiente exterior a la planta y el ambiente de sus células. En función de dichos mecanismos las plantas se pueden clasificar en dos estrategias para afrontar el estrés: 1) tolerantes, son aquellas que toleran que el estrés afecte sus tejidos; 2) evitadoras; son aquellas que desencadenan mecanismos de protección y amortiguación (Levitt, 1980; Larcher, 2003).

Los mecanismos funcionales que han desarrollado las distintas estrategias son muy variados y afectan todos los niveles de organización de la planta, desde la expresión genética hasta la configuración de los órganos en la planta. Las diferencias interespecíficas en la resistencia al estrés hídrico condicionan la distribución de las plantas (Baltzer et al, 2008; Kursar et al, 2009; Maréchaux et al, 2015).

1.4.1 Adaptaciones fisiológicas y morfológicas al estrés hídrico

Un buen número de plantas han desarrollados mecanismos para evitar (evitadores del estrés hídrico -avoiders-, según nomenclatura de Levitt, 1980) los efectos del estrés hídrico. Uno de estos mecanismo es evitar la deshidratación en los tejidos mediante mecanismos de ahorro de agua como el cierre de los estomas (Ehleringer y Cooper, 1992). Esto, sin embargo, limita la difusión del CO_2 y, por tanto, la fotosíntesis, lo que se traduce en un menor crecimiento (Fig. 6; Grant, 2012). También se puede evitar la deshidratación de los tejidos mediante la reducción de la superficie de transpiración con la abscisión de las hojas viejas durante la sequía, y almacenando agua en el xilema. En este sentido, algunas especies de pinos pierden entre el 30-50% del agua en las ramas y en los troncos antes de observarse reducciones significativas del potencial hídrico de las hojas (Larcher, 2000). Las raíces también juegan un papel importante en la capacidad de las plantas de evitar los efectos del estrés hídrico. La producción de raíces extensas y profundas permite la absorción de agua de horizontes profundos del suelo que permanecen húmedos durante los períodos secos (Jackson et al, 2000; Padilla et al, 2007; Comas et al, 2013; Andivia et al, 2017). Finalmente, los tejidos se pueden mantener hidratados mediante una elevada capacidad para conducir el agua desde las raíces al resto de la planta incrementando el área del xilema, el diámetro de los conductos del xilema y la densidad de las venas de las hojas (Zimmermann, 1983; Levitt 1980; Larcher, 2000).

Además de evitar que los tejidos se desequen, algunas plantas también pueden tolerar un cierto nivel de deshidratación del protoplasma sin sufrir mermas importantes en su funcionamiento. En casos más extremos de deshidratación, las plantas pueden detener casi por completo su metabolismo para evitar los daños por la falta de agua (Larcher, 2000). Este tipo de planta tolerante de la deshidratación, acumula algunos tipos de proteínas y carbohidratos que estabilizan las membranas celulares frente a su desnaturalización y hacen ajustes osmóticos activos, es decir, reducen el potencial osmótico celular. El ajuste osmótico permite mantener la turgencia de las células con elevadas pérdidas de agua y, por tanto, mantener una cierta capacidad de crecimiento y apertura estomática en situaciones de moderado déficit hídrico (Levitt, 1980; Larcher, 2000; Chavés et al, 2003).

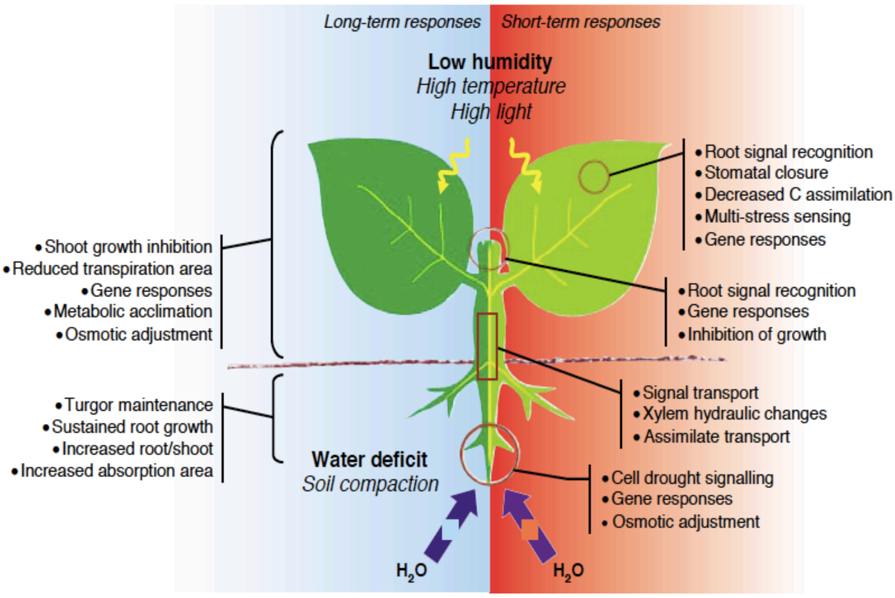


Fig. 6. Respuestas al estrés hídrico. Izquierda, respuestas a largo plazo; derecha, respuestas a corto plazo (tomado de Chavés et al, 2003).

1.4.2 Adaptaciones fisiológicas a las heladas

Las plantas de climas fríos y templados experimentan cambios estacionales en la temperatura de su ambiente. Para sobrevivir a las bajas temperaturas, desencadenan una serie de cambios fisiológicas antes de experimentar las heladas en un proceso denominado endurecimiento al frío (Levitt, 1980; Sakai and Larcher, 1987). El endurecimiento comienza al final del verano o principios del otoño y alcanza su máxima intensidad a mitad del invierno. Durante el endurecimiento, la concentración de solutos osmóticamente activos (especialmente azúcares solubles, aminoácido y pequeñas proteínas) se incrementa en el protoplasma lo que reduce levemente el punto de congelación (Li y Palta, 1978) y el potencial osmótico. Como se ha mencionado previamente, esto último permite mantener la turgencia celular en condiciones de deshidratación (Hinesley et al, 1992; Körner, 2006). Los azúcares solubles también aumentan la estabilidad de la membrana celular (Uemura y Steponkus 2003) y facilitan la reparación de las embolias (Zwieniecki y Holbrook, 2009). Finalmente, las plantas usan los azúcares solubles almacenados para llevar a cabo funciones metabólicas en el invierno y llevar a cabo el crecimiento en la primavera (Uscola et al. 2015). En consecuencia, muchas plantas de climas fríos y templados aumentan la concentración de azúcares durante la aclimatación a las heladas en el otoño, llegando a niveles máximos en pleno invierno, que luego se reducen en primavera y verano con la desaclimatación al frío (Sakai y Larcher 1987, Martínez-Vilalta et al. 2016). La magnitud de los cambios fisiológicos durante el endurecimiento difiere entre especies, lo que condiciona la capacidad de colonizar los distintos ambientes (Sakai and Larcher, 1987; Bigras y Colombo, 2000; Walther et al, 2002; Cavender-Bares, 2005).

1.5 Ecología, distribución y evolución de las especies de *Pinus* nativas de la Península Ibérica

Las coníferas ocupan ambientes muy diversos en la Tierra destacando su capacidad de colonizar los lugares más fríos del planeta donde la temperatura habitualmente pueden llegar a -30 ºC (Sakai, 1983; Bannister and Neuner, 2000). La familia *Pinaceae*, con 11 géneros, es la más grande en especies de las coníferas (Christenhusz et al, 2011). El género con más especies es *Pinus* que, de manera natural, se distribuye en el Hemisferio Norte. Se han descrito entre 110 y 120 especies de pinos (Farjon, 1998; Liston et al, 2003; Price et al. 1998) que colonizan ambientes muy variados, desde lugares áridos o muy fríos, hasta ecosistemas tropicales (Mirov, 1967).

Los registros fósiles sugieren que el antecesor de los pinos actuales, *Pinus belgica*, evolucionó en el actual oriente asiático durante el Jurásico, aunque se han encontrado registros poco fiables en el Triásico y el Cretácico (Millar, 1993; Mirov, 1967; Stewart y Rothwell, 1993). Durante el Cenozoico, el género *Pinus* extiendió su área de distribución y se diversificó. Se cree que entraron en Europa por Groenlandia, Islandia o las Islas Spitzbergen (Ruiz de la Torre y Ceballos, 1979; Gil-Sánchez et al, 1996). Los primeros restos fósiles de pinos en el área mediterránea datan del periodo comprendido entre el Cretácico y el Terciario (Klaus, 1989). La diversificación del género en el Mediterráneo se debió dar principalmente durante el Terciario (Gil-Sánchez et al, 1996).

En Europa, las especies nativas de pinos se pueden agrupar en tres grupos en función de la región que ocupan: la atlántica, la centroeuropea y la mediterránea (Richardson, 2000). La distribución espacial de los pinos europeos aparece segregada a lo largo de gradientes latitudinales y altitudinales de una manera predecible, lo que sugiere que algunos factores ambientales abióticos podrían controlar su distribución. Algunas especies, concretamente *Pinus halepensis* Mill, *Pinus pinea* L. y *Pinus pinaster* Aiton, se concentran en el sur de Europa, particularmente en sitios de clima mediterráneo con inviernos suaves y veranos calurosos y secos. Otras especies, como *Pinus nigra* subsp. *salzamannii* (Dunal) Franco, (en adelante *P. nigra*), *Pinus sylvestris* L. y *Pinus uncinata* Raymond ex A. D.C. se encuentran en sitios con inviernos fríos, ya sea por estar elevada latitud (en el centro y norte de Europa) o por estar a gran altitud (Europa meridional, incluida Península Ibérica) (Richardson, 2000).

Para España se han descrito siete especies con poblaciones naturales (Tabla 2, Fig. 7), que se separan en dos subsecciones. La primera es la subsección *Pinaster* formada por *Pinus canariensis* C. Sm. ex DC., *Pinus halepensis* Mill, *Pinus pinea* L. y *Pinus pinaster* Aiton. La segunda es la subsección *Pinus* constituida por *Pinus nigra* subsp. *salzamannii* (Dunal) Franco, (en adelante *P. nigra*), *Pinus sylvestris* L. y *Pinus uncinata* Raymond ex A. D.C. (Gernandt et al, 2005). Todas las especies tienen poblaciones naturales en la Península Ibérica (Fig. 6) excepto *P. canariensis* que es un endemismo de las Islas Canarias. Las masas naturales de estas especies se localizan principalmente en la cordillera Pirenaica e Ibérica, Serranía de Segura, montañas catalanas, sureste semiárido y su litoral (Ruiz de la Torre, 1990), a lo largo de un gradiente altitudinal entre 0 y 2700 msnm (Amaral, 1986). Cabe mencionar que la Península Ibérica es el límite latitudinal para las especies eurosiberianas *P. uncinata* (Ceballos, 1968) y *P. sylvestris* (Sinclair et al, 1999) ésta última con poblaciones muy grandes en Centroeuropa y Fenoscandia.

Las masas naturales de las distintas especies de pinos de la Península Ibérica se segregan a lo largo de un gradiente altitudinal entre el nivel del mar y 2700 m s.n.m. (Blanco et al, 2005). Las especies típicamente mediterráneas (*P. halepensis* y *P. pinea*) se distribuyen desde el nivel del mar hasta aproximadamente los 1000 m s.n.m. *Pinus pinaster*, que tiene poblaciones tanto en ambientes mediterráneos como atlánticos, se distribuyen desde el nivel del mar hasta los 1700 m s.n.m (Fig. 8). A bajas altitudes los inviernos son suaves y húmedos pero los veranos son cálidos y muy secos (Tabla 2) lo que ocasiona un fuerte déficit de agua en el suelo y en la atmósfera (Bolle, 2003). Dichas condiciones ocasionan un fuerte estrés hídrico y térmico a la vegetación. Por último, las especies de climas continentales húmedos y alpinos (*P. nigra*, *P. sylvestris* y *P. uncinata*) se localizan aproximadamente a partir de los (800)1000 m s.n.m. hasta los 2700 m s.n.m. En estos sitios de alta montaña el principal factor de estrés es el frío. La precipitación es más alta y la evapotranspiración menor y, por tanto, la sequía de verano es más corta y de intensidad moderada en relación con los sitios emplazados a menor altitud (Tabla 2, Fig. 8).

Por lo tanto, en la Península Ibérica y en el resto del sur de Europa las especies de pino se distribuyen a lo largo de un gradiente de aridez y de temperaturas que posiblemente refleje diferencias funcionales entre las especies.

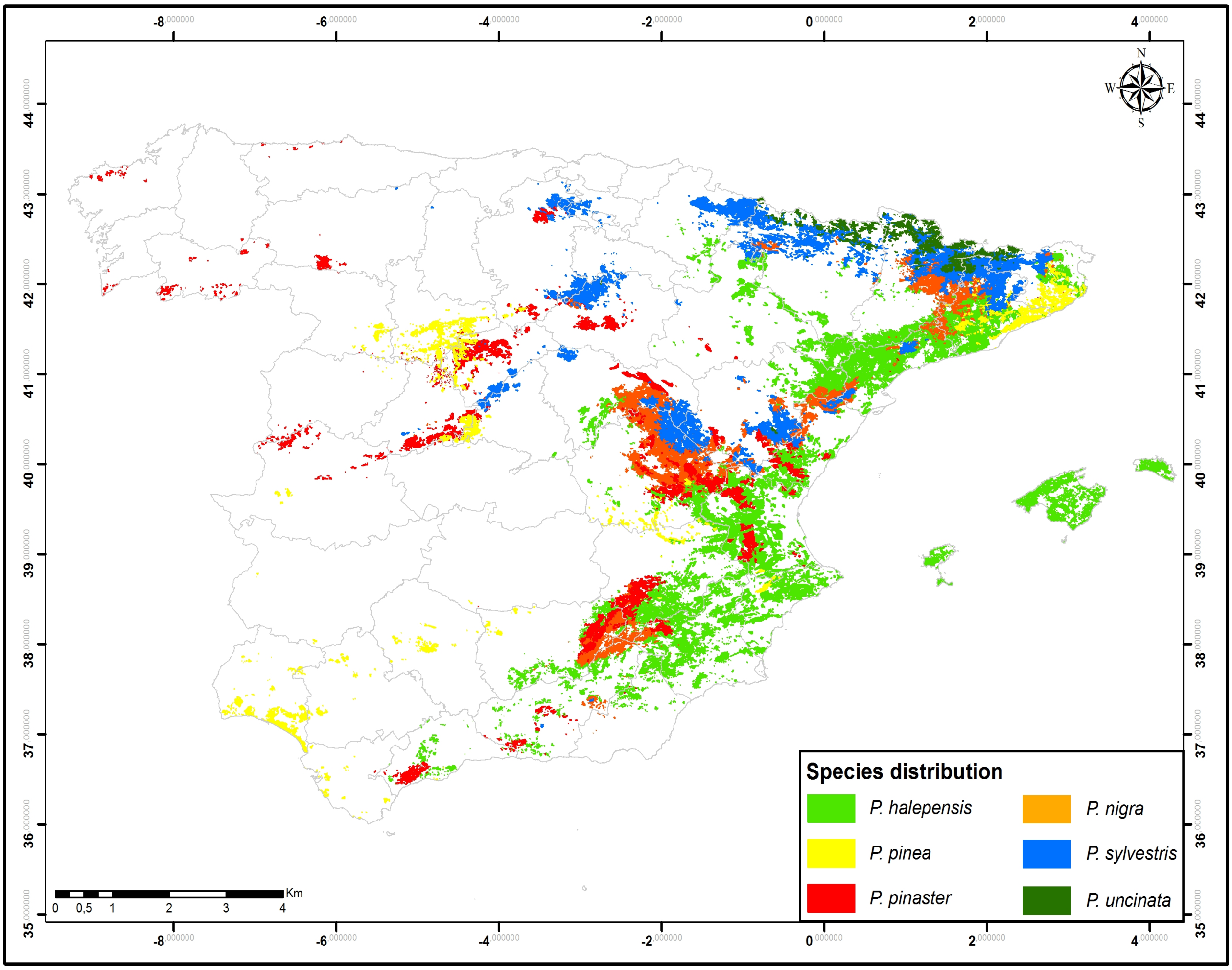


Fig. 7. Distribución natural de las masas forestales de las seis especies de pino en España. (Elaborado por Denis Conrado Da Cruz con datos obtenidos del IFN3).

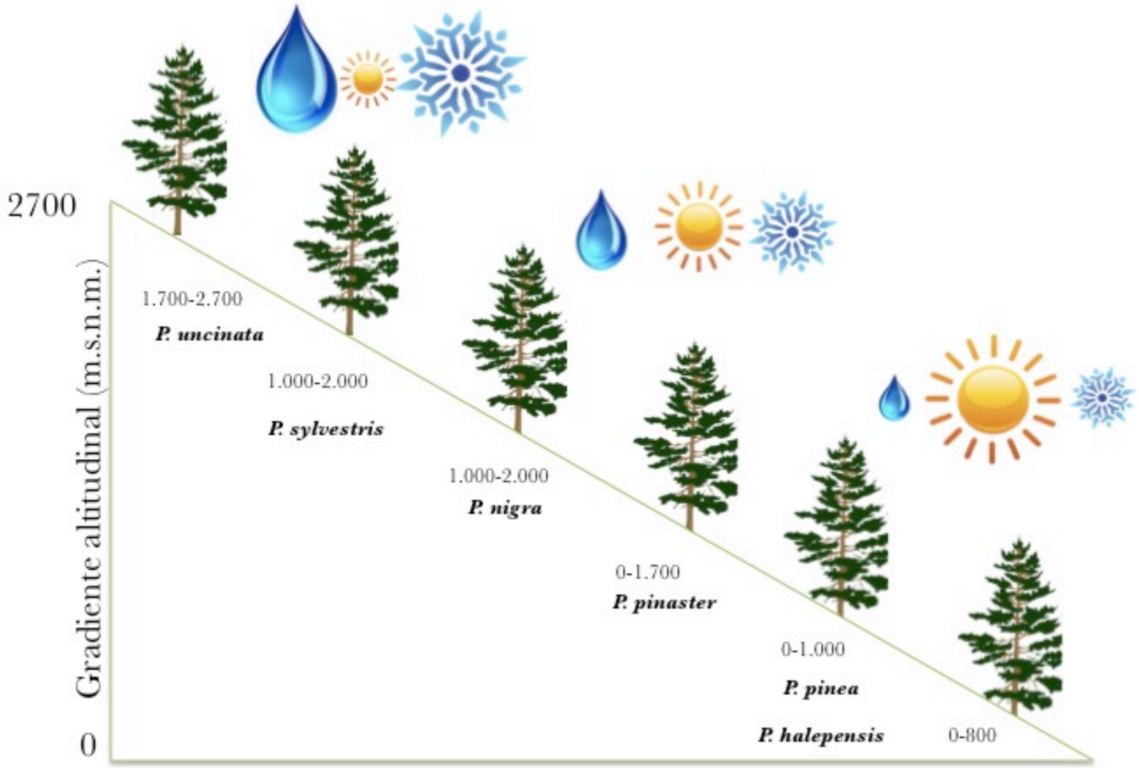


Fig. 8 Esquema de la distribución altitudinal de las especies del género *Pinus* en la Península Ibérica. Los símbolos representan la magnitud de los principales factores ambientales a los que están expuestas las especies en el gradiente altitudinal

El suelo también parece que también condiciona la distribución de los pinos. En concreto, las poblaciones naturales de *P. pinaster* y *P. pinea* son raras en suelos ricos en cal activa, mientras que las poblaciones naturales de *P. halepensis* son infrecuentes en suelos silíceos (Barbero et al, 1998; Costa et al, 1998; Ruiz de la Torre, 2006). El resto de las especies parece ser independiente de las propiedades químicas del suelo. Aunado a los factores abióticos, la distribución de algunas especies forestales se ha visto afectada por la actividad humana, en particular, el cambio de uso de la tierra, las reforestaciones y el abandono de las tierras agrícolas. Como resultado, algunas especies de pinos han aumentado su área de distribución en la Península Ibérica (Huntley y Birks, 1983; Delcourt y Delcourt, 1987; Ruiz de la Torre, 1990). Algunas poblaciones de los pinos ibéricos han presentado una alta mortalidad en los últimos 20 años, siendo las causas probables los cambios en el manejo forestal, cambios en el clima (aumento de temperatura) y la estructura de los bosques (Camarero et al, 2015), que a menudo presenta elevadas densidades ligadas a menudo a la forma de regeneración de las especies las repoblaciones forestales.

Tabla 2. Características ecológicas de las especies de *Pinus sp.* en la Península Ibérica (S/D: Sin determinar).

Especie	Antecesor	Época de aparición (1,2)	Tº media anual (ºC, 1,6)	Precipitación (mm)
P. halepensis	*P. herpios* (2)	Holoceno/ Eoceno	14	350 -700 (5)
P. pinea	S/D	Pal. medio	11.7-17.	623 (8)
P. pinaster	P. *praepinaster* (3)	Holoceno	11.8	390-1810 (7)
P. nigra	*P. laricioides* (4)	Mioceno y Plioceno	9.2-13	500-950 (8)
P. sylvestris	S/D	Plioceno	9.4	963 (8)
P. uncinata	S/D	S/D	2	1400 (8)

[1]Alía et al (2009); [2]Palamarev (1987); [3]Willis et al. (1998); [4]Vernet et al. (2005); [5]Gil et al. (1996); [6]Ruiz de la Torre y Ceballos (1979); [7]Alía et al. (1996); [8]Martín et al. (1998); [9]Martínez-García (1999).

En los últimos 30 años se ha avanzado enormemente en la comprensión de las estrategias funcionales de las especies mediterráneas (por ejemplo, Tenhunen et al 1987; Tretiach, 1993; Valladares et al, 2000; Martínez-Vilalta et al, 2002; Mediavilla y Escudero, 2003; Vilagrosa et al, 2003; Quero et al, 2006). Sin embargo, la mayor parte de estos trabajos se han centrado en frondosas habiendo muy poca información sobre las respuestas funcionales de los pinos ibéricos frente a los factores ambientales abióticos. La escasa información experimental sobre las diferencias comparadas de la ecofisiología de los pinos ibéricos se ha centrado sobre biología de las semillas (Escudero et al, 1999), respuesta de la fotosíntesis a la luz (Awada et al, 2003), hidráulica (Martínez-Vilalta et al, 2004) y, más recientemente, diferencias de resistencia a la helada y transpiración cuticular (Climent et al, 2009; Pardos et al, 2009), y la asignación de masa (Climent et al, 2011; Matías et al, 2017). Este conocimiento es muy importante para establecer una base ecológica sólida y fundamentada empíricamente de la distribución actual de las poblaciones naturales de las especies ibéricas de pino, predecir su potencial distribución y vulnerabilidad en escenarios climáticos y diseñar estrategias de mitigación contra el cambio climático.

1.6 Influencia del cambio global en la distribución de las especies

El cambio climático consiste en una desviación significativa y prolongada en el tiempo de la temperatura y/o la precipitación a escala regional y global con respecto a la media histórica previa. El último gran cambio climático ocurrido en la Tierra ha sido el calentamiento después de la última era glaciar. Estas condiciones climáticas post-glaciares han determinado en buena medida la distribución de la flora y el funcionamiento de las comunidades de plantas actuales. Durante el siglo XX, especialmente en los últimos 40 años, se ha producido un cambio a escala global en la temperatura y en la disponibilidad de algunos recursos claves para los organismos (agua, por cambios en el régimen de precipitación, CO_2 atmosférico y deposición atmosférica de nitrógeno -N-). La temperatura global ha aumentado en promedio 0.85 ºC (0.65-1.06 ºC) en el periodo 1880- al 2012 (IPCC, 2014). Estos cambios ambientales están en buena medida ligadas a la actividad del ser humano (Fig. 9; Christensen et al, 2007) y están afectando el funcionamiento y estructura de los ecosistemas forestales (Parmesan y Yohe, 2003; Churkina et al, 2010; IPCC, 2014, Pachauri et al, 2014).

En consecuencia, los estreses que sufren las plantas en algunos ecosistemas forestales se están agudizando (por ejemplo, el estrés hídrico), mientras que en otros se están relajando (estrés térmico por frío y nutricional). El cambio climático modifica la distribución de las especies (Parmesan y Yohe, 2003). En el norte y centro de Europa, algunas poblaciones de *P. sylvestris* han colonizado sitios a mayor latitud y altitud (Bolker et al, 1995; Koutavas, 2008; Lamarche et al, 1984), y los individuos han incrementado su crecimiento (Pretzsch et al, 2014). Se cree que ello ha podido deberse principalmente al aumento del CO_2 atmosférico, la deposición de N y de la temperatura (Briffa et al, 1995; Pretzsch et al, 2014). Además, el cese de algunas actividades humanas como la ganadería ha podido favorecer que las poblaciones de pinos han podido expandirse y recuperarse en los sitios localizados a mayores altitudes (Gehrig-Fasel et al, 2007). Por el contrario, otros estudios han evidenciado un decaimiento en el crecimiento de los árboles (Oberhuber, 2004). El patrón de respuesta fisiológica de las plantas a los factores ambientales asociados al cambio climático (CO_2 y T) no es claro. El aumento en la concentración de CO_2, la deposición de N y la temperatura pueden mejorar la fotosíntesis, pero tienen efectos variables en la conductancia estomática. Cuando la fotosíntesis aumenta más rápido que la conductancia estomática puede incrementarse la eficiencia en el uso del agua (Morison 1985; Norby et al, 1999; Ainsworth y Rogers 2007), como lo muestran algunos experimentos (Gunderson et al. 1993; Norby et al. 2005).

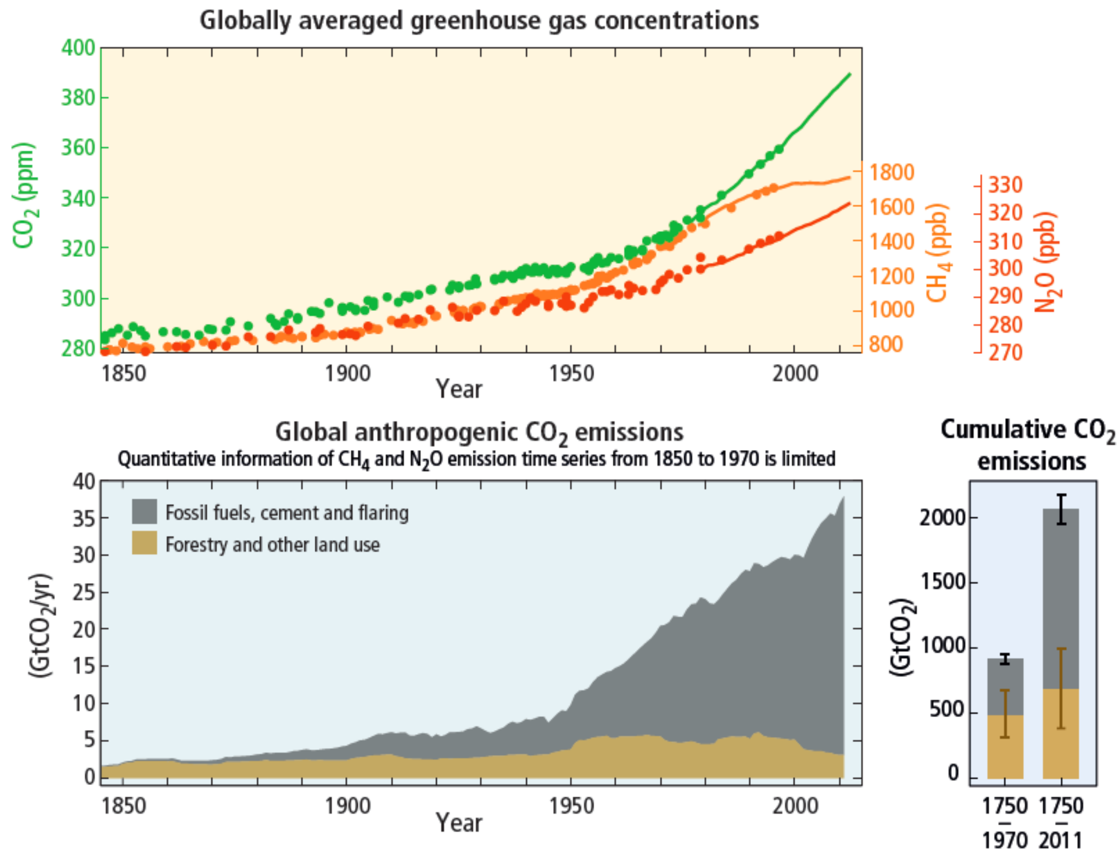


Fig. 9. Arriba: aumento en las concentraciones de gases de efecto invernadero: CO_2 (color verde), metano (color naranja), óxido nitroso (color rojo) determinados por núcleos de hielo (puntos) y medidas directas de la atmosfera (líneas). Panel derecho: efecto global de la acumulación de CH_4 y N_2O. Abajo. Emisiones antropogénicas globales de CO_2 por la selvicultura y otros cambios de uso de suelo, quema de combustibles fósiles, producción de cemento. Lado derecho: emisiones acumuladas por CO_2 por estas fuentes (barras) y sus incertidumbres (barras de significación) (Fuente: IPCC, 2014).

En paralelo al incremento de CO_2, la frecuencia de sequías ha aumentado durante de las últimas décadas del siglo XX (Resco De Dios et al, 2007; Wallace et al, 2007). Algunos estudios sugieren que el efecto del estrés hídrico puede anular los efectos positivos de la fertilización por CO_2 (Körner y Miglietta 1994; Camarero et al, 2015b). Por tanto, no existe un consenso claro si la “fertilización” por CO_2 y N pueden compensar los costos del incremento de la aridez. En concreto, en climas mediterráneos no hay un consenso sobre el efecto del cambio climático en el crecimiento de las especies forestales. Mientras que algunos estudios observacionales han reportado respuestas neutras (Peñuelas et al, 2011) e incluso negativas del crecimiento con el tiempo (que en este caso se usa como sustituto o correlato del incremento del CO_2) (Linares and Camarero 2012), otros apuntan a efectos positivos (Koutavas 2008; Madrigal-González et al. 2015; Marqués et al, 2016). Por ejemplo, se observó una reducción en el crecimiento asociado al aumento de la temperatura en *P. pinaster*, mientras que el incremento de CO_2 lo aumentó (Kramer et al, 2000).

En conclusión, el efecto de estreses ambientales abióticos en las respuestas fisiológicas, morfológicas y de crecimiento son variables entre las especies. Las respuestas variables entre las especies pueden estar condicionando su crecimiento, supervivencia y, por tanto, su distribución.

2. OBJETIVOS DE LA TESIS Y ESTRUCTURA DE LA MEMORIA DE LA TESIS DOCTORAL

El **objetivo general** de la tesis doctoral es estudiar las respuestas fisiológicas y de crecimiento a las bajas temperaturas y sequía en las especies de pinos nativas de la Península Ibérica. Las cuestiones que se plantean abarcan multitud de procesos ecológicos (supervivencia, crecimiento, tolerancia al frío y estrés hídrico) en diferentes niveles de organización (desde la bioquímica al individuo), escalas espaciales (local y regional), y estadios ontogenéticos (desde juveniles a árboles maduros).

La **hipótesis general** de la tesis es que las especies de pinos tienen respuestas funcionales distintas a los factores ambientales abióticos lo que condiciona su distribución y su capacidad de respuesta a factores de estrés.

La Memoria de esta tesis doctoral comprende siete capítulos. Tras el capítulo introductorio (**Capítulo 1**), se presentan cuatro capítulos con los resultados de un experimento y tres estudios observacionales (**Capítulos 2-5**). Tres de estos capítulos corresponden con artículos científicos publicados o en revisión en revistas científicas internacionales, mientras que el cuarto está prácticamente finalizado, pero todavía no se ha enviado para su publicación. La estructura de los capítulos se ha mantenido fiel a la versión publicada/enviada. En todos los casos están escritos en inglés excepto un resumen añadido en español. A continuación, se desarrolla una discusión general que integra los resultados de toda la investigación (**Capítulo**

6). Para terminar, se presentan las conclusiones más relevantes de esta tesis doctoral en el **Capítulo 7**.

Capítulo 2 "La distribución de los pinos en la Península Ibérica concuerda con las diferencias en la tolerancia a las heladas en hojas, no con la vulnerabilidad a la formación de embolias en el xilema causadas por las heladas". Las preguntas que se han formulado son: ¿Tienen las especies de pino que viven en lugares fríos una mayor tolerancia inherente a las heladas que las especies que viven en lugares templados? En caso de haber diferencias ¿Están determinadas por diferencias en la vulnerabilidad a la formación de embolias, la tolerancia a las heladas de las acículas y la concentración de azúcares solubles? ¿Cuál es el efecto del estrés hídrico sobre la tolerancia a las heladas? ¿lo incrementa? Para responder a estas preguntas Se llevo a cabo un experimento en el que se midieron la supervivencia a las heladas **(objetivo específico 1)**, las diferencias en la formación de embolias, el daño en las acículas y la concentración de azúcares solubles en plántulas de dos años de seis especies de pinos **(objetivo específico 2)** y la interacción entre el estrés hídrico y las heladas **(objetivo específico 3)**. La hipótesis de este trabajo es que vulnerabilidad a la formación de embolias, el daño en las acículas y el contenido de azúcares solubles entre las especies de pino son distintas para cada una y depende del estado hídrico de las plantas.

Capítulo 3. "La densidad mínima de la madera de las coníferas describe cambios en la precipitación durante la época temprana de crecimiento en regiones Eurasiáticas secas y frías" ¿Se incrementa la densidad mínima de la madera bajo condiciones de sequía temprana? ¿Cómo responden las características anatómicas de la madera (anchura de los anillos y densidad) a las precipitaciones? ¿Puede la densidad mínima de la madera ser un buen indicador de las precipitaciones de primavera? Para ello, se analizaron datos de densidad de la madera y la anchura de los anillos de crecimiento en dos géneros de coníferas (*Larix sibirica*, *Pinus nigra* y *Pinus sylvestris*) para determinar como el crecimiento estacional y la densidad de la madera responden al clima **(objetivo específico 1)**, y si la densidad mínima de la madera está relacionada negativamente con las precipitaciones de primavera **(objetivo específico 2)**. La hipótesis del trabajo es la densidad mínima de la madera aumenta en condiciones de sequía estacional (primavera).

Capítulo 4 "Crecimiento divergente del último siglo a lo largo de un gradiente altitudinal en poblaciones de *Pinus sylvestris* localizadas en el extremo seco de la distribución de la especie". Las preguntas de este capítulo han sido: ¿Ha cambiado el crecimiento de *Pinus sylvestris* en paralelo al aumento en la concentración de CO_2 y las deposiciones de nitrógeno en el último siglo? En caso de haber diferencias, ¿las condiciones de crecimiento a lo largo de un gradiente altitudinal y la edad de los árboles pueden modular dicho patrón de crecimiento? Para contestar dichas preguntas, se examinaron datos de crecimiento radial decadal desde 1930 hasta 2010 para determinar si el crecimiento de los pinos se ha acelerado durante el último siglo y si esta aceleración cambia a lo largo de gradientes ambientales y ontogenéticos (**objetivo específico 1**). La hipótesis de este trabajo es que el crecimiento de los árboles se ha modificado diferencialmente a lo largo del gradiente altitudinal a consecuencia del cambio climático y estos cambios serán distintos dependiendo de la edad de los árboles.

Capítulo 5. "Factores que determinan la alometría aérea de los árboles de *Pinus sylvestris*". ¿Han cambiado las relaciones alométricas de *Pinus sylvestris* por efecto del cambio climático durante el último siglo? ¿Existen cambios en las relaciones alométricas dependiendo de la edad de los árboles? ¿Las relaciones alométricas son diferentes a través del gradiente altitudinal? Se examinaron las relaciones alométricas entre la altura y el diámetro a la altura del pecho (DBH), así como la relación entre el DBH y la copa de los árboles desde 1930 hasta 2010. Lo anterior se realizó para determinar si algunas relaciones alométricas de los árboles relacionadas con la economía hídrica y de carbono han cambiado durante el último siglo y si estos cambios ocurren a lo largo de gradientes ambientales y ontogenéticos (**objetivo específico 1**). La hipótesis de este trabajo es que las relaciones entre la altura y el DBH, así como las relaciones entre el DBH y la copa de los árboles se han modificado diferencialmente debido al cambio climático.

REFERENCIAS

Ainsworth E, Rogers A (2007) The response of photosynthesis and stomatal conductance to rising [CO_2]: Mechanisms and environmental interactions. Plant, Cell and Environment. 30:258–270.

Alía MR, Martín AS, De Miguel DAJ, Galera PRM, Agúndez LD, Gordo AJ, Salvador NL, Catalán BG, Gil SLA (1996) Regiones de procedencia *Pinus pinaster* Aiton. Dirección General de Conservación de la Naturaleza, Madrid. 75 p.

Alía R, Garcia del Barrio JM, Iglesias S, Mancha JA, De Miguel J, Nicolas JL, Sánchez de Ron D (2009) Regiones de procedencia de especies forestales en España. Organismo Autónomo Parques Nacionales, Madrid, 363 pp.

Amaral FJ (1986) *Pinus* L. En: Castroviejo S et al, (eds). Flora Ibérica. 1: 168-174. Madrid.

Andivia E, Zuccarini P, Grau B, De Herralde F, Villar-Salvador P, Savé R (2017). Rooting big and deep rapidly: the ecological roots of pine species distribution in southern Europe. Functional Ecology. En revisión.

Awada T, Radoglou K, Fotelli MN, Constantinidou HIA (2003) Ecophysiology of seedlings of three Mediterranean pine species in contrasting light regimes. Tree Physiology. 23:33-41.

Bannister P, Neuner G (2000) Frost resistance and the distribution of Conifers. In: Bigras, F. Colombo, S. Conifer Cold Hardiness. Springer Sciece and Business Media. 3-21 pp.

Barbero M, Loisel R, Quézel P, Richardson DM, Romane F (2006). Pines in Mediterranean Basin. En: Richardson R (ed). Ecology and Biogeography of *Pinus*. 153-170.

Baltzer JL, Davies SJ, Bunyavejchewin S, Noor NSM (2008) The role of desiccation tolerance in determining tree species distributions along the Malay–Thai Peninsula. Functional Ecology. 22:221-231.

Beck E, Heim R, Hansen J (2004) Plant resistance to cold stress: mechanisms and environmental signals triggering frost hardening and dehardening. Journal of Bioscience. 29, 449–59.

Beck EH, Fetting S, Knake C, Hartig K, Bhattarai T (2007) Specific and unespecific response of plants to cold and drought stress. Journal of Biosciences. 32(3):501-510.

Bigras FJ, Colombo SJ (2000) Conifer Cold Hardiness. Springer Science and Business Media, B.V. 1189 pp.

Blanco CE, Casado GMA, Costa-Tenorio M, Escribano BR, García AM, Génova FM, Gómez MA, Gómez MF, Moreno SJC, Morla JC, Regato PP, Sainz OH (2005) Los bosques ibéricos. Una interpretación geobotánica. Tercera reimpresión. Ed. Planeta, Barcelona. 597 pp.

Bolker B, Pacala S, Bazzaz F, Canhams CD, Levin SA (1995) Species diversity and ecosystem response to carbon dioxide fertilization: conclusions from a temperate forest model. Global Change Biology. 1:373–381.

Bolle H (2003) Mediterranean Climate Variabilty and trends. Springer. 36 p.

Bouche PS, Larter M, Domec JC, Burlett R, Gasson P, Jansen S, Delzon S (2014) A broad survey of hydraulic and mechanical safety in the xylem of conifers. Journal of Experimental Botany. 65(15):4419-4431.

Bouriaud O, Leban JM, Bert D, Deleuze C (2005) Intra-annual variations in climate influence growth and wood density of Norway spruce. Tree Physiology. 25: 651-600.

Bräuning A, Mantwill B (2004) Summer temperature and summer monsoon history on the Tibetan Plateau during the last 400 years recorded by tree rings. Geophysical Research Letters. 31, L24205.

Briffa KR, Jones PD, Schweingruber FH, Shiyatov SG, Cook ER (1995) Unusual twentieth-century summer warmth in a 1000-yr temperature record from Siberia. Nature, 376: 156-159.

Briffa KR, Osborn TJ, Schweingruber FH (2004). Large-scale temperature inferences from tree rings: a review. Global Planetary Change, 40(1–2): 11–26.

Broadmeadow MSJ, Jackson SB (200) Growth responses of *Quercus petraea*, *Fraxinus excelsior* and *Pinus sylvestris* to elevated carbon dioxide, ozone and water supply. New Phytologist. 146(3): 437-451.

Camarero JJ, Gazol A, Sancho-Benages S, Sangüesa-Barreda G (2015a) Know your limits? climate extremes impact the range of Scots pine in unexpected places. Annals of Botany. 1-11.

Camarero J, Gazol A, Tardif J, Conciatori F (2015b) Attributing forest responses to global-change drivers: Limited evidence of a CO_2-fertilization effect in Iberian pine growth. Journal of Biogeography. 42:2220–2233.

Cavender-Bares J, Cortes P, Rambal S, Joffre R, Miles B, Rocheteau A (2005) Summer and winter sensitivity of leaves and xylem to minimum freezing temperatures: A comparison of co-occurring Mediterranean oaks that differ in leaf lifespan. New Phytologist. 168:597–612.

Ceballos L (1968) Una nueva localidad española de *Pinus uncinata* Ram. Collectane Botanica (VII, I, 9), 213-220.

Charrier G, Charra-Vaskou K, Kasuga J, Cochard H, Mayr S, Améglio T (2014) Freeze-thaw stress: effects of temperature on hydraulic conductivity and ultrasonic activity in ten woody angiosperms. Plant Physiology. 164, 992–8.

Chave J, Coomes D, Jansen S, Lewis SL, Swenson NG, Zanne AE (2009) Towards a worldwide wood economics spectrum. Ecology Letters, 12:351-366.

Chavés MM, Maroco JP, Pereira JS (2003) Understanding plant responses to drought-drom genes to the whole plant. Functional Plant Biology. 30(3): 239-264.

Christenhusz M, Reveal J, Farjon A, Gardner M, Mill R, Chase M (2011) A new classification and linear sequence of extant gymnosperms. Phytotaxa 19, 55–70.

Christensen JH, Hewitson B, Busuioc A, Chen A, Gao X, Held I, Jones R, Kolli RK, Kwon W-T, Laprise R, Magaña-Rueda V, Mearns L, Menéndez CG, Räisänen J, Rinke A, Sarr A, Whetton P (2007) Regional Climate Projections. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds.) Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Churkina G, Zaehl S, Hughes J, Viovy N, Chen Y, Jung M, Heumann BW, Ramankutty N, Heimann M, Jones C (2010) Interactions between nitrogen deposition, land cver conversion, and climate change determine the contemporary carbon balance of Europe. Biogeosciences, 7:2749-2764.

Climent J, Costa e Silva F, Chambel M, Pardos M, Almeida M (2009) Freezing injury in primary and secondary needles of Mediterranean pine species of contrasting ecological niches. Annals of Forest Science. 66, 407–407.

Climent J, Chambel MR, Pardos M, Lario F, Villar-Salvador P (2011). Biomass allocation and foliage heteroblasty in hard pine species respond differentially to reduction in rooting volume. European Journal of Forest Research.

Comas LH, Becker SR, Cruz VMV, Byrne PF, Dierig DA (2013). Root traits contributing to plant productivity under drought. Frontiers in Plant Science, 4(November), 442.

Costa TMC, Morla C, Sainz H (1997) Los bosques ibéricos. Una interpretación geobotánica. Planeta. Barcelona. 600p.

Dalla-Sala G, Martínez-Mier A, Cochard H, Rozenberg P (2009) Variation of wood density and hydraulic properties of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) clones related to a heat and drought wave in France. Forest Ecology and Management. 257:182-189.

Davis SD, Sperry JS, Hacke UG (1999) The relationship between xylem conduit diameter and cavitation caused by freezing. American Journal of Botany. 86(10):1367-1372.

Delcourt PA, Delcourt HR (1987) Long-term forest dynamics of the temperate zone: a case of study of Late-Quaternary forests in eastern North America. Ecological Studies 63. Springer, New York.

Ehleringer JR, Cooper TA (1992) On the role of orientation in reducing photoinhibitory damage in photosynthetic-twig desert shrubs. Plant, Cell and Environment. 15(3):301-306.

Escudero A, Sanz MV, Pita JM, Pérez-García, F (1999). Probability of germination after heat treatment of native Spanish pines. Annales des Sciences Forestières. 56:511-520.

Evert F (2006) Esau Anatomia vegetal. Mersitemas, células y tehidos de las plantas: su estructura, función y desarrollo. Tercera edición

Fajardo A (2016) Wood density is a poor predictor of competitive ability among individuals of the same species. Forest ecology and Management. 372: 217-225.

Farjon A (1998) World Checklist and Bibliography of Conifers. Royal Botanic Gardens, Kew.

Feild T, Brodribb T (2001) Stem water transport and freeze-thaw xylem embolism in conifers and angiosperms in a Tasmanian treeline heath. Oecologia. 127, 314–320.

Frédéric D, González G, Céline M (2009) Phenotypic plasticity of *Pinus pinaster* to water stress: biomass allocation and root architecture. International Symposium "Root Research and Applications". Vienna, Austria .

Fritts HC (1976). Tree Rings and Climate. New York: Academic Press. 567 pp.

Gehrig-Fasel J, Guisan A, Zimmermann NE (2007) Tree line shifts in the Swiss Alps: Climate change or land abandonment? Journal of Vegetation Science. 18(4):571-582.

Gernandt D, Geada L, Ortíz G, Liston A (2005) Phylogeny and classification of *Pinus*. Taxon. 54, 29–42.

Gil-Sánchez L, Díaz-Fernández PM, Jiménez MP, Roldan M, Alía R, De Miguel J, Martín S, De Tuero M (1996) Regiones de procedencia de *Pinus halepensis* Mill. en España. Organismo Autónomo Parques Nacionales, Madrid. 97 pp.

Goodman RC, Phillips OL, Baker TR (2014) The importance of crown dimensions to improve tropical tree biomass estimates. Ecological Applications. 24(4): 680-698.

Grabner M, Cherubini P, Rozenberg P, Hannrup B (2006) Summer drought and low earlywood density induce intra-annual radial cracks in conifers cracks in conifers. Scandinavian Journal of Forest Research. 21:151-157.

Grant RF, Baldocchi D, Ma S (2012) Ecological controls on net ecosystem productivity of a seasonally dry annual grassland under current and future climates: modelling with ecosys. Agricultural and Forest Meteorology. 152(1):189-200.

Gunderson C, Norby R, Wullschleger S (1993) Foliar gas exchange responses of two deciduous hardwoods during 3 years of growth in elevated CO_2: no loss of photosynthetic enhancement. Plant, Cell and Environment. 16:797–807.

Hacke UG, Sperry JS (2001) Functional and ecological xylem anatomy. Perspectives in Plant Ecology, Evolution and Systematics. 4(2):97-115.

Hacke UG, Sperry JS, Pockman WT, Davis SD, McCulloh KA (2001) Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. Oecologia. 126:457-461.

Hinesley LE, Pharr DM, Snelling LK, Funderburk SR (1992) Foliar raffinose and sucrose in four conifer species: relationship to seasonal temperature. Ecological Applications. 117(5):852-855.

Hsiao TC (1973) Plant responses to water stress. Annual Review of Plant Physiology. 24:519-570.

IPCC (2014) Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.

Jackson RB, Sperry JS, Dawson TE (2000) Root water uptake and transport: using physiological processes in global predictions. Trend in Plant Science. 5(11):1360-1385.

Jacoby GC, D'Arrigo RD (1995). Tree ring width and density evidence of climatic and potential forest change in Alaska. Global Biogeochemical Cycles. 9(2): 227-234.

Klaus W (1989) Mediterranean pines and their history. Plant Systematics and Evolution. 162, 133–163.

Körner C, Miglietta F (1994) Long term effects of naturally elevated CO_2 on mediterranean grassland and forest trees. Oecologia 99:343–351.

Körner C (2006) Significance of temperature in plant life. In: Plant growth and climate change. Ed. Morison JIL, Morecroft MD. Blackwell publishing. 48-69 pp.

Koutavas A (2008) Late 20th century growth acceleration in greek firs (*Abies cephalonica*) from Cephalonia Island, Greece: A CO_2 fertilization effect? Dendrochronologia 26:13–19.

Kozlowski TT (1971) Growth and development of trees. Academic Press, New York.

Kozlowski T, Kramer P, Pallardy S (1991) The Physiological Ecology of Woody Plants. Academic Press, Inc. 657 p.

Kozlowski TT, Pallardy S (1997) Growth control in woody plants. Academic Press . 641 pp.

Kramer PJ, Kozlowski TT (1979) Physiology of woody plants. Academy Press. Nueva york. 1979.

Kramer K, Leinonen I, Loustau D (2000) The importance of phenology for the evaluation of impact of climate change on growth of boreal, temperate and Mediterranean forest ecosystems: an overview. International Journal of Biometeorology. 44:67-75.

Kursar TA, Engelbrecht BMJ, Burke A, Tyree MT, EI Omari B, Giraldo JP (2009) Tolerance to low leaf water status of tropical tree seedlings is related to drought performance and distribution. Functional Ecology. 23:93–102.

Lamarche VC, Graybill DA, Fritts HC, Rose MR (1984) Increasing atmospheric carbon dioxide: tree ring evidence for growth enhancement in natural vegetation. Science. 225(4666):1019-1021.

Lambers H, Chapin FS, Pons TL (2008) Plant Physiological Ecology. Springer New York, New York, NY.

Larcher W (2000) Temperature stress and survival ability of Mediterranean sclerophyllous plants. Plant Biosystems. 134(3):279-295.

Larcher W (2003) Physiological Plant Ecology. Ecophysiology ans stress physiology of Functional groups. Springer. Cuarta edición. 517 pp.

Larcher W (2005) Climatic constraints drive the evolution of low temperature resistance in woody plants. Journal of Agricultural Meteorology. 61, 189–202.

Larsson PR (1994). The vascular cambium. Development and structure. Springer-Verlag, Heidelberg, Germany.

Levitt J (1980) Responses of plants to environmental stresses. Volume II. Water, radiation, salt, and other stresses. Ed. 2. Academic Press, Nueva York. 697 p.

Li PH, Palta JP (1978) Frost hardening and freezing stress in tuber-bearing solanum species. pp. 49–71, in: PH Li and A Sakai (eds). Plant Cold Hardiness and Freezing Stress. Vol. I, New York, NY: Academic Press.

Lichtenthaler HK, Rinderle U (1988) The role of chlorophyll fluorescence in the detection of stress conditions in plants. CRC Critical Reviews in Analytical Chemistty 19, Suppl. I, 29-85.

Linares J, Camarero J (2012) From pattern to process: linking intrinsic water-use efficiency to drought-induced forest decline. Global Change Biology. 18:1000–1015.

Liston A, Vining T, Campbell C, Gernandt D, Piñero D (2003) Molecular phylogeny of Pinaceae and Pinus. Acta Horticulturae. 107–114.

Luckman BH, Wilson RJS (2005). Summer temperature in the Canadian Rockies during the last millennium: a revised record. Climate Dynamics. 24, 131–144.

Madrigal-González J, Hantson S, Yue C, Poulter B, Ciais P, Zavala MA (2015) Long-term wood production in water-limited forests: evaluating potential CO_2 fertilization along with historical confounding factors. Ecosystems 18:1043–1055.

Maherali H, Williams BL, Paige KN, Delucia EH (2002) Hydraulic differentiation of Ponderosa pune populations along a climate gradient is no associated with ecotypic divergence. Functional Ecology. 16(4):510-521.

Maréchaux I, Bartlett MK, Sack L, Baraloto C, Engel J, Joetzjer E, Chave J (2015). Drought tolerance as predicted by leaf water potential at turgor loss point varies strongly across species within an Amazonian forest. Functional Ecology. 29:1268-1277.

Markesteijn L, Poorter L (2009). Seedling root morphology and biomass allocation of 62 tropical tree species in relation to drought- and shade-tolerance. Journal of Ecology. 97(2), 311–325.

Marqués L, Camarero JJ, Gazol A, Zavala MA (2016) Drought impacts on tree growth of two pin species along an altitudinal gradient and their use as early-warning signals of potential shifts in tree species distributions. Forest Ecology and Management. 381:157-167.

Martín AS, Díaz-Fernández PM, De Miguel, Del Ángel J (1998) Regiones de procedencia de las especies forestales españolas. Géneros *Abies*, *Fagus*, *Pinus* y *Quercus*. Servicio de Material Genético. Dirección General de Conservación de la Naturaleza. Ministerio de Medio Ambiente. Organismo Autónomo Parques Nacionales. 89 p.

Martín-Benito D, Beeckman H, Cañellas I (2013). Influence of drought on tree rings and tracheid features of *Pinus nigra* and *Pinus sylvestris* in a mesic Mediterranean forest. European Journal of Forest Research. 132:33-45.

Martínez-García F (1999). Los bosques de *Pinus sylvestris* L. del Sistema Central Español. Distribución, historia, composición florística y tipología. Tesis doctoral. Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA). 253 pp.

Martínez-Meier A, Sánchez L, Pastorino M, Gallo L, Rozenberg P (2008) What is hot in tree rings? The wood density of surviving Douglas-firs to the 2003 drought and heat wave. Forest Ecology and Management. 256:837-843.

Martínez-Vilalta J, Prat E, Oliveras I, Piñol J (2002) Hydraulic properties of roots and stems of nine woody species from a holm oak forest in NE Spain. Oecologia. 133:19-29.

Martínez-Vilalta J, Sala A, Piñol J (2004) The hydraulic architecture of Pinaceae- a review. Plant Ecology. 171:3-13.

Martínez-Vilalta J, Cochard H, Mencuccioni M, Sterck F, Herrero A, Korhonen JFJ, Llorens P, Nikinmaa E, Nolè A, Poyatos R, Ripullone F, Sass-Klaassen U, Zweifel R (2009)

Hydraulic adjustmen of Scots pine across Europe. New Phytologist. 184(2):353-364.

Martínez-Vilalta J, Sala A, Asensio D, Galiano L, Hoch G, Palacio S, Piper F, Lloret F (2016) Dynamics of non-structural carbohydrates in terrestrial plants: A global synthesis across Europe. Ecological Monographs. 86(4): 495-516.

Matías L, Castro J, Villar-Salvador P, Quero JJL, Jump ASA (2017). Differential impact of hotter drought on seedling performance of five ecologically distinct pine species. Plant Ecology. 218: 201–212.

Mayr S, Gruber A, Bauer H (2003) Repeated freeze-thaw cycles induce embolism in drought stressed conifers (Norway spruce, stone pine). Planta. 217: 436–441.

McCulloh K, Johnson D, Meinzer F, Lachenbruch B (2011) An annual pattern of native embolism in upper branches of four tall conifer species. American Jorunal of Botany. 98: 1007–1015.

Mediavilla S, Escudero A (2003). Stomatal responses to drought at a Mediterranean site: a comparative study of co-occurring woody species differing in leaf longevity. Tree Physiology. 23:987–996.

Millar CI (1993) Impact of the Eocene on the evolution of *Pinus* L. Annals of the Missouri Botanical Garden, 80: 471-498.

Mirov NT (1967) The genus *Pinus*. Ronald, New York, 602 pp.

Morison J (1985) Sensitivity of stomata and water use efficiency to high CO_2. Plant, Cell and Environment. 8:467–474.

Muller-Landau HC (2004) Interespecific and inter-site variation in wood specific gravity of Tropical trees. Biotropica. 36(1): 20-32.

Norby R, Wullschleger S, Gunderson C, Johnson DW, Ceulemans R (1999) Tree responses to rising CO_2 in field experiments: Implications for the future forest. Plant, Cell and Environment. 22:683–714.

Norby R, DeLucia E, Gielen B, Calfapietra C, Giardina CP, King JS, Ledford J, McCarthy HR, Moore DJP, Ceulemans R, De Angelis P, Finzi AC, Karnosky DF, Kubiske ME, Lukac M, Pregitzer KS, Scarascia-Mugnozza GE, Schlesinger WH, Oren R (2005) Forest response to elevated CO_2 is conserved across a broad range of productivity. Proceedings of the NAtional Academy of Science. 2:18052–18056.

Oberhuber W (2004) Influence of climate on radial growth of *Pinus cembra* within the alpine timberline ecotone. Tree Physiology. 24: 291-301.

Olano JM, Eugenio M, García-Cervigón AI, Folch M, Rozas V (2012). Quantitive tracheid anatomy reveals a complex environmental control of wood structure in continental

Mediterranean climate. International Journal of Plant Sciences, 173: 137-149.

Pachauri RK, Allen MR, Barros VR, Broome J, Cramer W, Christ R, Church JA, Clarke L, Dahe Q, Dasgupta P, Dubash NK, Edenhofer O, Elgizouli I, Field CB, Forster P, Friedlingstein P, Fuglestvedt J, Gomez-Echeverri L, Hallegatte S, Hegerl G, Howden M, Jiang K, Jimenez Cisneroz B, Kattsov V, Lee H, Mach KJ, Marotzke J, Mastrandrea MD, Meyer L, Minx J, Mulugetta Y, O'Brien K, Oppenheimer M, Pereira JJ, Pichs-Madruga R, Plattner GK, Pörtner HO, Power SB, Preston B, Ravindranath NH, Reisinger A, Riahi K, Rusticucci M, Scholes R, Seyboth K, Sokona Y, Stavins R, Stocker TF, Tschakert P, van Vuuren D, van Ypserle JP (2014) Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.

Padilla FM, Pugnaire FI (2007). Rooting depth and soil moisture control Mediterranean woody seedling survival during drought. Functional Ecology. 21: 489–495.

Palamarev E (1987) Paleobotanical evidences of the Tertiary history and origin of the Mediterranean sclerophyll dendroflora. Plant Systematics and Evolution. 162: 93-107.

Pardos M, Calama R, Climent J (2009) Difference in cuticular transpiration and sclerophylly in juvenile and adult pine needles relates to the species-specific rates of development. Trees. 23: 501–508.

Parmensan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. Nature. 421: 37-42.

Pearce R (2001) Plant freezing and damage. Annals of Botany. 87: 417–424.

Peguero-Pina J, Alquézar-Alquézar J, Mayr S, Cochard H, Gil-Pelegrín E (2011) Embolism induced by winter drought may be critical for the survival of *Pinus sylvestris* L. near its southern distribution limit. Ann For Sci 68:565–574.

Peñuelas J, Lloret F, Montoya R (2001) Severe drought effects on mediterranean woody flora in Spain. Forest Science. 47: 214–218.

Pittermann J, Sperry J (2006) Analysis of freeze-thaw embolism in conifers. The interaction between cavitation pressure and tracheid size. Plant Physiology. 140: 374–382.

Pompa-García M, Camarero-Martínez JJ (2015) Potencial dendroclimático de la madera temprana y tardía de *Pinus cooperi* Blanco. Agrociencia. 49:177-187.

Poorter H, Niinemets U, Poorter L, Wright IJ, Villar R (2009) Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. New Phytologist. 182(3):565-588.

Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L (2012). Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. The New Phytologist. 193(1): 30–50.

Prentice I, Cramer W, Harrison S, Leemans R, Monserud R, Solomon A (1992) A global biome model based on plant physiology and pominance, soil properties and climate. Journal of Biogeography. 19: 117–134.

Pretzsch H, Biber P, Schütze G, Uhl E, Rötzer T (2014) Forest stand growth dynamics in Central Europe have accelerated since 1870. Nature communications. 1-10.

Price RA, Liston A, Strauss SH (1998) Phylogeny and systematics of *Pinus*. En: D.M. Richardson (ed.), Ecology and Biogeography of Pinus. Cambridge University Press, Cambridge, UK. 527 pp.

Quero JL, Villar R, Marañón T, Zamora R (2006) Interactions of drought and shade effects on seedlings of four *Quercus* species: physiological and structural leaf responses. The New Phytologist. 170:819–834.

Reich PB, Leksyn J, Tjoelker MG (1994) Relationship of aluminium and calcium to net CO_2 exchange among diverse Scots pine provenances under pollution stress in Poland. Oecologia. 97:82-92.

Resco De Dios V, Fischer C, Colinas C (2007) Climate change effects on mediterranean forests and preventive measures. New Forests. 33:29–40.

Richardson DM (2000). Ecology and biogeography of Pinus. Cambridge University Press. 527 pp.

Rigling A, Bräker O, Schneiter G, Schweingruber F (2002) Intra-annual tree-ring parameters indicating differences in drought stress of *Pinus sylvestris* forests within the Erico-Pinion in the Valais (Switzerland). Plant Ecology. 163:105-121.

Ruiz de la Torre J, Ceballos L (1979) Árboles y arbustos de la España Peninsular. Escuela Tecnológica Superior de Ingenieros, Madrid, 512 pp.

Ruiz de la Torre J (1990) Distribución y características de las masas forestales españolas. Ecología, Fuera de Serie. 1: 11–30.

Ruiz de la Torre J (2006) Flora Mayor. En: Organismo Autónomo de Parques Nacionales. Dirección General para la Biodiversidad. Madrid.

Sakai A (1983) Comparative study on freezing resistance of conifers with special reference to cold adaptation and its evolutive aspects. Canadian Journal of Botany. 61, 2323–2332.

Sakai A, Larcher W (1987) Frost Survival of Plants. Responses and Adaptation to freezing stress. Springer-Verlag.

Saranpää P (2003) Wood density and growth. In: Wood quality and its biological basis (Barnett J, Jeronimidis G) Blackwell Publishing. 87-117.

Sinclair WT, Morman JD, Ennos RA (1999) The postglacial history of Scots pine (*Pinus sylvestis L.)* in Western Europe: evidence from mitochondrial DNA variation. Molecular Ecology. 8:83-88.

Sperry JS, Tyree MT (1990) Water-stress-induced xylem embolism in three species of conifers. Plant, Cell and Environment. 13: 427–436.

Sperry J, Sullivan J (1992) Xylem embolism in response to freeze-thaw cycles and water stress in ring-porous, diffuse-porous, and conifer species. Plant Physiology. 100: 605–13.

Sperry JS (1993) Winter embolism and spring recovery in *Betula cordifolia, Fagus grandifolia, Abies balsamifera,* and *Picea rubens*. pp. 86-98 En: Water Transport in Plants Under Climatic Stress. Cambridge University Press, Cambridge, UK.

Sperry JS, Hacke UG, Pitterman J (2006) Size and function in conifer tracheids and angiosperm vessels. American Journal of Botany. 93(10):1490-1500.

Stewart WN, Rothwell GW (1993) Paleobotany and the evolution of plants. Cambridge: Cambridge University Press. 405 pp.

Tenhunen JD, Beyschlag W, Lange OL Harley PC (1987). Changes during summer drought in leaf CO_2 uptake rates of macchia shrubs growing in Portugal: Limitations due to photosynthetic capacity, carboxylation efficiency, and stomatal conductance. *In* Plant response to stress. Functional analysis in Mediterranean ecosystems Eds. J.D. Tenhunen, F.M. Catarino, O.L. Lange and W.C. Oechel. Springer-Verlag, Berlin, pp. 305-327.

Tognetti R, Longobucco A, Raschi A (1998) Vulnerability of xylem to embolism in relation to plant hydraulic resistance in *Quercus pubescens* and *Quercus ilex* co-ocurring in a Mediterranean coppice stand in central Italy. New Phytologist. 139:437-447.

Tretiach, M. 1993. Photosynthesis and transpiration of evergreen Mediterranean and deciduous trees in an ecotone during a growing season. Acta Oecologica. 14:341-360.

Tyree MT, Ewers FW (1991) The hydraulic architecture of trees and other woody plants. New Phytologist 119:345-360.

Uemura M, Steponkus PL (2002) Modification of the intracellular sugar content alters the incidence of freeze-induced membrane lesions of protoplast isolated from *Arabidopsis thaliana* leaves. Plant, Cell and Environment. 26:1083-1096.

Uscola M, Villar-Salvador P, Gross P, Maillard P (2015) Fast growth involves high dependence on stored resources in seedlings of Mediterranean evergreen trees. Annals of Botany. 115:1001–1013.

Valladares F, Martínez-Ferri E, Balaguer L, Pérez-Corona E, Manrique E (2000) Low leaf-level response to light and nutrients in Mediterranean evergreen oaks: a conservative resource-use strategy? New Phytologist. 148:79-91.

Vanninen P, Ylitalo H, Sievänen R, Mäkelä A (1996) Effects of age and site quality on the distribution of biomass in Scots pine (*Pinus sylvestris* L.). Trees. 10:231-238.

Vernet JL, Meter A, Zéraïa L (2005). Premières datations des feux holocénes dans les monts de Saint- Guilhem-le-Déssert (Hérault, France), contribution a l'histoire de la forêt relique de *Pinus nigra* Arnold ssp. *salzmannii* (Dun.) Franco. Geoscience. 337: 533-537.

Vilagrosa A, Bellot J, Vallejo VR Gil Pelegrín E (2003) Cavitation, stomatal conductance, and leaf dieback in seedlings of two co-occurring Mediterranean shrubs during an intense drought. Journal of Experimental Botany. 54:2015-2024.

Villar-Salvador P (2000) Estrategias ecológicas y evolutivas del xilema en plantas leñosas mediterráneas. Tesis Doctoral. Universidad de Valencia.

Vizcaino-Palomar N, Ibánez I, González-Martínez SC, Zavala MA, Alía R (2016) Adaptation and plasticity in aboveground allometry variation of four pine species along environmental gradients. Ecology and Evolution. 1-13.

Wallace Z, Lovett G, Hart J, Machona B (2007) Effects of nitrogen saturation on tree growth and death in a mixed-oak forest. Forest Ecology and Management. 243:210–218.

Walther G, Post E, Convey P, Menzel A, Parmesan C, Beebee T, Fromentin J, Hoegh-Guldberg O, Bairlein F (2002) Ecological responses to recent climate change. Nature 416, 389–95.

Weiner J (2004) Allocation, plasticity and allometry in plants. Perspectives in Plant Ecology, Evolution and Systematics. 6(4): 207-215.

Wesley-Smith J, Walters C, Pammenter N, Berjak P (2015) Why is intracellular ice lethal? A microscopical study showing evidence of programmed cell death in cryo-exposed embryonic axes of recalcitrant seeds of Acer saccharinum. Ann. Bot. 1–10.

Willis KJ, Bennett KD, Birks JHB (1998). The Late Quaternary dynamic of pines in Europe. En: Ecology and Biogeography of *Pinus*, Richardson, D.M. 107-121 p.

Zimmermann MH (1983) Xylem Structure and the ascent of sap. Springer Series in Wood Science. Springer-Verlag, Berlin.

Zobel BJ, van Buijtenen JP (1989) Wood variation- its causes and control. Springer Verlag, Berlin. 383 pp.

Zwieniecki MA, Holbrook NM (2009) Confronting Maxwell's demon: biophysics of xylem embolism repair. Trends Plant Science 14: 530–534.

CAPÍTULO 2

Distribution of pines in the Iberian Peninsula agrees with species differences in foliage frost tolerance, not with vulnerability to freezing-induced xylem embolism

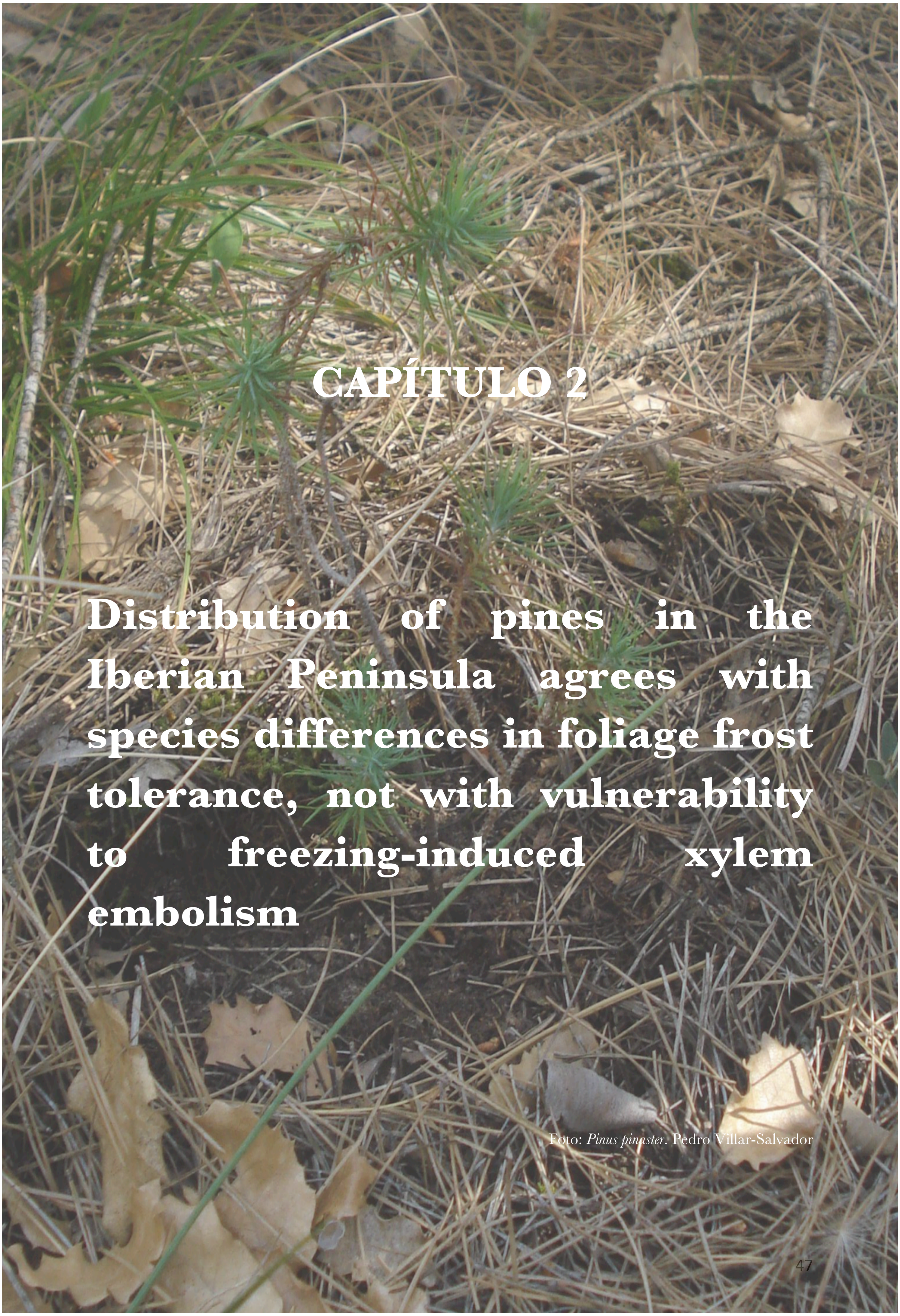


Foto: *Pinus pinaster*. Pedro Villar-Salvador

Distribution of pines in the Iberian Peninsula agrees with species differences in foliage frost tolerance, not with vulnerability to freezing-induced xylem embolism

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RESUMEN

La sequía y las heladas son los principales determinantes en el funcionamiento y la distribución de las plantas. Ambos estreses pueden causar embolias en el xilema y daños en el follaje. El objetivo de este estudio fue analizar si la distribución altitudinal y latitudinal de seis especies comunes en Europa está relacionada con sus diferencias interespecíficas en la tolerancia a las heladas, así como determinar los mecanismos fisiológicos que subyacen dicha tolerancia. También evaluamos si la tolerancia a las heladas depende del estado hídrico de las plantas. Se estudió la supervivencia en plantas de 2 años en un rango de temperaturas de congelación y evaluamos el porcentaje de pérdida de conductividad hidráulica (PLC) por la formación de embolias en el xilema, así como el daño foliar por la liberación de electrolitos en las acículas (EL) después de un ciclo de helada a -15 ° C y en un rango de valores de potencial hídrico antes del amanecer (ψ_{pd}). Las especies que experimentan inviernos fríos en su área de distribución (*Pinus nigra* J.F. Arnold, *Pinus sylvestris* L. y *Pinus uncinata* Raymond ex A. DC.) tuvieron las mayores tasas de supervivencia a las heladas, y los menores EL y concentración de azucares solubles (SS). Por el contrario, los pinos que habitaban lugares templados o fríos en invierno (especialmente *Pinus halepensis* Mill. y *Pinus pinea* L. y, en menor medida, *Pinus pinaster* Ait.) tuvieron una supervivencia baja y valores elevados de EL y SS. El PLC inducido por la helada fue muy bajo y las diferencias entre las especies no se relacionaron con los daños sufridos en las acículas por la helada. La reducción del ψ_{pd} disminuyó el daño en las acículas por las heladas en *P. pinea* y *P. sylvestris*, lo aumentó en *P. uncinata* y tuvo un efecto neutral en el resto de la especies. Este estudio demuestra que las temperaturas de congelación son un importante condicionante ambiental para la distribución de los pinos y sugiere que las diferencias interespecíficas en la sensibilidad del follaje a las heladas, y no la vulnerabilidad a las embolias por heladas o SS explican la supervivencia de las plántulas de pino a las heladas

Palabras clave: azúcares solubles, diámetro de las traqueidas. ecofisiología, embolias, estrés hídrico, liberación de electrolitos, *Pinus*.

ABSTRACT

Drought and frosts are major determinants of plant functioning and distribution. Both stresses can cause xylem embolism and foliage damage. The objective of this study was to analyse if the distribution of six common pine species along latitudinal and altitudinal gradients in Europe is related to their interspecific differences in frost tolerance and to the physiological mechanisms underlying species-specific frost tolerance. We also evaluate if frost tolerance depends on plant water status. We studied survival to a range of freezing temperatures in 2-year-old plants and assessed the percentage loss of hydraulic conductivity (PLC) due xylem embolism formation and foliage damage determined by needle electrolyte leakage (EL) after a single frost cycle to −15 °C and over a range of predawn water potential (ψ_{pd}) values. Species experiencing cold winters in their range (*Pinus nigra* J.F. Arnold, *Pinus sylvestris* L. and *Pinus uncinata* Raymond ex A. DC.) had the highest frost survival rates and lowest needle EL and soluble sugar (SS) concentration. In contrast, the pines inhabiting mild or cool winter locations (especially *Pinus halepensis* Mill. and *Pinus pinea* L. and, to a lesser extent, *Pinus pinaster* Ait.) had the lowest frost survival and highest needle EL and SS values. Freezing induced PLC was very low and differences among species were not related to frost damage. Reduction in ψ_{pd} decreased leaf frost damage in *P. pinea* and *P. sylvestris*, increased it in *P. uncinata* and had a neutral effect on the rest of the species. This study demonstrates that freezing temperatures are a major environmental driver for pine distribution and suggests that interspecific differences in leaf frost sensitivity rather than vulnerability to freezing-induced embolism or SS explain pine juvenile frost survival.

Keywords: drought stress, ecophysiology, electrolyte leakage, embolism, *Pinus*, soluble sugars, tracheid diameter.

1. INTRODUCTION

Drought and frosts are major drivers of plant evolution and distribution (Woodward and Williams 1987, Pockman and Sperry 1997, Choat et al. 2012). Both stress factors affect important plant physiological processes (Sakai and Larcher 1987, Mayr et al. 2006). Particularly, water transport and gas exchange are usually reduced due to frost-induced embolism and drought-induced stomatal closure (Sperry and Sullivan 1992, Davis et al. 1999, Willson and Jackson 2006). These physiological responses reduce the productive capacity and hydration of plants, and might damage the plant and eventually cause their death (McCulloh et al. 2011, McDowell 2011, Peguero-Pina et al. 2011). Most comparative studies on frost tolerance use foliage EL or decrease in fluorescence activity of photosystems to assess tissue damage (Strand and Öquist 1988, Climent et al. 2009), while few studies report plant survival (Hawkins et al. 2003), which is key for plant fitness. However, while simple and fast, EL or fluorescence of photosystems do not inform on the capacity of individuals to remain alive after a stress event. The mechanism by which drought and frosts cause xylem embolism is different (Zimmermann 1983), but in both cases the amount of embolism is expected to increase with declining xylem water potential (ψ) (Davis et al. 1999, Mayr et al. 2003). Drought-induced embolism occurs when low ψ causes the air contained in air filled cells to be sucked through the pits of water-filled xylem conduits, forming bubbles that block the conduit (Zimmermann 1983). Frost-induced embolism occurs when the bubbles formed after thawing of frozen water inside the xylem conduits expand and block xylem conduits (Robson et al. 1988, Langan et al. 1997). Frosts can also damage plants by causing the loss of cell membrane functional integrity (Uemura et al. 2006), which mainly occurs in the most exposed organs. Freezing of apoplast causes the water to move outside the cell, producing large changes in protoplasm volume, which can damage the cell membrane and hinder metabolism (Sakai and Larcher 1987, Wesley-Smith et al. 2015). Soluble sugars (SS) play an important role in plant frost tolerance. Soluble sugars increase the stability of cell membranes to frost damage (Uemura and Steponkus 2003), may contribute to embolism repair (Zwieniecki and Holbrook 2009) and reduce the protoplasm osmotic potential, which increases cell desiccation tolerance and reduces the freezing point (Hinesley et al. 1992). Finally, plants use stored SS to support winter metabolism and early spring growth (Uscola et al. 2015). Consequently, many plants from the cold and temperate biomes increase their SS concentration during frost acclimation in the fall to a maximum level in midwinter, which is then reduced in spring and summer with cold deacclimation (Sakai and Larcher 1987, Martínez-Vilalta et al. 2016). Distribution of pine species native to Europe appears segregated along latitudinal and altitudinal gradients in a predictable manner, suggesting the existence of environmental factors that differentially influence the species. Some species, namely *Pinus halepensis* Mill., *Pinus pinaster* Ait. and *Pinus pinea* L., are concentrated in southern of Europe, particularly at Mediterranean climate sites with mild to cool winters and hot and dry summers (see Figure S1 available as Supplementary Material). Other species, such as *Pinus nigra* J.F. Arnold, *Pinus sylvestris* L. and *Pinus uncinata* Raymond ex A. DC., are located in cold winter sites, either at high latitude (in central and northern Europe) or at high altitude (southern Europe including the Iberian Peninsula; see Figure S1 available as Supplementary Material) (Richardson 1998). In these high-mountain locations rainfall can be higher, evapotranspiration is lower and thus summer drought is shorter and of moderate intensity relative to lower altitude sites. Intra- and inter-specific variability to drought-induced embolism is low in pines (Martínez-Vilalta and Piñol 2002, Martínez-Vilalta et al. 2004, Martínez-Vilalta et al. 2009, Lamy et al. 2014) which make differences in drought-induced embolism an unlikely explanation for differences in species distribution. We hypothesize that this latitudinal and altitudinal distribution pattern of European pines could be driven by species differences in cold tolerance. The Mediterranean climate has a dry season concentrated in the summer but occasionally winters can also be dry. High altitude locations in the Mediterranean basin such as the plateaus and mountain ranges of the Iberian Peninsula have cold winters. The combination of cold temperatures in winter and summer drought is an important determinant for plant life in these environments (Mitrakos 1980). Besides, in dry winter years, plants at Mediterranean cold winter sites can suffer drought stress (Peguero-Pina et al. 2011), which can enhanced the negative effects of frost-induced embolism (Willson and Jackson 2006). This phenomenon has been related to pine dieback in high elevations in the Mediterranean mountains and boreal forests (Kullman 1991, Peguero-Pina et al. 2011). At the same time, drought stress can also increase frost tolerance in some plant organs (Medeiros and Pockman 2011, Villar-Salvador et al. 2013, Sperling et al. 2017). It is possible that drought-induced physiological acclimation responses such as changes in SS concentration, cell membrane stability or the reduction of cell osmotic potential (Serrano et al. 2005, Beck et al. 2007, Villar-Salvador et al. 2013) may also enhance cold tolerance. Numerous studies have addressed the effect of drought and frost independently on plant physiological performance (see Beck et al. 2004, Dobbertin et al. 2005), but relatively few studies have analysed experimentally how drought affects frost tolerance of plants (Grossnickle et al. 1991, Medeiros and Pockman 2011). The general objective of this study is to assess whether interspecific differences in frost tolerance are related to the distribution of the six-pine species native to the Iberian Peninsula and the physiological mechanisms underlying species differences in frost tolerance. These pine species (*P. halepensis*, *P. pinea*, *P. pinaster*, *P. nigra*, *P. sylvestris* and *P. uncinata*) are also common throughout Europe. Specifically, we asked: (i) Do pine species inhabiting cold sites have higher inherent cold tolerance than pines inhabiting mild or cool winter locations? (ii) Are differences in frost tolerance explained by differences in frost-induced embolism, needle frost tolerance or tissue SS? (iii) What is the effect of drought stress on frost tolerance? To answer these questions, we conducted an experiment in which the frost survival, and the interaction between drought and frost on xylem embolism and needle damage were assessed in 2-year-old plants. We used juveniles as early life stages are important bottlenecks in pine population dynamics (Castro 1999, Herrero et al. 2013).

2. MATERIALS AND METHODS

2.1 Plant material

Seeds of pine species were collected in populations of the Iberian Range (Eastern Spain), except for *P. uncinata*, whose seeds were collected in the Pyrenees (Table S1). Seeding was done in 2012 on different dates to synchronize seedling emergence. *Pinus halepensis* was seeded on 30 January, while *P. pinaster* and *P. pinea* were seeded 7 and 11 days later, respectively, than *P. halepensis*. *Pinus nigra* subsp. *salzmannii* (here after *P. nigra*) and *P. uncinata* were seeded on 15 February, while *P. sylvestris* was seeded on 20 February. Seedlings were cultivated in Plasnor trays (190/300-45, Plasnor, Legazpi, Spain) with 45 cavities of 300 ml. Growing medium was fertilized peat (White 420 F6 Kekkilä, Vantaa, Finland; pH 4.7, containing 0.8–1 kgm^{-3} of a slow-release fertilizer NPK 16-10-20). Seedlings were initially grown in a greenhouse of the Centro Nacional de Recursos Genéticos Forestales 'El Serranillo' (Guadalajara, Spain, 40°40'N, 3°10'W, 650 m above sea level) to avoid frost damage. In mid May 2012, seedlings were moved outdoorsand were kept well-watered, periodically fertilized and exposed to the natural seasonal changes in temperature, radiation and day length until January 2014. Testing differences in seedling frost survival Frost tolerance in the experiment was assessed by seedling survival, xylem embolism and needle damage. Survival was assessed after freezing batches of seedlings at the following target temperatures: −5, −8, −12, −16, −20, −25 and −30 °C. For each temperature, we used 10 seedlings per species, which were transplanted into styrofoam containers to isolate the roots. Plants were placed in a programmable freezing chamber (Dycometal, CCK, Viladecans, Barcelona, Spain) at the INIA (Madrid, Spain), and subjected to one frost cycle. Temperature was reduced from 5 to 0 °C in 1 h. Then, the temperature decreased at a ~3 °C h−1 rate to the target temperature, which was maintained for 3 h. Finally, temperature was increased at a rate of 5 °C h−1 up to 5 °C, where temperature was maintained for 1 h. Then, seedlings were moved to a greenhouse and were well-watered to favour their recovery. After 2 months, we verified seedling status and we considered a seedling to be dead when it had not resumed growth and >95% of needles were brown.

2.2 Foliage electrolyte leakage and xylem embolism measurements

In early March 2014, we performed an experiment in which seedlings of all species were randomly distributed into four treatments: (i) frost and well irrigated, (ii) frost with restricted watering, (iii) unfrozen and well irrigated and (iv) unfrozen with restricted watering. We used 1418 seedlings per species and treatment. The sizes of shoot fractions of the seedlings used in the experiment are presented in Table 2. Restricted watering was achieved by stopping watering 7–10 days before physiological measurements. The remaining seedlings were irrigated every 2–3 days to keep them well-watered. For the frost treatments, seedlings were subjected to a single −15 °C frost cycle using a programmable freezing chamber (A.S.L. Aralab International®-CON-550-20, Madrid, Spain), using the same protocol explained in the previous section. Before frost exposure, we measured seedling predawn water potential (ψ_{pd}) using a Scholander Pressure Chamber (SKPM 1400, Skye Instruments, Llandrindod Wells, UK). In most cases, the ψ_{pd} was measured on lateral twigs, but in a few cases we used needles attached to brachiblasts. Seedlings were covered with opaque plastic bags overnight to reduce seedling transpiration before ψ_{pd} measurements. ψ_{pd} ranged between −0.23 and −1.68 MPa in *P. nigra*, *P. sylvestris* and *P. uncinata*, and from −0.16 to −2.0 MPa in *P. halepensis*, *P. pinaster* and *P. pinea*. We measured damage in secondary needles and xylem embolism after the −15 °C frost to assess the importance of the interaction of frost and drought on species physiological performance. Needle frost damage was assessed by EL (Earnshaw1993), while xylem frost-induced embolism was measured as the percentage loss in stem hydraulic conductivity (PLC) (Charrier et al. 2013). For needle EL measurements, secondary needles were cut in small pieces (fresh weight ~0.30 g) after the −15 °C treatment, and washed twice in distilled water for 20 min and left in a vial with 20 ml of deionized water that was gently shaken (Orbital shaker, Selecta, Abrera, Barcelona, Spain) on an illuminated bench under laboratory temperature (20–22 °C). After 24 h, the electrical conductivity (C_i) of the water bathing the needles was measured with an electro conductivity meter (Crison® CM 35+, L'Hospitalet, Barcelona, Spain). Then, the needles were autoclaved at 120 kg cm^2 for 10 min.

After cooling the samples at room temperature, electrical conductivity was remeasured (C_f). Electrolyte leakage (EL) was calculated as:

$$EL= \left(\frac{C_i}{C_f} \text{ x } 100\right)\text{...(Equation 1).}$$

PLC was measured in stem segments 3 cm long excised from the lower part of the stem (immediately above the cotyledon insertion point). Working under water to prevent air entry into the tracheids, we cut the stem segments, removed the bark in the 3–4 mm extremes of the segments and then recut the stems to remove resin remains with a fresh razor blade under water. Water flow through the stem segments was measured using the Xyl'em Plus apparatus (Embolism Meter, INRA Licensed Instrumentec, Bronkhorst France S. A.S., Montigny-Les-Cormeilles, France, Version 2.1, Cochard 2002). Previously, the extremes of the stems segments were wrapped with Teflon tape before connection to the tubes to prevent lateral leaks. We calculated the PLC as:

$$PLC = \frac{K_m\text{-}K_i}{K_m} \text{ x } 100 \text{ ....(Equation 2),}$$

where K_i (mmol ms^{-1} MPa^{-1}) is the hydraulic conductivity of the segment after seedling freezing and before embolism removal and K_m is the maximum hydraulic conductivity, measured after removing embolism by immersing the stem segments in the same solution used for the flow measurements in a vacuum chamber (BR116, Selecta) for at least 12 h at a suction of 90 kPa. Both conductivities were calculated as the ratio between the flow of a 10 mmol KCl + 1 mmol of $CaCl_2$ solution through the segment divided by the pressure gradient (pressure difference = 0.49–0.52 kPa). In all cases, the solution used for measurements was previously filtered with a 0.45 µm nylon syringe filter membrane (Filter-Lab, Barcelona, Spain) and degassed in the mentioned vacuum chamber for 24 h at a suction of 90 kPa. Measurement of tracheid diameter Tracheid diameter was measured only in the unfrozen, well watered plants. Cross-sections 20 µm in thickness were cut from six individuals per species using a cryostat microtome (Microm HM 505 E, Ramsey, MN, USA). We used the same stem segments used for PLC. Cross-sections were bathed in bleach for 30 min, rinsed in tap water and finally stained with safranin (1 g in 50% ethanol). Tracheid diameter was measured using the ImageJ software on all the tracheids located in three randomly selected radial rows, from the pit to the outer border of the section. A minimum of 160 tracheids per individual was measured.

2.3 Soluble sugars determination

Soluble sugars were determined only in unfrozen well-watered plants. Needles of six seedlings per species were stored frozen immediately after EL measurements. When SS were determined, needles were thawed and dried at 50 °C for 24 h. Soluble sugars were extracted following the methodology in Chow and Landhäusser (2004). Briefly, needles were ground in a ball mill (PM100, Retsch, Haan, Germany) and 50 mg of ground needles was used for SS extraction with 5 ml of ethanol 80% at 90 °C for 10 min. Then, the tubes were centrifuged at 2500 rpm for 5 min, and the supernatant was preserved. We repeated the extraction three times and the supernatant of the three extractions was combined and oven-dried at 50 °C until complete evaporation of the ethanol. Then, the residue was dissolved in 1 ml hot deionized water and filtered with 0.45 μm nylon syringe filter membrane (Filter-Lab). We analysed the concentration of main SS (see Table 2) using a high performance liquid chromatography system (Agilent Technologies, 1100 series, Palo Alto, CA, USA) coupled to the refractive index detector (HPLC-RI) equipped with a quaternary pump, degasser, auto injector and HP-1047A RI detector. The chromatographic data were acquired using the ChemStation software. The samples were analysed using a Supelcogel column Ca-59305U (30 cm × 7.8 mm) under isocratic condition with ultrapure water (Milli-Q). The injection volume was 20 μl and the flow rate was 0.5 ml min^{-1}. Column and detector temperature were 80 and 50 °C, respectively. Identification and quantification of sugars were determined by comparing the peak retention time and area of the samples with pure standard solutions of each sugar that were made by us in the laboratory.

2.4 Data analysis

To compare the frost survival of pine species, we compared the temperature at which survival is 50% (LT$_{50}$). We used a logistic model to fit survival data for each species against freezing temperatures (see Figure 1). LT$_{50}$ is the inflection point of the logistic model. We used a logistic model to fit survival data for each species against freezing temperatures (see Figure 1). For each LT$_{50}$ value, we calculated its 95% confidence intervals following Collett (1991).

A generalized linear model (GLM) was performed to test the effect of species (six levels), frost (two levels, unfrozen and −15 °C frost) and ψ_{pd} on EL and PLC, testing all the interactions. As the Species × Frost × ψ_{pd} was significant for EL, the least square means and standard errors were calculated at specific ψ_{pd} values, −0.4, −1.2 and −1.5 MPa (see Figure 2). Selected values are a compromise between choosing values within the range of water potential values measured in all species and the water potential values that these species show in the field under dry winter conditions (Martinez-Ferri et al. 2004, Poyatos et al. 2008, Peguero-Pina et al. 2011). A general linear mixed model (GLMM) was performed to compare the tracheid diameter among species. Data of tracheids in a row were nested within the individual, which was included as a random effect. Species differences in SS were analysed using one-way ANOVA. Tukey's honest significance difference (HSD) test ($\alpha = 0.05$) was used for multiple comparisons of mean values. All data were checked for residual normal distribution (Shapiro test) and variance homoscedasticity (Levene's test). Statistical analyses were performed in R platform and Statistica 7.0 (StatSoft, Inc., Tulsa, OK, USA).

3. RESULTS

3.1 Survival

In all species, fitted curves of survival against freezing temperatures were highly significant ($P < 0.001$). Frost survival significantly differed among species (Figure 1) with a two-fold LT_{50} variation between *P. halepensis*, the species with the lowest LT_{50}, and P. nigra, the species with the highest LT_{50}. *Pinus nigra*, *P. sylvestris* and *P. uncinata* did not show any mortality until −15 °C, while at this temperature survival of *P. halepensis* and *P. pinea* was only around 20%. Four groups according to their LT_{50} values and confidence intervals were distinguished: *P. halepensis* = *P. pinea* > *P. pinaster* > *P. sylvestris* = *P. uncinata* > *P. nigra*. The survival curve was similar for *P. sylvestris* and *P. uncinata*, and their LT_{50} values were slightly higher than those estimated for *P. nigra*. *Pinus pinaster* had lower frost survival than *P. uncinata*, *P. sylvestris* and *P. nigra*, but higher frost survival than the Mediterranean pines *P. halepensis* and *P. pinea*, which showed little frost survival differences between them.

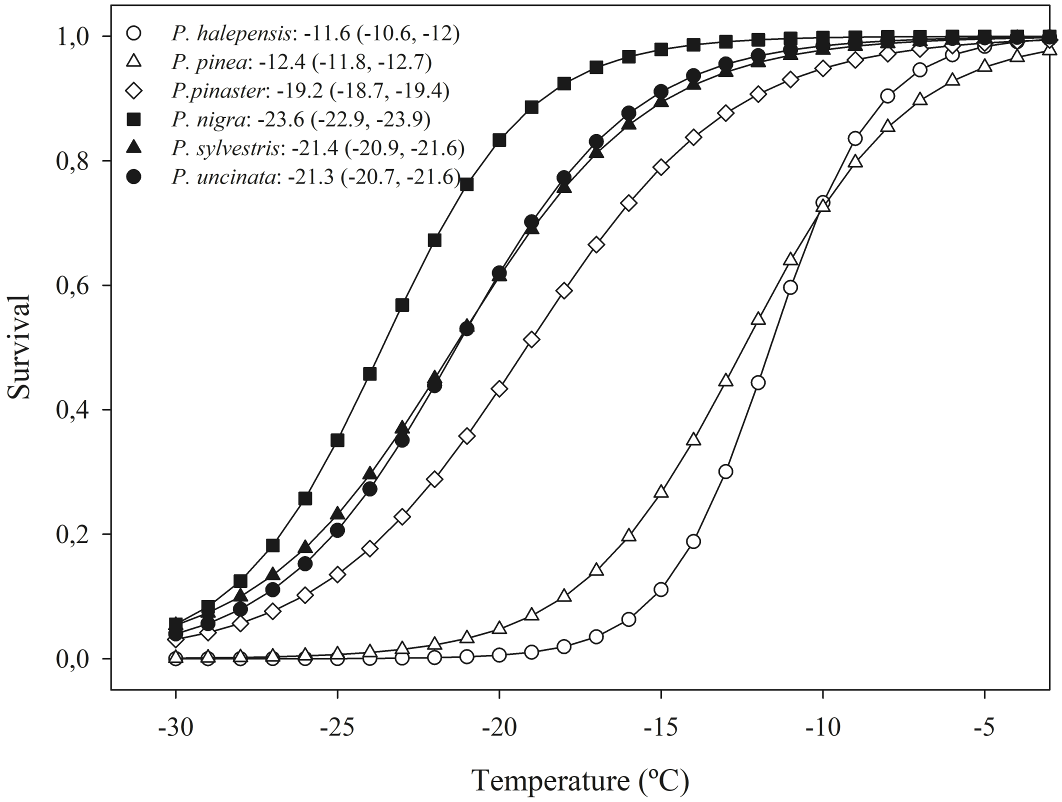

Fig. 1 Relationship between survival and freezing temperatures among six pine species distributed in Europe. Data inserted in the figure are the mean LT50 values (ºC) and the 95% confidence interval (in brackets). See Materials and methods for data fitting details.

3.2 Foliage electrolyte leakage

Across species, a single −15 °C frost increased foliage EL relative to unfrozen plants, particularly in *P. halepensis* and *P. pinea* (Figure 2). However, the effect of the frost on EL depended on seedling ψ_{pd} and the species (Species × Frost × ψ_{pd} interaction, Table 1). Thus, among the unfrozen seedlings, reduction in ψ_{pd} increased EL in *P. nigra* and *P. sylvestris*, while EL remained low and was unaffected by ψ_{pd} in the remaining species. Among the frozen plants, species differences in EL depended on seedling water status. Frozen *P. halepensis* seedlings had the highest EL values and reduction in ψ_{pd} did not affect EL. Reduction in seedling ψ_{pd} strongly decreased EL after frost in *P. pinea* and to a lesser extent in *P. sylvestris*. In contrast, reduction of seedling ψ_{pd} increased foliage EL after frost in *P. uncinata*. Finally, reduction in ψ_{pd} had no effect on EL after frost in *P. pinaster* and *P. nigra*.

3.3 Percentage loss in stem hydraulic conductivity and tracheid diameter

Frost and ψ_{pd} did not affect PLC (Table 1). However, *P. uncinata*, *P. nigra* and to a less extent *P. sylvestris*, tended to have slightly higher PLC values than the rest of the species (Table 2). Species differed in tracheid diameter ($P < 0.001$; Table 2). *Pinus uncinata* had the narrowest tracheids followed by *P. halepensis*. In contrast, *P. pinaster* and *P. pinea* had the widest tracheids, while *P. nigra* and *P. sylvestris* had intermediate tracheid diameter. We did not find any significant correlation between PLC and ψ_{pd} (*P. halepensis*: $r = -0.17$, $P = 0.35$; *P. pinea*: $r = 0.064$, $P = 0.72$; *P. pinaster*: $r = -0.06$, $P = 0.73$; *P. nigra*: $r = 0.18$, $P = 0.33$; *P. sylvestris*: $r = 0.16$, $P = 0.36$; *P. uncinata*: $r = -0.33$, $P = 0.05$).

Table 1. Model results of the effect of predawn water potential, frost, species and their interactions on the needle electrolyte leakage (EL) and percentage loss in stem hydraulic conductivity (PLC) in six pine species.

Effect	Needle EL			PLC		
	DF	c^2	*p*-value	DF	F value	*p*-value
Predawn water potential (ψ_{pd})	1	5.43	0.020	1	0.0119	0.91
Frost	1	168	< 0.0001	1	0.881	0.35
Species	5	39.4	< 0.0001	5	2.26	0.050
ψ_{pd} × Frost	1	4.45	0.035	1	1.66	0.20
Frost × Species	5	56.0	< 0.0001	5	0.615	0.69
ψ_{pd} × Species	5	10.7	0.060	5	1.35	0.24
ψ_{pd} × Frost × Species	5	25.2	< 0.0001	5	0.693	0.63

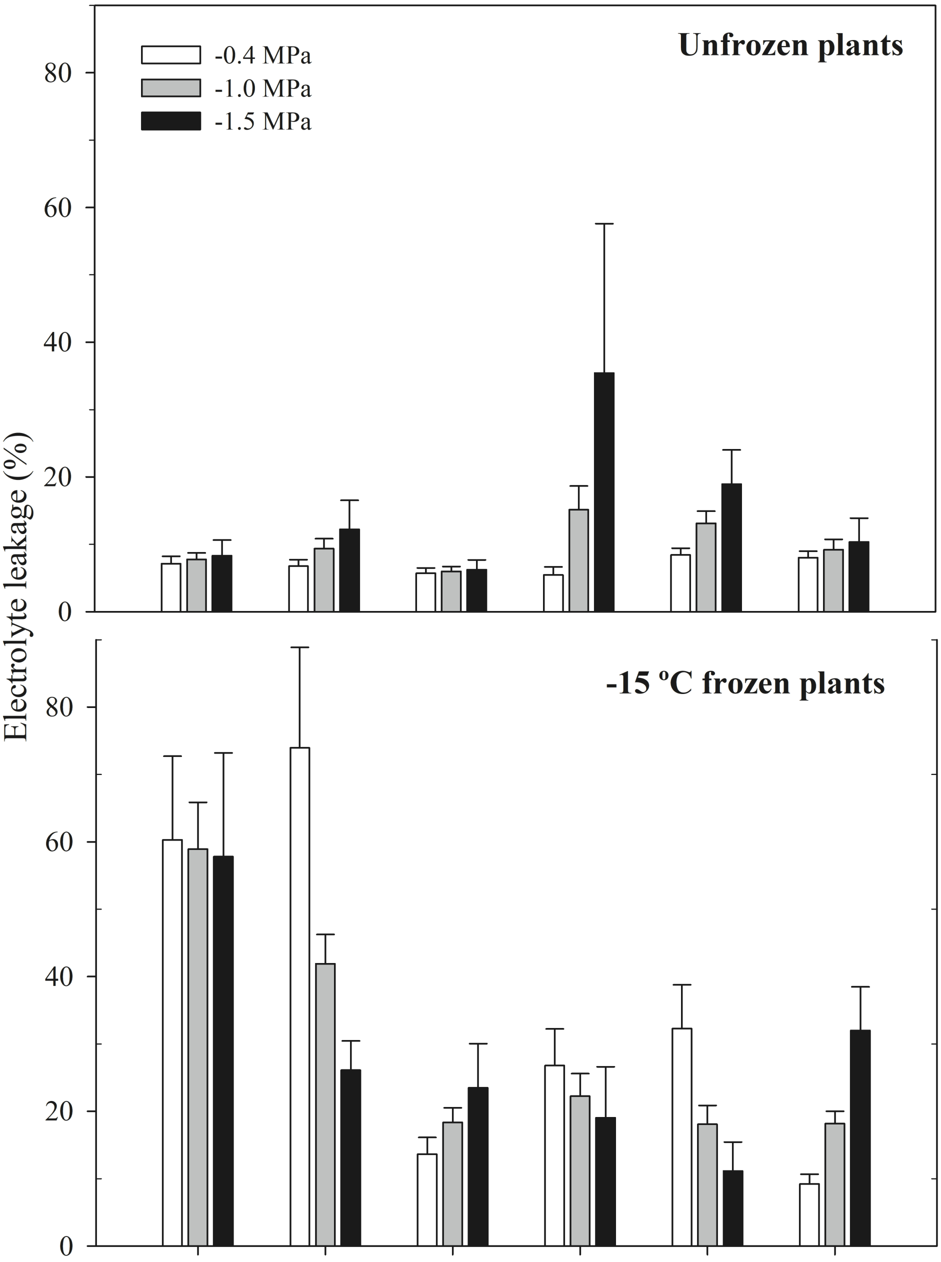

Fig. 2 Variation of foliage electrolyte leakage with seedling ψ_{pd} in unfrozen (upper panel) and frozen seedlings (subjected to a single -15°C frost, lower panel) in six pine species. Data are least square means ±SE, estimated at -0.4, -1.0 and -1.5 MPa after ANCOVA.

Table 2. Percent loss in stem hydraulic conductivity (PLC) after a single -15 ºC treatment, and tracheid diameters and concentration of different foliage soluble sugars of unfrozen, well-watered plants of six Iberian pine species. Data are mean ± SE. Same letter indicates not significant differences. SML: sucrose+maltose+lactose.

Specie	**PLC (%)**	**Tracheid diameter (mm)**	**Soluble sugars (mg g^{-1})**					
			Raffinose	**Glucose**	**Fructose**	**Fructose**	**SML**	**Total**
P. halepensis	15.0±1.24 c	7.6±0.44 bc	0.53±0.42	41.0±5.9 a	30.2±6.3 a	1.8±0.4 a	1.8±0.4 a	73.5±11.3 ab
P. pinea	16.6±1.28 bc	10.5±0.3 a	0.31±0.49	34.7±5.2 ab	27.9±3.9 ab	0.9±0.3 b	0.9±0.3 b	63.8±8.4 abc
P. pinaster	16.0±0.89 bc	10.6±0.63 a	Not detected	41.0±11 a	30.6±4.2 a	0.9±0.3 b	0.9±0.3 b	80.0±12.3 a
P. nigra	21.8±1.35 a	9.5±0.72 ab	0.61±0.66	26.3±8.9 ab	23.1±6.1 ab	0.2±0.3 c	0.2±0.3 c	50.2±14 c
P. sylvestris	20.0±1.22 ab	9.6±0.32 ab	0.80±0.57	22.4±8.0 b	19.9±4.2 b	0.1±0.2 c	0.1±0.2 c	43.2±10.5 c
P. uncinata	23.8±1.24 a	6.6±0.37 c	0.33±0.47	31.5±8.5 ab	24.7±5.2 ab	0.1±0.2 c	0.1±0.2 c	56.6±11.7 bc

3.4 Soluble sugars

Four SS were detected in the foliage in the following concentration trend: glucose > fructose > galactose > raffinose. A complex of sucrose, maltose and lactose (SML) was also detected in all species. Raffinose occurred at a very low concentration, it was found in all species but *P. pinaster* and no species differences in raffinose were observed (Table 2). Galactose was only found in *P. pinaster* (7.51 ± 3.9 mg g^{-1}). The total concentration of SS, SML and glucose and fructose showed significant differences among species ($P < 0.0001$): *P. halepensis* and *P. pinaster* had overall the highest concentration while *P. nigra* and *P. sylvestris* had the lowest concentrations. Across species, total concentration of SS tended to increase with LT_{50}, but the correlation was not statistically significant ($r = 0.59$, $P = 0.22$).

4. DISCUSSION

4.1 Differences in seedling frost survival are related to the natural distribution of pine species in Europe

Juveniles of the pine species studied showed remarkable differences in their ability to survive freezing temperatures. These differences in survival are largely consistent with their range in the Iberian Peninsula, suggesting that the distribution of studied pine species is in part controlled by low temperatures. Our frost survival results agree with the distribution of theses species at European scale; but the use of one provenance per species restricted to the Iberian Peninsula requires some caution. *Pinus sylvestris* reaches very high latitudes and together with *P. uncinata* and *P. nigra* constitute the tree line in many mountains in the Iberian Peninsula and in other areas in southern Europe (Barbero et al. 1998, Strimbeck and Schaberg 2009). These pine species had LT_{50} values <−21 °C. On the contrary, *P. halepensis* and *P. pinea* showed almost two-fold higher LT_{50} values than the pines of the cold winter zones; a single −10 °C frost may cause 30% mortality in both species. The low frost tolerance of *P. halepensis* and *P. pinea* is consistent with their distribution in mild to cool-winter locations in southern Europe and indicate that strong frosts likely limit the colonization of these Mediterranean pines at higher latitude and altitude locations. Consistent with our findings, Climent et al. (2009) observed a negative relationship between the needle frost damage and the mean temperature of the coldest month of the seed source across several pine species.

Similarly, comparing several provenances, Bachofen et al. (2016) observed that *P. halepensis* needles were less frost tolerant than *P. sylvestris* and *P. nigra* needles. An unexpected result was that *P. nigra* had a slightly higher frost tolerance than *P. sylvestris* and *P. uncinata*. Climent et al. (2009) also observed that *P. nigra* secondary needles were less frost damaged than *P. sylvestris* needles. In the high mountains of southern Europe, *P. nigra* is distributed slightly below the *P. sylvestris* and clearly below *P. uncinata* altitudinal belts (Tapias et al. 2011), which would imply that *P. nigra* should be less frost tolerant than *P. sylvestris* and *P. uncinata*. This conflict between frost tolerance and the altitudinal distribution of *P. nigra* may be explained by provenances used in the study or species differences in competitive capacity at the coldest sites and/or the lower capacity of *P. sylvestris* and *P. uncinata* to withstand summer drought (Herrero et al. 2013, Matias et al. 2017, Tíscar et al. 2017). Drought usually increases at lower altitudes in the mountains of southern Europe (Barbero et al. 1998). Frost tolerance was significantly higher in *P. pinaster* than in two species with which it frequently coexists, *P. halepensis* and *P. pinea*. *Pinus pinaster* has remarkable interpopulation differences in frost tolerance with the provenances of cold continental climates having higher frost tolerance than the mild winter sources (Corcuera et al. 2011). In this study, the seeds used to cultivate *P. pinaster plants* come from a cold winter site (see Table S1 available as Supplementary Material), which may explain the observed high frost tolerance of this species. Similarly, we selected rather cold and continental provenances for *P. halepensis* and *P. pinea* and provenances for *P. sylvestris* and *P. nigra* near the rear edge of their southern distribution in Europe, which might have contributed to reduce species differences in frost tolerance. Consequently, results in this study probably show a conservative measurement of the cold tolerance differences among studied species.

4.2 Frost tolerance differences among pine species is explained by needle frost.

Our results provide insights into the mechanisms underlying the differences in frost survival among pines. Electrolyte leakage is a measure of cell membrane integrity after subjecting plants to stress (Earnshaw 1993). We found that the two Mediterranean pines, *P. halepensis* and *P. pinea*, which showed the lowest frost survival presented the highest needle EL values after a frost of −15 °C (particularly at high ψ_{pd}, Figure 2). This indicates that the low frost survival of the Mediterranean pines is mostly due to differences in the vulnerability of foliage to low temperature. We cannot exclude that differences in other organs, such as roots also contribute to the observed species differences in frost survival. Toca et al. (2018) reported that root frost tolerance in *P. halepensis* and *P. pinea* is lower than in *P. pinaster* and much lower than in *P. nigra*.

Glucose and fructose were the most important sugars in the SS pool in all species, consistent with previous findings in other conifers (Hoch et al. 2003). In contrast, raffinose concentration remained low in all species, which contrasts with results in Strimbeck and Schaberg (2009), who reported high leaf concentrations of raffinose and that seasonal increase in raffinose was positively associated to higher frost tolerance in several conifer species. Similar to our findings, Hoch et al. (2003) observed very low levels of raffinose at the end of the winter in *P. sylvestris*. The concentration of SS in plant tissues increases during cold acclimation of perennial plants in temperate and cold biomes to a maximum in the middle of the winter, coinciding with the period of greatest frost tolerance (Chomba et al. 1993, Cavender-Bares et al. 2005, Tjoelker et al. 2008). Soluble sugars increase the fluidity and stability of the cellular membranes, which prevents tissue freezing damage (Sakai and Larcher 1987, Uemura and Steponkus 2003). Contrary to our expectation we did not find SS concentration to increase with frost tolerance across pine species. Rather, pines that live in mild or cool winter sites tended to have higher SS concentration than cold winter pines (Table 2). Most of the studies that have demonstrated a relationship between frost tolerance and SS are at the intraspecific scale (Hinesley et al. 1992, Ögren et al. 1997, Charrier et al. 2013). For example, Charrier et al. (2013) observed SS to increase with frost tolerance in 9 out of 11 tree species. In contrast to intraspecific comparisons, interspecific studies on the relation between frost tolerance and tissue SS are relatively scarce. Among *Quercus* species no clear relationship was observed between frost tolerance and tissue SS (Cavender-Bares et al. 2005, Morin et al. 2007). Overall, these results suggest that SS do not necessarily explain interspecific differences in frost tolerance. It is possible that compounds other than SS, such as specific proteins (Kontunen-Soppela et al. 2000), might be more important to explain frost tolerance across species.

Plants from cold climates tend to have higher respiration rates than their counterparts from mild climates (Mariko and Koizumi 1993, Reich et al. 1996, Heskel et al. 2016), which may lead to higher non-structural carbohydrate consumption under cool conditions. As the plants used in our study were cultivated in an open nursery, where the mean air temperature varied between 0 and 8 °C most of the winter, species differences in respiration and probably in photosynthesis may explain the lower SS concentration attained by the most frost tolerant species (*P. uncinata*, *P. sylvestris* and *P. nigra*) relative to frost intolerant ones (*P. halepensis* and *P. pinea*). Similarly, growth differences among species were large (see Table S2 available as Supplementary Material), which may have affected the species SS differences.

In contrast to needle frost sensitivity differences, the very low PLC values and the lack of species differences in PLC after exposure to −15 °C indicate that freezing-induced xylem embolism does not explain frost survival differences among pine species. Vulnerability to freezing-induced embolism is directly related to the diameter of xylem conduits and increases with decreasing xylem water potential (Sperry 1995, Davis et al. 1999, Pittermann and Sperry 2003). Xylem conduits in conifers are much narrower and consequently safer than angiosperm vessels (Cavender-Bares 2005, Pittermann and Sperry 2006). We observed that species differences in tracheid diameter were relative small, with average values ranging from 6.6 to 10.6 μm and unrelated to native PLC (Table 2). For frost-induced embolisms to occur in such narrow tracheids, xylem water potential must be <-2.0 MPa (Pittermann and Sperry 2006), which is significantly lower than both the lowest ψ_{pd} to which the plants were subjected in our study and the ψ_{pd} that these species commonly experience in the field during winter (Martinez-Ferri et al. 2004, Poyatos et al. 2008, Peguero-Pina et al. 2011).

A very interesting result of our study was that a moderate increase in drought stress (ψ_{pd} up to −1.5 MPa) modulated the effect of frost on foliage damage. However, this effect differed among species (Figure 2) and, apparently, it was not associated with species ecology. Drought stress significantly reduced frost damage in *P. sylvestris* and, especially, in *P. pinea*. However, it slightly increased it in *P. uncinata* and had minor effects in the rest of the species. A similar reduction in frost damage in drought-stressed *P. sylvestris* plants was reported by Sutinen et al. (1992). However, our results for *P. sylvestris* are not consistent with the interpretation by Peguero-Pina et al. (2011). They mentioned that defoliation in *P. silvestris* population at the southern edge of the species range during a dry winter could be a result of the fact that drought-stressed trees were more predisposed to frost damage than relatively hydrated trees.

It is important to note that the EL values after freezing to −15 °C in *P. pinea* seedlings subjected to $\psi_{pd} <-1$ MPa were similar to the EL values of the most frost tolerant pine species at high ψ_{pd}. Similarly, Villar-Salvador et al. (2013) observed that *P. pinea* seedlings subjected to moderate drought stress cycles showed higher tolerance to frost than well-watered plants. The increase in frost tolerance when plants are subjected to moderate drought stress may explain that *P. pinea* can develop in sandy soils in the north of the Iberian Peninsula where the winters are colder than those experienced by *P. pinea* throughout much its range (Campelo et al. 2006). Increases in frost tolerance with drought can be due to activation of generic stress tolerance physiological mechanisms such as abscisic acid or changes in cell membrane composition in lipids and proteins induced by either frost or drought stress (Mäntylä et al. 1995, Pearce 2001, Shinozaki et al. 2003).

5. CONCLUSIONS

Our study evidences remarkable differences in the ability of juveniles of six common pines in Europe to survive to frosts, which were related to the winter climatic conditions within their Iberian range. This supports the idea that frost is an important filter for the distribution of the studied pine species in the Iberian Peninsula and likely in the rest of Europe. The Mediterranean pines, *P. halepensis* and *P. pinea*, were the least frost tolerant species, while *P. sylvestris*, *P. uncinata* and *P. nigra*, which inhabit the coldest locations, were the most frost tolerant species. Interspecific differences in frost tolerance were due to differences in the frost tolerance of foliage, and not to different vulnerability to freezing-induced xylem embolism. Drought increased frost tolerance in *P. pinea* and *P. sylvestris*, but decreased it in *P. uncinata*. Species showing high frost survival had similar or lower concentration of SS than species having a low frost tolerance, indicating that other elements are more important than SS in determining differences in frost tolerance across pine species. Our results suggest that the expected increase in winter temperatures associated with climate change will reduce the cold limitations of Mediterranean pines to colonize sites at higher latitude and altitude in Europe.

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8. REFERENCES

Bachofen C, Wohlgemuth T, Ghazoul J, Moser B (2016) Cold temperature extremes during spring do not limit the range shift of Mediterranean pines into regions with intermittent frost. Funct Ecol 30:856–865.

Barbero M, Loisel R, Quezel P, Richardson DM, Romane F (1998) Pines of the Mediterranean basin. In: Richardson DM (ed) Ecology and biogeography of Pinus. Cambridge University Press, Cambridge, UK, pp 153–170.

Beck E, Heim R, Hansen J (2004) Plant resistance to cold stress: mechanisms and environmental signals triggering frost hardening and dehardening. J Biosci 29:449–459.

Beck E, Fetting S, Knake C, Hartig K, Bhattarai T (2007) Specific and unspecific responses of plants to cold and drought stress. J Biosci 32: 501–510.

Campelo F, Nabais C, Freitas H, Gutierrez E (2006) Climatic significance of tree-ring width and intra-annual density fluctuations in Pinus pinea from a dry Mediterranean area in Portugal. Ann For Sci 64:229–238.

Castro J (1999) Seed mass versus seedling performance in Scots pine: a maternally dependent trait. New Phytol 144:153–161.

Cavender-Bares J (2005) Impacts of freezing on long distance transport in woody plants. In: Holbrook N, Zwieniecki M (eds) Vascular transport in plants. Academic Press, Elservier, pp 401–424.

Cavender-Bares J, Cortes P, Rambal S, Joffre R, Miles B, Rocheteau A (2005) Summer and winter sensitivity of leaves and xylem to minimum freezing temperatures: a comparison of co-occurring Mediterranean oaks that differ in leaf lifespan. New Phytol 168: 597–612.

Charrier G, Cochard H, Ameglio T (2013) Evaluation of the impact of frost resistances on potential altitudinal limit of trees. Tree Physiol 33: 891–902.

Choat B, Jansen S, Brodribb T et al. (2012) Global convergence in the vulnerability of forests to drought. Nature 491:752–755.

Chomba B, Guy R, Weger H (1993) Carbohydrate reserve accumulation and depletion in Engelmann spruce (Picea engelmannii Parry): effects of cold storage and pre-storage CO2 enrichment. Tree Physiol 13: 351–364.

Chow P, Landhäusser S (2004) A method for routine measurements of total sugar and starch content in woody plant tissues. Tree Physiol 24: 1129–1136.

Climent J, Costa e Silva F, Chambel M, Pardos M, Almeida M (2009) Freezing injury in primary and secondary needles of Mediterranean pine species of contrasting ecological niches. Ann For Sci 66: 407–407.

Cochard H (2002) A technique for measuring xylem hydraulic conductance under high negative pressures. Plant Cell Environ 25:815–819.

Collett D (1991) Modeling binary data. Chapman and Hall, London.

Corcuera L, Gil-Pelegrin E, Notivol E (2011) Intraspecific variation in Pinus pinaster PSII photochemical efficiency in response to winter stress and freezing temperatures. PLoS One 6:1–12.

Davis S, Sperry J, Hacke U (1999) The relationship between xylem conduit diameter and cavitation caused by freezing. Am J Bot 86: 1367–1372.

Dobbertin M, Mayer P, Wohlgemuth T, Feldmeyer-Christe E, Graf U, Zimmermann N, Rigling A (2005) The decline of *Pinus sylvestris* L. forests in the Swiss Rhone valley—a result of drought stress? Phyton Ann Rei Bot 45:153–156.

Earnshaw MJ (1993) Stress indicators: electrolyte leakage. In: Hendry GAF, Grime JP (eds) Methods in comparative plant ecology. A laboratory manual. Chapman and Hall, London, UK, pp 152–154.

Grossnickle S, Arnott J, Major J, Tschaplinski T (1991) Influence of dormancy induction treatments on western hemlock seedlings. I. Seedling development and stock quality assessment. Can J For Res 21: 175–185.

Hawkins B, Guest H, Kolotelo D (2003) Freezing tolerance of conifer seeds and germinants. Tree Physiol 23:1237–1246.

Herrero A, Castro J, Zamora R, Delgado-Huertas A, Querejeta J (2013) Growth and stable isotope signals associated with drought-related mortality in saplings of two coexisting pine species. Oecologia 173: 1613–1624.

Heskel M, O'Sullivan O, Reich P et al. (2016) Convergence in the temperature response of leaf respiration across biomes and plant functional types. Proc Natl Acad Sci USA 113:3832–3837.

Hinesley L, Pharr D, Snelling L, Funderburk S (1992) Foliar raffinose and sucrose in four conifer species: Relationship to seasonal temperature. J Am Soc Hortic Sci 117:852–855.

Hoch G, Richter A, Körner C (2003) Non-structural carbon compounds in temperate forest trees. Plant Cell Environ 26:1067–1081.

Kontunen-Soppela S, Taulavuori K, Taulavuori E, Lahdesmaki P, Laine K (2000) Soluble proteins and dehydrins in nitrogen-fertilized Scots pine seedlings during deacclimation and the onset of growth. Physiol Plant 109:404–409.

Kullman L (1991) Cataclysmic response to recent cooling of a natural boreal pine (*Pinus sylvestris* L.) forest in northern Sweden. New Phytol 117:351–360.

Lamy JB, Delzon S, Bouche PS, Alia R, Vendramin GG, Cochard H, Plomion C (2014) Limited genetic variability and phenotypic plasticity detected for cavitation resistance in a Mediterranean pine. New Phytol 201:874–886.

Langan SJ, Ewers FW, Davis SD (1997) Differential susceptibility to xylem embolism caused by freezing and water stress in two species of chaparral shrubs. Plant Cell Environ 20:425–437.

Mäntylä E, Lang V, Palva E (1995) Role of abscisic acid in droughtinduced freezing tolerance, cold acclimation, and accumulation of LT178 and RAB18 Proteins in Arabidopsis thaliana. Plant Physiol 107: 141–148.

Mariko S, Koizumi H (1993) Respiration for maintenance and growth in Reynoutria japonica ecotypes from different altitudes on Mt Fuji. Ecol Res 8:241–246.

Martinez-Ferri E, Manrique E, Valladares F, Balaguer L (2004) Winter photoinhibition in the field involves different processes in four cooccurring Mediterranean tree species. Tree Physiol 24:981–990.

Martínez-Vilalta J, Piñol J (2002) Drought-induced mortality and hydraulic architecture in pine populations of the NE Iberian Peninsula. For Ecol Manage 161:247–256.

Martínez-Vilalta J, Sala A, Piñol J (2004) The hydraulic architecture of Pinaceae – a review. Pflant Ecol 171:3–13.

Martínez-Vilalta J, Cochard H, Mencuccini M et al. (2009) Hydraulic adjustment of Scots pine across Europe. New Phytol 184:353–364.

Martínez-Vilalta J, Sala A, Asensio D, Galiano L, Hoch G, Palacio S, Piper F, Lloret F (2016) Dynamics of non-structural carbohydrates in terrestrial plants: a global synthesis across Europe. Ecol Monogr 86: 495–516.

Matias L, Castro J, Villar-Salvador P, Quero J, Jump A (2017) Differential impact of hotter drought on seedling performance of five ecologically distinct pine species. Plant Ecol 218:201–212.

Mayr S, Schwienbacher F, Bauer H (2003) Winter at the alpine timberline. Why does embolism occur in norway spruce but not in Stone pine? Plant Physiol 131:780–792.

Mayr S, Hacke U, Schmid P, Schwienbacher F, Gruber A (2006) Frost drought in conifers at the alpine timberline: xylem dysfunction and adaptations. Ecology 87:3175–3185.

McCulloh K, Johnson D, Meinzer F, Lachenbruch B (2011) An annual pattern of native embolism in upper branches of four tall conifer species. Am J Bot 98:1007–1015.

McDowell N (2011) Mechanisms linking drought, hydraulics, carbón metabolism, and vegetation mortality. Plant Physiol 155: 1051–1059.

Medeiros J, Pockman W (2011) Drought increases freezing tolerance of both leaves and xylem of Larrea tridentata. Plant Cell Environ 34: 43–51.

Mitrakos KA (1980) A theory for Mediterranean plant life. Acta Oecol 1: 245–252.

Morin X, Améglio T, Ahas R, Kurz-Besson C, Lanta V, Lebourgeois F, Miglietta F, Chuine I (2007) Variation in cold hardiness and carbohydrate concentration from dormancy induction to bud burst among provenances of three European oak species. Tree Physiol 27: 817–825.

Ögren E, Nilsson T, Sundblad L (1997) Relationship between respiratory depletion of sugars and loss of cold hardiness in coniferous seedlings over-wintering at raised temperatures: indications of different sensitivities of spruce and pine. Plant Cell Environ 20:247–253.

Pearce RS (2001) Plant freezing and damage. Ann Bot (Lond) 87: 417–424.

Peguero-Pina J, Alquézar-Alquézar J, Mayr S, Cochard H, Gil-Pelegrín E (2011) Embolism induced by winter drought may be critical for the survival of *Pinus sylvestris* L. near its southern distribution limit. Ann For Sci 68:565–574.

Pittermann J, Sperry J (2003) Tracheid diameter is the key trait determining the extent of freezing-induced embolism in conifers. Tree Physiol 23:907–914.

Pittermann J, Sperry J (2006) Analysis of freeze-thaw embolism in conifers. The interaction between cavitation pressure and tracheid size. Plant Physiol 140:374–382.

Pockman W, Sperry J (1997) Freezing-induced xylem cavitation and the northern limit of Larrea tridentata. Oecologia 109:19–27.

Poyatos R, Llorens P, Piñol J, Rubio C (2008) Response of Scots pine (*Pinus sylvestris* L.) and pubescent oak (Quercus pubescens Willd.) to soil and atmospheric water deficits under Mediterranean mountain climate. Ann For Sci 65:306.

Reich P, Oleksyn J, Tjoelker M (1996) Needle respiration and nitrogen concentration in Scots Pine populations from a broad latitudinal range: A common garden test with field-grown trees. Funct Ecol 10:768–776.

Richardson DM (1998) Ecology and biogeography of Pinus. Cambridge University Press, Cambridge, UK.

Robson DJ, McHardy WJ, Petty JA (1988) Freezing in conifer xylem: II. Pit aspiration and bubble formation. J Exp Bot 39:1617–1621.

Sakai A, Larcher W (1987) Frost survival of plants: responses and adaptation to freezing stress. Springer-Verlag, Berlin, Heidelberg.

Serrano L, Peñuelas J, Ogaya R, Savé R (2005) Tissue-water relations of two co-occurring evergreen Mediterranean species in response to seasonal and experimental drought conditions. J Plant Res 118: 263–269.

Shinozaki K, Yamaguchi-Shinozaki K, Seki M (2003) Regulatory network of gene expression in the drought and cold stress responses. Curr Opin Plant Biol 6:410–417.

Sperling O, Secchi F, Godfrey J, Zwieniecki M (2017) Acclimation of Pistacia integerrima trees to frost in semi-arid environments depends on autumn's drought. Planta 245:671–679.

Sperry JS (1995) Limitations on stem water transport and their consequences. In: Gartner B (ed) Plant stems. Academic Press, London, UK, pp 105–124.

Sperry J, Sullivan J (1992) Xylem embolism in response to freeze-thaw cycles and water stress in ring-porous, diffuse-porous, and conifer species. Plant Physiol 100:605–613.

Strand M, Öquist G (1988) Effects of frost hardening, dehardening and freezing stress on in vivo chlorophyll fluorescence of seedlings of Scots pine (*Pinus sylvestris* L.). Plant Cell Environ 11:231–238.

Strimbeck G, Schaberg P (2009) Going to extremes: low temperature tolerance and acclimation in temperate and boreal conifers. In: Gusta LV, Wisniewski ME, Tanino KK (eds) Plant cold hardiness: from the laboratory to the field. CABI Publishing, Oxfordshire, pp 226–239.

Sutinen M, Palta J, Reich P (1992) Seasonal differences in freezing stress resistance of needles of Pinus nigra and Pinus resinosa: evaluation of the electrolyte leakage method. Tree Physiol 11:241–254.

Tapias R, Climent J, Pardos J, Gil L (2011) Life histories of Mediterranean pines. Plant Ecol 171:53–68.

Tíscar P, Candel-Pérez D, Estrany J, Balandier P, Gómez R, Lucas-Borja M (2017) Regeneration of three pine species in a Mediterranean forest: a study to test predictions from species distribution models under changing climates. Sci Total Environ 584–585:78–87.

Tjoelker M, Oleksyn J, Reich P, Zytkowiak R (2008) Coupling of respiration, nitrogen, and sugars underlies convergent temperature acclimation in Pinus banksiana across wide-ranging sites and populations. Glob Chang Biol 14:782–797.

Toca A, Oliet J, Villar-Salvador P, Maroto J, Jacobs DF (2018) Species ecology determines the role of nitrogen nutrition on the frost tolerance of pine seedlings. Tree Physiol (in press) doi:10.1093/treephys/tpx165.

Uemura M, Steponkus P (2003) Modification of the intracellular sugar content alters the incidence of freeze-induced membrane lesions of protoplasts isolated from Arabidopsis thaliana leaves. Plant Cell Environ 26:1083–1096.

Uemura M, Tominaga Y, Nakagawara C, Shigematsu S, Minami A, Kawamura Y (2006) Responses of the plasma membrane to low temperatures. Physiol Plant 126:81–89.

Uscola M, Villar-Salvador P, Gross P, Maillard P (2015) Fast growth involves high dependence on stored resources in seedlings of Mediterranean evergreen trees. Ann Bot 115:1001–1013.

Villar-Salvador P, Peñuelas J, Jacobs D (2013) Nitrogen nutrition and drought hardening exert opposite effects on the stress tolerance of Pinus pinea L. seedlings. Tree Physiol 33:221–232.

Wesley-Smith J, Walters C, Pammenter N, Berjak P (2015) Why is intracellular ice lethal? A microscopical study showing evidence of programmed cell death in cryo-exposed embryonic axes of recalcitrant seeds of Acer saccharinum. Ann Bot 115:991–1000.

Willson C, Jackson R (2006) Xylem cavitation caused by drought and freezing stress in four co-occurring Juniperus species. Physiol Plant 127:374–382.

Woodward F, Williams B (1987) Climate and plant distribution at global and local scales. Vegetatio 69:189–197.

Zimmermann MH (1983) Xylem structure and the ascent of sap. Springer, Berlin.

Zwieniecki MA, Holbrook NM (2009) Confronting Maxwell's demon: biophysics of xylem embolism repair. Trends Plant Sci 14:530–534.

9. SUPLEMENTARY DATA

Figure S1. Range and altitudinal segregation (inserted pictograph) of the natural stands of pine species native to Spain. Yellow symbols represent the species in the pictograph and the location of the seed sources of each species used to cultivate the seedlings. Source: Third National Forestry Inventory of the Spanish government http://www.mapama.gob.es/es/biodiversidad/servicios/banco-datos-naturaleza/informacion-disponible/ifn3.aspx

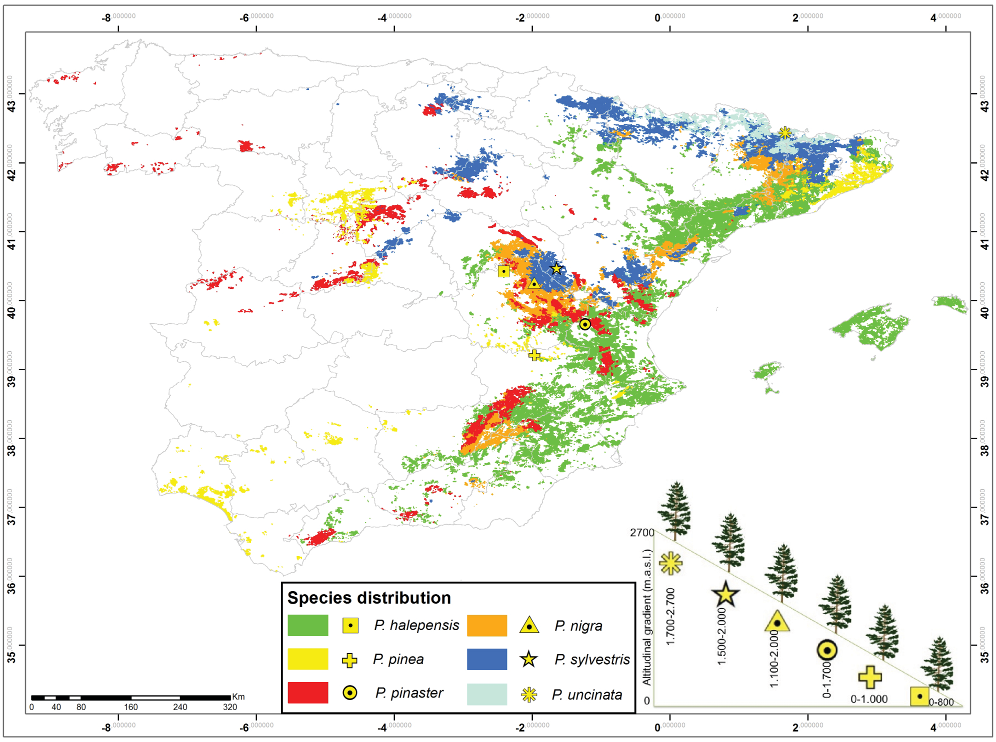
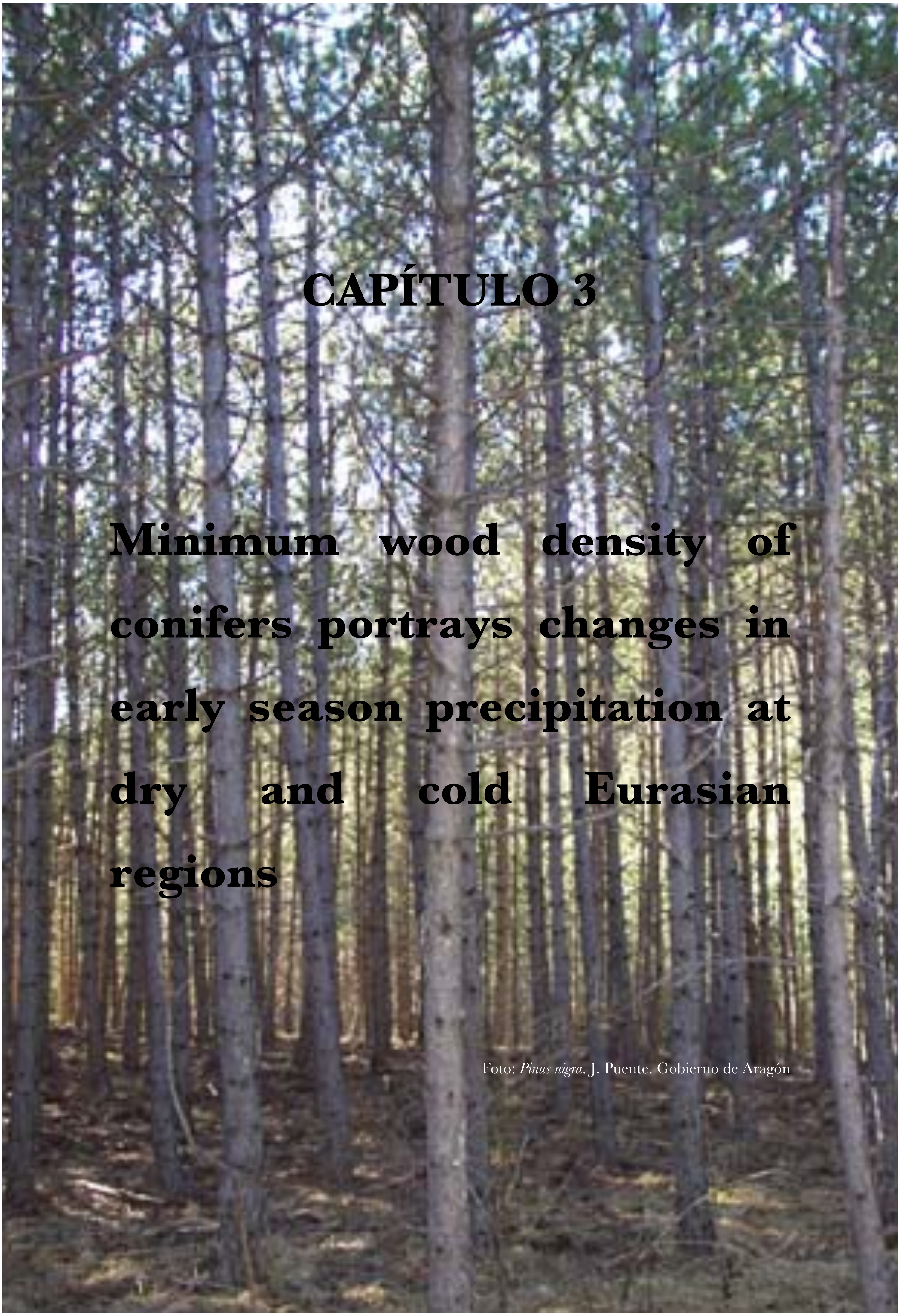


Table S1. Geographic location and climatic characteristics of the provenances where seeds of the study were collected. MAT= mean annual temperature, TCM= mean of the minimum temperatures of the coldest month. Provenance names follow nomenclature in Alía et al (2009)*.

Species	**Provenance**	**Latitude (N)**	**Longitude (W)**	**Altitude (masl)**	**MAT (°C)**	**TCM (°C)**	**Annual rainfall (mm)**
P. halepensis	Alcarria	40°24’52”	2°24’33”	860	12.6	-0.6	580
P. pinea	La Mancha	39°12’02”	1°57’59”	675	14.2	0.7	397
P. pinaster	Cuenca	39°38’44”	1°13’52”	1135	12	-1.5	540
P. nigra subsp. *salzmanii*	Sistema Ibérico Meridional	40°15’16”	1°58’22”	1515	10.4	-3.2	617
P. sylvestris	Montes Universales	40°28’09”	1°38’42”	1725	9.2	-4.8	894
P. uncinata	Pirineo Central	42°25’59”	1°40’18”	2050	5.3	-7.5	888

* Alía Miranda R, García del Barrio, J.M. Iglesias Sauce S, Mancha Núñez JA, de Miguel y del Ángel J, Nicolás Peragón JL, Pérez Martín F, Sánchez de Ron D (2009) Regiones de procedencia de especies forestales españolas. Organismo Autónomo de Parques Nacionales, Madrid, Spain.



CAPÍTULO 3

Minimum wood density of conifers portrays changes in early season precipitation at dry and cold Eurasian regions

Foto: *Pinus nigra*. J. Puente. Gobierno de Aragón

CAPÍTULO 3

Minimum wood density of conifers portrays changes in early season precipitation at dry and cold Eurasian regions

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Key message Minimum wood density exhibits strong responses to precipitation and, thus, it is a robust proxy of early season water availability.

RESUMEN

En las coníferas, las traqueidas cumplen la mayoría de las funciones de la madera (soporte mecánico y transporte de agua). La mayor parte de la conductividad hidráulica la llevan a cabo las traqueidas de la madera temprana dentro del anillo de crecimiento anual del árbol. Eventos de sequía durante la temporada de crecimiento, cuando se forma la madera temprana, pueden conducir a la formación de lúmenes estrechos en las traqueidas y una madera temprana densa. En este artículo evaluamos si existe una relación negativa entre la densidad mínima de madera y la precipitación de la estación de crecimiento temprana (primavera). Usando dendrocronología, estudiamos los datos de crecimiento y densidad en nueve rodales forestales de tres especies de Pinaceae (*Larix sibirica*, *Pinus nigra* y *Pinus sylvestris*) ampliamente distribuidas en tres regiones eurasiáticas frescas y secas de la estepa forestal (Rusia, Mongolia) y del Mediterráneo (España). Medimos para cada anillo anual de árbol y el período común de 1950-2002 las siguientes variables: madera temprana y ancho de madera tardía, y densidad de madera mínima y máxima. Como era de esperar, las condiciones secas durante la estación de crecimiento temprana (primavera) se asociaron con una baja producción de madera temprana pero, lo que es más importante, con una alta densidad mínima en las tres especies de coníferas. Las asociaciones entre la densidad mínima y la precipitación primaveral fueron más fuertes (r = -0.65) que las observadas con el ancho de la madera temprana (r = 0.57). Interpretamos la relación entre la disponibilidad de agua y la alta densidad mínima como una reducción inducida por la sequía del diámetro del lumen, la conductividad hidráulica y el crecimiento. En consecuencia, las condiciones secas pronosticadas en la estación de crecimiento se traducirían en un aumento de la densidad mínima de la madera y reflejarían una reducción en la conductividad hidráulica, el crecimiento radial y la formación de la madera. Dada la naturaleza del estudio de este trabajo, se necesitan más investigaciones sobre otros sitios secos y fríos con especies de coníferas adicionales para probar si la densidad mínima de madera es un indicador robusto de la disponibilidad de agua a principios de la temporada de crecimiento.

Palabras clave: Alerce siberiano, dendrocronología, densidad de la madera, disponibilidad de agua, pino negro, pino silvestre.

ABSTRACT

Tracheids fulfill most wood functions in conifers (mechanical support and water transport) and earlywood tracheids account for most hydraulic conductivity within the annual tree ring. Dry conditions during the early growing season, when earlywood is formed, could lead to the formation of narrow tracheid lumens and a dense earlywood. Here, we assessed if there is a negative association between minimum wood density and early growing-season (spring) precipitation. Using dendrochronology, we studied growth and density data at nine forest stands of three Pinaceae species (*Larix sibirica*, *Pinus nigra*, and *Pinus sylvestris*) widely distributed in three cool–dry Eurasian regions from the forest-steppe (Russia, Mongolia) and Mediterranean (Spain) biomes. We measured for each annual tree ring and the common 1950–2002 period the following variables: earlywood and latewood width, and minimum and maximum wood density. As expected, dry early growing season (spring) conditions were associated with low earlywood production but, most importantly, to high minimum density in the three conifer species. The associations between minimum density and spring precipitation were stronger (r=-0.65) than those observed with earlywood width (r=0.57). We interpret the relationship between spring water availability and high minimum density as a drought-induced reduction in lumen diameter, hydraulic conductivity, and growth. Consequently, forecasted growing-season drier conditions would translate into increased minimum wood density and reflect a reduction in hydraulic conductivity, radial growth, and wood formation. Given the case-study-like nature of this work, more research on other cold–dry sites with additional conifer species is needed to test if minimum wood density is a robust proxy of early season water availability.

Keywords: Black pine, Scots pine, Siberian larch, Dendroecology, Water availability, Wood density.

1. INTRODUCTION

In trees, wood fulfils multiple functions including mechanical support, transport, storage, and defense against biotic agents (Zobel and van Buijtenen 1989). The balances between these functions are reflected in changes of wood density between (Muller-Landau 2004) and within tree species (Martínez-Vilalta et al. 2009; Fajardo 2016). Trade-offs determining wood density result from the conflict between xylem filling with carbon-rich walls and parenchyma vs. leaving open conduit spaces (Carlquist 1975). A higher wood density provides greater strength but also entails higher construction costs (Niklas 1992). It is common that tree species forming narrow conduits and having high wood density show low growth rates (Chave et al. 2009). Conversely, conduit diameter increases in tree species with softer wood (Hacke and Sperry 2001; Bouche et al. 2014), thus providing a high hydraulic conductivity. However, presumed conflicts in wood functions may reflect evolutionary paths of co-varying traits, and caution must be taken to interpret such assumed trade-offs (Larjavaara and Muller-Landau 2010). In addition, wood density is a very conservative trait showing low variation along climatic gradients (Zhang et al. 2011). Nevertheless, since both radial growth and density determine forest carbon uptake, assessments of their responses to climate together with a better characterization of climate-growth-wood density associations are required (Bouriaud et al. 2015).

Conifers subjected to double stress during seasonal wood formation (i.e., growing under cold and dry conditions) are particularly suited to perform such assessments, because wood functions are mostly carried out by tracheids which are formed during a short growing season (Larson 1994). In conifers, wood density may be defined as the ratio of cell-wall thickness to transversal lumen diameter, which is a proxy of absolute wood density (Yasue et al. 2000). This thickness–span ratio is considered a surrogate of xylem hydraulic functions, since a large tracheid lumen (low thickness–span ratio) provides more hydraulic conductivity but increases the risk of frost- or drought-induced embolism (Hacke et al. 2001, 2015). Under dry conditions during the early growing season, conifers produce narrow earlywood tracheid lumens, which account for most hydraulic conductivity within the annual tree ring (Domec et al. 2009) and, therefore, a dense earlywood. However, large tracheid lumens have also been reported in Scots pine (*Pinus sylvestris*) provenances from dry sites (Martín et al. 2010) or in trees subjected to imposed dry conditions (Eilmann et al. 2011). These contradictory patterns require a better interpretation. Therefore, more research is needed to ascertain how density responds to changes in water availability during the growing season in conifers thriving in cold and seasonally dry climates. A negative association between minimum wood density and early growing-season (spring) precipitation was already observed in the Cupressaceae Spanish juniper (*Juniperus thurifera* L.) subjected to cool–dry conditions (Camarero et al. 2014). Nevertheless, this relationship has to be investigated in other species as pines and larches which are widely distributed in cool–dry regions and form the world's largest conifer forests (Richardson 1998). A reduction in hydraulic conductivity would be linked to narrower tracheid lumens and higher minimum density values (Pittermann et al. 2006). Thus, we hypothesize that dry spring conditions would increase minimum wood density. Specifically, we aim to: (1) analyse how seasonal growth (earlywood and latewood widths) and wood density components (minimum and maximum density) respond to climate (mean monthly and seasonal temperatures, and total precipitation); and (2) assess whether minimum wood density is consistently (negatively) associated with spring precipitation in Pinaceae species of two genera (*Larix* and *Pinus*) across a broad range of environmental conditions in Eurasia.

2. MATERIALS AND METHODS

2.1 Study sites and species

Sampling regions were selected to include Pinaceae species whose radial growth and wood formation were constrained by two stress types, namely low temperatures shortening the growing-season length and drought reducing the growth rates during the growing season. We selected four Eurasian regions from three countries subjected to cold and dry climatic conditions: southern Siberia and southern Urals in Russia, Khangai in Mongolia, and Sierra de Gúdar in eastern Spain (Table 1; Supporting Information, Fig. S1). Continental climate conditions (cold winters, ample temperature range) and warm conditions and low precipitation during the growing season constrain tree radial growth in all the study areas (Block et al. 2004, Dulamsuren et al. 2009, Velisevich and Kozlov 2006, Devi et al. 2008, Knorre et al. 2010, Camarero et al. 2015). In southern Siberia and Mongolia, we sampled Siberian larch (*Larix sibirica* Ledeb.), whilst Scots pine (*Pinus sylvestris* L.) was sampled in the Urals and Spain. In Spain, we also sampled Black pine (*Pinus nigra* Arn. subsp. *salzmannii* (Dunal) Franco), a typical Mediterranean tree species (Richardson 1998).

The northernmost study region is located in southern Siberian forest-steppe zone where open larch forests are typical (Dylis 1961, Knorre et al, 2010). The forest sampled in the Urals is situated in the Aldan plateau where open conifer forests predominate up to 1250 m (Devi et al. 2008). In Mongolian lowlands, sampled trees formed forest-steppe ecotones which constitute the lower treeline (Treter 2000, Dulamsuren et al. 2010). The southernmost study region is located in Sierra de Gu´dar (southern Iberian Range, eastern Spain) and includes two Scots pine stands and two Black pine stands. In this region, the study sites were distributed along an altitudinal gradient, because Scots pine dominates at cold sub-Mediterranean sites situated at higher elevation, whereas Black pine is dominant at mid-elevation sites experiencing Mediterranean climate (Camarero et al. 2015). We sampled one site (LR in Table 1) where the two species co-occur. The selected study stands were not impacted by local anthropogenic disturbances (grazing, fires, and logging) since the 1960s. The understory is dominated by shrubs in most of the study sites (e.g., *Juniperus communis* L.). Soils are brown in the Russian and Mongolian sites, whereas basic and clayey soils appear in Spain.

2.2 Field sampling

We randomly selected and sampled 15–30 dominant trees per site in ca. 1-ha large sampling areas (Table 1). A 10-mm wide core per tree was extracted at 1.3 m for densitometry analyses using Pressler increment borers. We took special care for sampling this last core perpendicular to the main stem so as to capture the main fibre direction. We also measured diameter at 1.3 m and total height of each tree using tapes and clinometers, respectively. Trees selected for sampling had diameters ranging between 39.0 and 47.0 cm, heights between 9.3 and 10.5 m, and ages (estimated at 1.3 m) between 79 and 318 years (Table 1). Stands are relatively open, with basal area values ranging between 12 and 38 m2 ha-1. We took two additional 5-mm wide cores from each tree for obtaining tree-ring width data so as to cross-date the densitometry samples. These cores were glued onto wooden mounts, sanded, visually cross-dated, and checked for dating accuracy using dendrochronology (Fritts 2001).

2.3 Width and density measurements

One radial X-ray density profile was obtained from each tree using indirect X-ray densitometry. Prior to further treatment, resin was extracted from the wood samples with alcohol in a Soxhlet extractor. Then, each core was cut carefully using a double-bladed saw to obtain ca. 1.5-mm thick laths. These samples were air dried to moisture equilibrium and then subjected to X-ray exposure. The resulting X-ray films were scanned with a resolution of 10 µm using a microdensitometer DENDRO-2003 (Walesch Electronics Ltd., Switzerland). The measured grey levels of the X-ray films were transferred to density values by comparing them to a standard of known physical and optical density also exposed on the same film. For each annual ring, the following variables were obtained from the tree-ring density profiles: earlywood (EW hereafter) and latewood widths (LW hereafter), and minimum (MN hereafter) and maximum wood densities (MX hereafter). Note that MN and MX are tightly related to earlywood and latewood mean densities, so we only analysed the former two variables, because they are easier to define and show a stronger response to climate variables (Camarero et al. 2014). To define the earlywood–latewood transition, we used the 50% level between the MN and MX values of each ring following Polge (1978), and confirmed this separation with a visual checking of the tree rings (Mäkinen and Hynynen 2014).

Table 1. Characteristics of the study sites. Age values are means ± SD.

Region (country)	**Species (code)**	**Site (code)**	**Latitude(N)**	**Longitude**	**Elevation (m a.s.l.)**	**No. trees**	**Age at 1.3 m (years)**	**Timespan with > 5 trees**
Siberia (Russia)	*Larix sibirica* (Ls)	Efremkino (EF)	54º 29’	89º 28’ E	520	17	194 ± 49	1766-2002
		Shira (KH)	54º 24’	89º 58’ E	590	24	142 ± 40	1877-2005
Southern Urals (Russia)	*Pinus sylvestri*s (Ps)	Aldanskoye (CA)	52º 11’	59º 44’ E	740	16	80 ± 9	1902-2005
Khangai (Mongolia)	*Larix sibirica* (Ls)	Khangai 2 (M7)	47º 24’	100º 43’ E	1920	24	137 ± 75	1838-2002
		Khangai 1 (M0)	46º 44’	102º 40’ E	1870	30	228 ± 80	1695-2002
Sierra de Gúdar (Spain)	*Pinus sylvestri*s (Ps)	Peñarroya (PN)	42º 23’	0º 39’ W	2020	16	79 ± 19	1906-2011
		Las Roquetas (LR)	40º 19’	0º 43’ W	1615	15	103 ± 20	1875-2011
	Pinus nigra (Pn)	Las Roquetas (LR)	40º 19’	0º 43’ W	1615	15	131 ± 33	1847-2011
		Alto de Cabra (AC)	40º 20’	0º 48’ W	1090	16	120 ± 21	1859-2011

2.4 Chronology building

The accurately dated tree-ring series produced in the previous step (EW, LW, MN, and MX) were individually detrended to remove non-climatic biological growth trends (Cook and Kairiukstis 1990). Prior to trend removal, however, a power transformation was applied to the raw density data. A 2/3 cubic smoothing spline with 50% frequency–response cutoff was fitted to the individual records and indexed values were calculated. Then, indexed tree-ring series were subjected to autoregressive modelling to remove the first-order autocorrelation and produce residual indices. This autoregressive (pre-whitening) method was applied, because instrumental target data may reflect white noise behaviour (Bu¨ntgen et al. 2010). Finally, site chronologies for each species and variable were obtained by averaging the residual indices on a yearly basis using a bi-weight robust mean. These procedures were performed using the library dplR in the R platform (Bunn 2008, Bunn et al. 2016). To compare the resulting chronologies, several statistics were calculated for the common period 1950–2002 considering either the raw site chronologies (mean; SD, standard deviation; AR1, first-order autocorrelation) or the residual chronologies (rbt, mean inter-series correlation; MSx, mean sensitivity, a measure of the relative variability between consecutive years; cf. Fritts 2011).

2.5 Climate data

Local climate data (monthly total precipitation and mean temperature) were retrieved from the nearest meteorological stations for all study sites (Table 2; Supporting Information, Fig. S2), excepting Mongolian sites which are located far away from climate stations with long and homogeneous precipitation records. There, precipitation was obtained from the nearest 0.5_ grid box to each sampling site of the high-resolution gridded climate data set (Climatic Research Unit, CRU TS 3.22; Harris et al. 2014). In Spain, local data from ten stations situated in the study area were converted into a regional climate series and elevation differences were corrected by calculating regressions between the station elevation and mean annual temperature or total annual precipitation. To take into account the elevation difference between climate stations and sampling sites, we corrected the regional mean temperature and precipitation data considering a mean lapse rate of -7.8 ºC km^{-1} and +420 mm km^{-1}, respectively (see more details in Sangüesa-Barreda et al. 2014). Climate data were obtained for the common period 1950–2002 when tree-ring data were available for all sites (Table 1).

To estimate the water balance at each study site, we calculated the potential evapotranspiration (PET) following the Hargreaves–Samani method (Hargreaves and Samani 1982). Then, we calculated the annual water balance as the sum of the monthly differences between precipitation and PET. We also calculated the Conrad continentality index to characterize the temperature range at each site (Tuhkanen 1980).

Table 2. Climatic characterization of the study sites calculated for the common period 1950-2002. Sites' and species' codes are as in Table 1. In climate-growth analyses, CRU gridded climate data (0.5° resolution) were used for Mongolian sites.

Region (country)	Study sites (species)	Closest meteorological station (coordinates)	Mean annual temperature (°C)	Annual precipitation (mm)	Annual water balance (mm)	Continentality index (type)
Siberia (Russia)	EF (Ls), KH (Ls)	Shira (54° 29' N, 89° 58' E, 448 m a.s.l.)	-1.9 °	316	-161	63 (Continental)
S. Urals (Russia)	CA (Ps)	Bredy (52° 25' N, 60° 20' E, 305 m a.s.l.)	2.4 °	352	-240	60 (Continental)
Khangai (Mongolia)	M7 (Ls), M0 (Ls)	Tsetserleg (47° 24' N, 101° 01' E, 1691 m a.s.l.)	0.4 °	331	-124	50 (Sub-continental)
Gúdar (Spain)	PN (Ps), LR (Ps), LR (Pn), AC (Pn)	Mora de Rubielos (40° 15' N, 0° 45' W, 1038 m a.s.l.)	9.5	533	-849	26 (Oceanic)

2.6 Statistical analyses

To compare tree-ring statistics (means, rbt, MSx) of width and density variables, we performed one-way analysis of variance (ANOVA) in cases when data were normal (means, rbt) or Kruskal–Wallis test when normality could not be assumed (MSx). These comparisons were followed by Tukey's Honest Significant Difference test for normally distributed data or Mann–Whitney U test otherwise. To evaluate how tree-ring variables were related, we calculated Pearson correlation coefficients between site chronologies for each species considering the common 1950–2002 period. We also performed separate Principal Component Analysis (PCA) on variance–covariance matrices of width and density chronologies to summarize their variability into a few principal components (Jolliffe 2002). Then, we used Pearson correlations to characterize climate–growth associations by relating either tree-ring width or density variables or the first (PC1) and second (PC2) principal components of their corresponding PCAs (summarizing their variability) with monthly climate data (mean temperature and precipitation). The first two PCA axes were retained as they accounted for at least 50% of the total variance (Jolliffe 2002). Climate-growth relationships were analysed from September prior to tree-ring formation to October of the growth year based on previous studies in the study regions (Kirdyanov et al. 2007, Camarero et al. 2010, 2015). Partial correlation analyses were calculated to discern if some variables (EW and MN) were more related to early season precipitation than to temperature data in sites where significant ($P<0.05$) correlations were found for those variables. Analyses were done using the vegan package (Oksanen et al. 2013) in the R environment (R Development Core Team, 2015).

3. RESULTS

3.1 General features of width and density site chronologies

Overall, Scots pine showed higher EW and LW, followed by Siberian larch and Black pine (Table 3). Conversely, Black pine presented the highest MN values, whereas larch showed the highest MX values. On average, MN showed the lowest mean autocorrelation (0.35), whilst EW showed the highest one (0.64). The coherence between trees (rbt) was significantly ($F = 2.53$, $P = 0.03$) higher in the case of seasonal width variables (EW 0.49; LW 0.51) than in the case of density variables (MN, 0.31; MX, 0.36). Note that the highest rbt values for MN were observed for the Mediterranean Black pine. Finally, the year-to-year variability (MSx) was significantly ($K = 0.99$, P\0.001) higher in the case of width variables (EW 0.44; LW 0.55) as compared with density variables (MN 0.14; MX 0.13).

Table 3. Comparison of the dendrochronological statistics calculated for the standard (mean, SD, AC) and residual site chronologies (r_{bt} and MSx) of the three tree species considering four variables (EW, earlywood width; LW, latewood width; MN, minimum wood density; MX, maximum wood density). Values were calculated for the common 1950-2002 period. Statistics abbreviations: AR1, first-order autocorrelation; r_{bt}, mean inter-sites correlation; MSx, mean sensitivity. Significant ($P < 0.05$) differences between sites and species are indicated by different letters according to Tukey's Honest Significant Difference (r_{bt}) or Mann-Whitney *U* tests (MSx). Site's codes are as in Table 1.

Species	**Site**	**EW**				**LW**			
		Mean ± SD (mm)	**AR1**	$\mathbf{r_{bt}}$	**MSx**	**Mean ± SD (mm)**	**AC**	$\mathbf{r_{bt}}$	**MSx**
Pinus sylvestris	CA	0.97 ± 0.17 b	0.52	0.41	0.39	0.22 ± 0.08 ab	0.32	0.49	0.62
	LR	0.80 ± 0.16 ab	0.65	0.46	0.35	0.20 ± 0.06 ab	0.41	0.54	0.58
	PN	1.05 ± 0.18 b	0.58	0.62	0.48	0.26 ± 0.08 b	0.60	0.46	0.46
Pinus nigra	AC	0.47 ± 0.07 a	0.62	0.55	0.52	0.15 ± 0.04 a	0.50	0.48	0.55
	LR	0.56 ± 0.13 a	0.69	0.48	0.38	0.18 ± 0.06 a	0.39	0.55	0.60
Larix sibirica	EF	0.58 ± 0.15 a	0.71	0.63	0.54	0.24 ± 0.09 ab	0.63	0.52	0.59
	KH	0.36 ± 0.17 a	0.54	0.66	0.63	0.10 ± 0.02 a	0.51	0.51	0.60
	M7	0.37 ± 0.06 a	0.78	0.33	0.26	0.13 ± 0.03 a	0.41	0.48	0.54
	M0	0.78 ± 0.15 ab	0.68	0.45	0.38	0.21 ± 0.05 ab	0.61	0.36	0.42
		MN				**MX**			
		Mean ± SD (g cm^{-3})	**AR1**	$\mathbf{r_{bt}}$	**MSx**	**Mean ± SD (g cm^{-3})**	**AC**	$\mathbf{r_{bt}}$	**MSx**
Pinus sylvestris	CA	0.28 ± 0.01 a	0.39	0.31	0.27	0.82 ± 0.03 a	0.41	0.26	0.14
	LR	0.33 ± 0.02 ab	0.30	0.34	0.11	0.89 ± 0.05 ab	0.36	0.35	0.11
	PN	0.30 ± 0.02 ab	0.41	0.25	0.09	0.82 ± 0.03 a	0.39	0.32	0.12
Pinus nigra	AC	0.39 ± 0.02 b	0.22	0.40	0.16	0.88 ± 0.04 ab	0.32	0.39	0.13
	LR	0.35 ± 0.01 ab	0.35	0.38	0.11	0.85 ± 0.04 a	0.43	0.38	0.10
Larix sibirica	EF	0.28 ± 0.02 a	0.37	0.31	0.15	0.93 ± 0.04 b	0.40	0.37	0.17
	KH	0.32 ± 0.02 ab	0.34	0.32	0.15	0.90 ± 0.03 ab	0.34	0.43	0.18
	M7	0.27 ± 0.01 a	0.46	0.25	0.10	0.93 ± 0.05 b	0.51	0.34	0.10
	M0	0.28 ± 0.01 a	0.35	0.26	0.14	0.94 ± 0.06 b	0.41	0.37	0.13

3.2 Associations between tree-ring variables

EW and LW records were positively associated for all species and sites, being stronger in the case of larch (r = 0.70–0.87), followed by Black pine (r = 0.45–0.60) and Scots pine (r = 0.28–0.39) (Table 4). In contrast, EW and MN records were negatively related with stronger associations found for Black pine (r = -0.73 to -0.81), followed by larch (r = -0.66 to -0.75) and Scots pine (r = -0.38 to -0.71) (Table 4). More latewood production (higher LW) was also related to heavy latewood (higher MX), but this association was again particularly strong for larch and Black pine, which explains the strong positive EW-MX and the negative LW-MN associations observed for these species. MN and MX records were negatively associated, with this relationship being significant in all larch forests and one Black pine (AC site) and one Scots pine (CA site) sites (Table 4).

Table 4. Pearson correlations calculated between tree ring variables (EW, earlywood width; LW, latewood width; MN, minimum wood density; MX, maximum wood density) for each site-species combination. Correlations were calculated based on residual tree-ring chronologies for the common 1950-2002 period and values with P > 0.05 are in bold. Sites' codes are as in Table 1.

Species	**Site**	EW-LW	EW-MN	EW-MX	LW-MN	LW-MX	MN-MX
Pinus sylvestris	CA	**0.39**	**-0.41**	**0.55**	**-0.32**	**0.43**	**-0.36**
	LR	**0.34**	**-0.71**	0.22	-0.23	**0.55**	-0.14
	PN	**0.28**	**-0.38**	0.02	-0.19	**0.28**	-0.04
Pinus nigra	AC	**0.60**	**-0.81**	**0.65**	**-0.34**	**0.64**	**-0.49**
	LR	**0.45**	**-0.73**	**0.44**	**-0.34**	**0.53**	-0.19
Larix sibirica	EF	**0.70**	**-0.75**	**0.81**	**-0.45**	**0.72**	**-0.73**
	KH	**0.75**	**-0.70**	**0.76**	**-0.41**	**0.80**	**-0.45**
	M7	**0.81**	**-0.69**	**0.31**	**-0.45**	**0.68**	**-0.34**
	M0	**0.87**	**-0.66**	**0.80**	**-0.52**	**0.74**	**-0.68**

3.3 Common patterns in width and density data between sites and species

According to the Principal Component Analyses (PCA), the variance captured by the first two axes was about the same for width and density variables, with the first principal component (PC1) accounting for about 35% of the total variance (Fig. 1). The second principal component (PC2) accounted for more variance in width than in density variables (21 vs. 15%; Fig. 1). The loadings of EW and LW data of the same species and sites were grouped together in the PCA diagram with maximum loadings on the PC1 corresponding to LW data from the low-elevation Spanish sites (Fig. 1a). In turn, EW-LW data for the larch sites had the highest loadings on the PC2 (Fig. 1a). Considering density data, larch MX and MN chronologies showed the highest and lowest PC1 loadings, respectively (Fig. 1b). The minimum and maximum PC2 loadings were observed for MN chronologies from the Black pine in AC site and Siberian larch in M7 site, respectively.

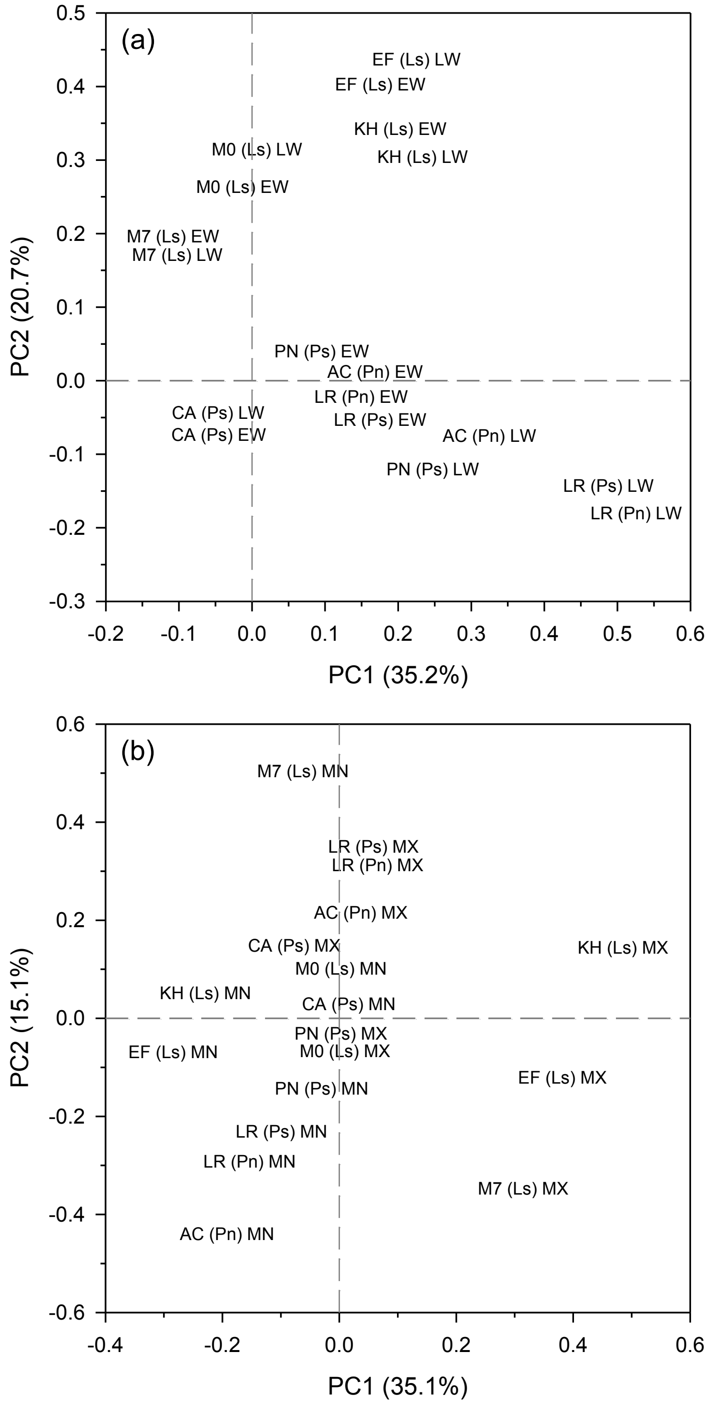

Figure 1. Scatter plots showing the loadings of the first (PC1) and second (PC2) principal components obtained by calculating Principal Component Analyses on the variance-covariance matrices of residual **a** width and **b** density site chronologies. **a** Earlywood and latewood widths are abbreviated as EW and LW, respectively. **b** Minimum and maximum wood densities are abbreviated as MN and MX, Sites are abbreviated as in Table 1. Tree species are abbreviated by the codes written between parentheses (Ls, *Larix sibirica*; Ps, *Pinus sylvestris*; Pn, *Pinus nigra*).

Regarding the width chronologies, low EW and LW values of the driest Black pine site (the low-elevation AC site) correspond to severe droughts (e.g., 1986 and 1994; Fig. 2). Low EW and LW values were also observed during the 1980s in Russian and Mongolian sites (Fig. 2). Regarding density data, high MN was found during dry years (e.g., 1994 in Spain, 1979–1980 in Russia and Mongolia, 1986–1987 in Mongolia), and this pattern was most evident in dry sites as the Spanish AC site (Fig. 3a). Low MX values were observed during cool summers in cold sites (e.g., 1972 in the high-elevation PN Scots pine site; 1976 in Russian and Mongolian sites; Fig. 3b).

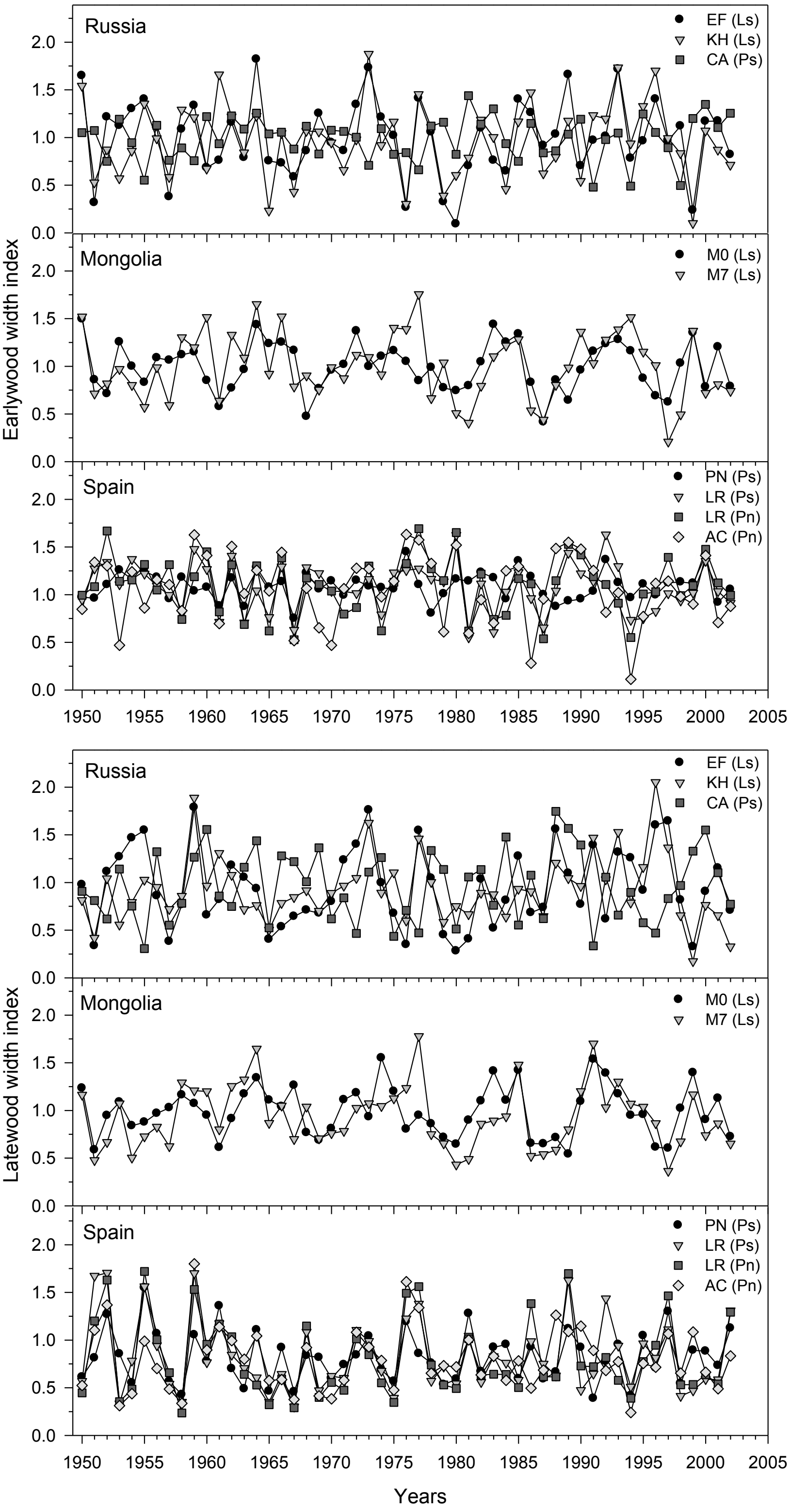

Figure 2. Residual earlywood (upper panels) and latewood chronologies (lower panel) for the study sites abbreviated as in Table 1. Graphs are plotted for each country. Tree species are abbreviated by the codes written between parentheses (Ls, *Larix sibirica*; Ps, *Pinus sylvestris*; Pn, *Pinus nigra*).

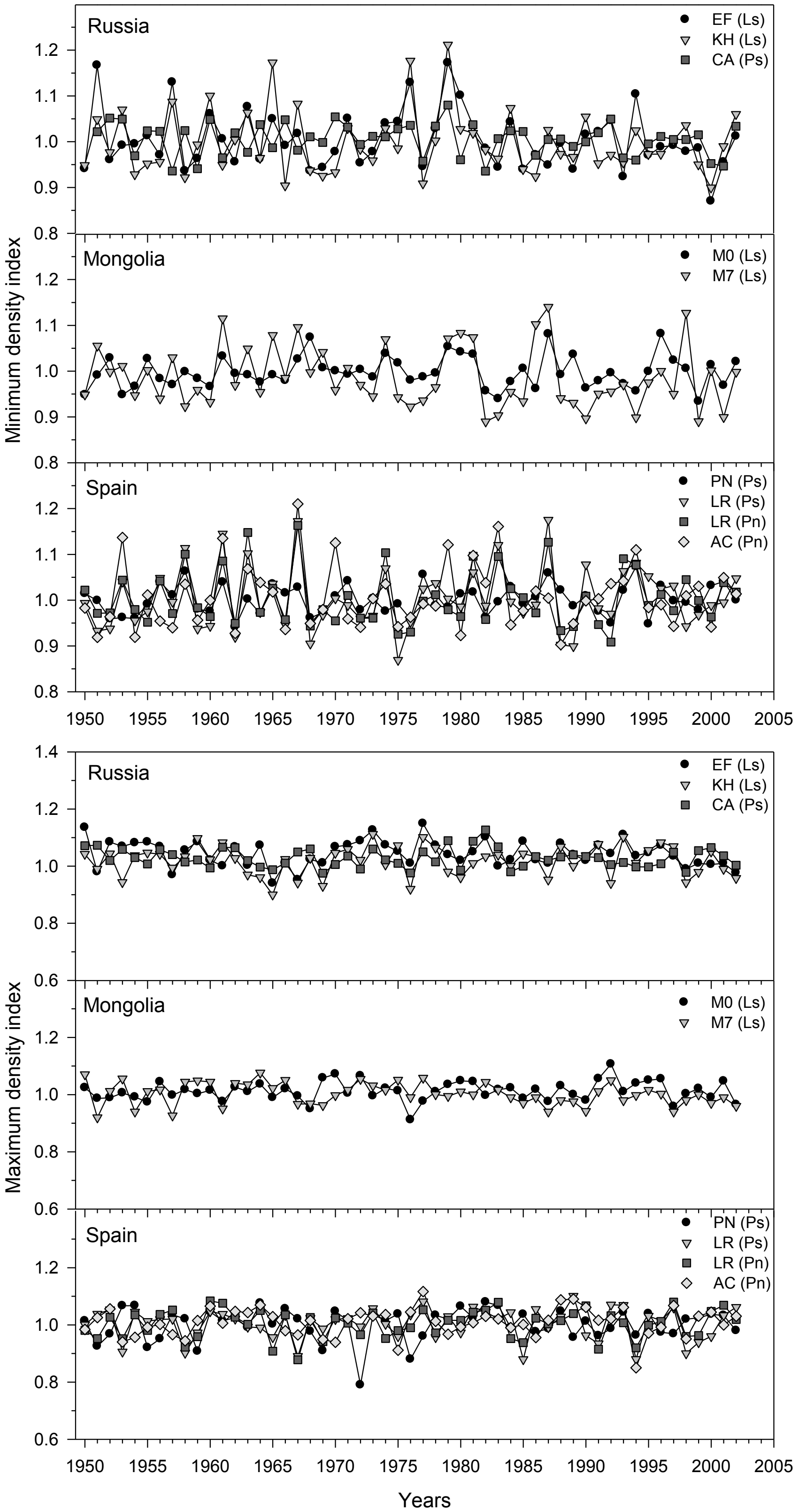

Figure 3. Residual minimum (upper panels) and maximum density chronologies (lower panels) for the study sites abbreviated as in Table 1. More details are shown in Figure 2.

3.4 Relationships between climate, width, and density variables

The first axis of the PCA based on climate–growth or climate–density relationships accounted for a high common variance in the case of LW (50%) and MN (46%) (see Supporting Information, Fig. S3). In both cases, Spanish sites grouped together and showed the highest PC1 scores, albeit the high-elevation PN Scots pine site showed a low PC1 score in the case of MN. Mongolian and Russian larch sites also grouped together and showed high PC2 loadings in the LW and MN. In the PCAs based on EW and MX data, the PC1 separated the PN site from the rest of Spanish sites suggesting a different impact of climate on the determination of EW and MX at this high-elevation location. Similarly, Siberian larch chronologies showed higher PC2 loadings compared to Mongolian sites.

Wet and cool spring (May to June) conditions enhanced EW formation but lead to low MN values, i.e., dry spring conditions were associated with low EW and high MN across sites and species (Fig. 4; see also Supporting Information, Table S1). LW formation was enhanced by wet–cool conditions during the growing season (May–September), particularly in Spanish pinewoods (excluding the high-elevation PN site) and Mongolian larch sites. MX increased in response to high May–June precipitation values and low June–July temperatures (Fig. 4; Table S1).

The strongest associations between climate and width or density variables were found for May or June precipitation and MN (Supporting Information, Table S1). The negative precipitation–MN relationship was consistently observed across species and countries. The strongest correlations were detected either in May (Spain, Black pine AC site; Russia, Scots pine CA site) or in June (e.g., Mongolia Siberian larch M7 site) (Fig. 5). May or June precipitation explained ca. 32–42% of the MN variance in these drought-prone sites (Fig. 5). Finally, partial correlation analyses confirmed that EW and MN responded more strongly to May–June precipitation than to temperatures. Partial correlations of EW and MN with May–June precipitation remained significant at the 0.01 significance after controlling for temperature effects (Supporting Information, Table S2), whilst partial correlations with May–June temperatures after controlling for precipitation effects were not significant ($P>0.05$).

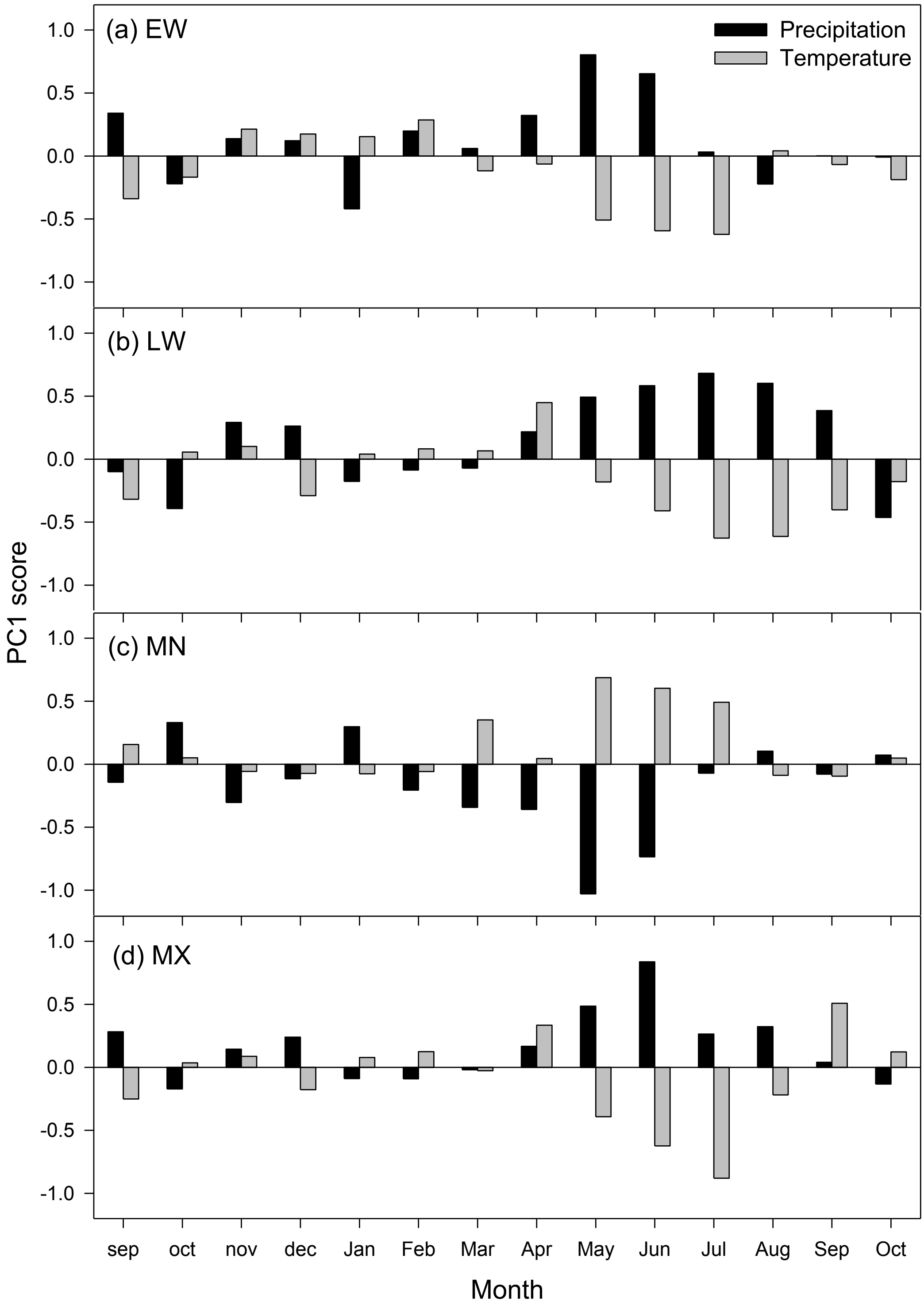

Figure 4. Associations between climate variables and scores of the first principal component (PC1) of Principal Component Analyses calculated on the indices of earlywood (EW, **a**) and latewood width (LW, **b**) data and minimum (MN, **c**) and maximum (MX, **d**) wood density data. Bars correspond to the correlations of summarized tree-ring variables (PC1 and PC2 scores) with monthly precipitation (black bars) and temperature (grey bars) from September prior to tree-ring formation to October of the growth year (previous- and current-year months are abbreviated by lowercase and uppercase letters, respectively).

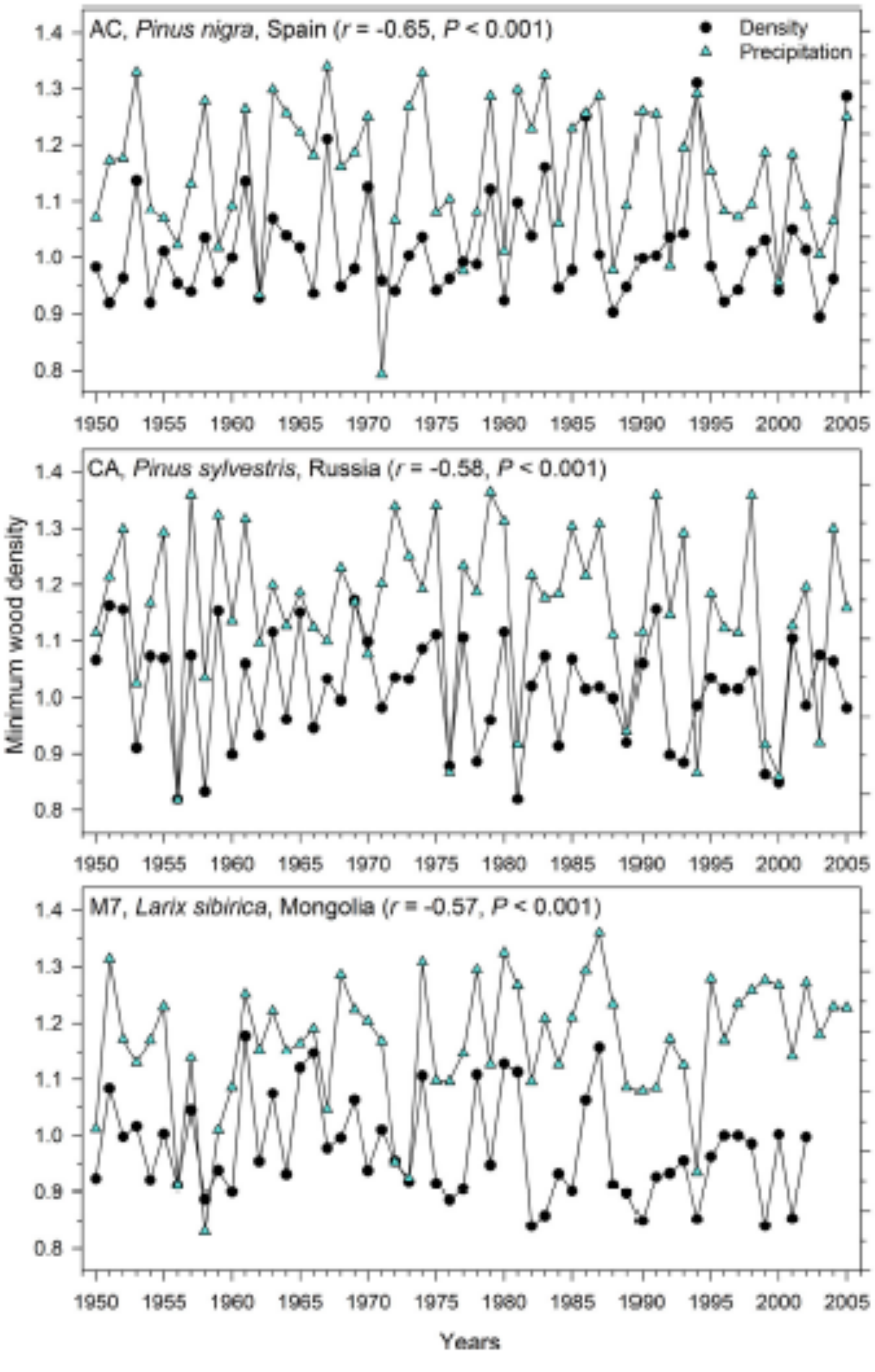

Figure 5. Main relationships observed between climate variables (May and June precipitation) and the residual Chronologies of minimum wood density in each country. In each plot, the Pearson correlation (*r*) between precipitation and wood density values is shown with its corresponding significance levels (*P*). Note the reverse precipitation scales. Sites' codes in Table 1.

4. DISCUSSION

In agreement with our hypothesis, dry spring conditions were associated with high MN values and, consequently, to a dense earlywood across species and sites in Eurasia. Such strong response of MN to precipitation indicates that it is a robust proxy of early season water availability at the beginning of the growing season, when the earlywood is formed, leads to a dense earlywood (Vaganov et al. 2009). Such dense earlywood is characterized by tracheids with narrow lumens and a decreased hydraulic conductivity (Domec et al. 2009).

Our results confirm the previous studies showing that: (1) seasonal density data better reflect the moisture status of conifer species during the growing season than width variables, and (2) this difference is noticeable in drought-prone sites (Cleaveland 1986). Such precipitation-density coupling, possibly mediated by adjustments in hydraulic conductivity, could also explain the decrease in earlywood production observed in response to water deficit in drought-prone sites as showed for forest-steppe and Mediterranean biomes (e.g., Dulamsuren et al. 2010; Camarero et al. 2015). According to ecophysiological studies, photosynthesis rates decrease in both winter and summer in these cool–dry regions, and growing-season drought stress caused by elevated atmospheric vapour pressure deficit and low soil water availability leads to radial growth reduction (Dulamsuren et al. 2009; Gimeno et al. 2012). Nevertheless, the correlations between spring precipitation and MN were always stronger, in absolute terms, than those detected between precipitation and EW (Fig. 4; Table S1). Consequently, MN exhibits strong response to precipitation and, thus, it is a robust proxy of early season water availability. This agrees with empirical approaches demonstrating that an improved water status is linked to a lower density in Norway spruce (*Picea abies*) (Lundgren 2004).

The correlations found between MN and spring precipitation in pine and larch (Fig. 5) were similar to those observed in Spanish juniper (*Juniperus thurifera*) (Camarero et al. 2014), explaining on average 37% of variability in minimum density. Indeed, further investigations should explore the nature of climate–MN associations in more detail. This research could explicitly consider wood anatomy to disentangle whether MN changes are mainly due to lumen modifications, as we assumed here, or to changes in cell-wall thickness. For instance, in drought exposed Norway spruce trees, wood density augmented as a consequence of the formation of thicker cell walls (Jyske et al. 2010), whilst increased tracheid lignin content was observed in drought-prone Austrian

Black pine forests (*Pinus nigra*) (Gindl 2001). Climate–MN associations could also reflect the extent of plastic responses to drought stress. For instance, Douglas fir (*Pseudotsuga menziesii*) trees showing high resistance to xylem cavitation and lower drought-induced mortality also presented the highest minimum wood density values (Dalla-Salda et al. 2009; Ruiz Diaz Britez et al. 2014), which could reflect narrower tracheid lumens. Nevertheless, other parameters affecting cavitation and hydraulic conductivity should be also considered, including conduit length, pit size, and the numbers of tracheids per unit area (Carlquist 1975).

Tree-ring width and density data often provide redundant information (Kirdyanov et al. 2007; Büntgen et al. 2010; Vaganov and Kirdyanov 2010, Galván et al. 2015). This redundancy could apply to MN, which is negatively related to EW (Tables 4) and, in turn, both variables show opposite associations with precipitation (Fig. 4). Note that we opted for analysing residual growth and density chronologies without removing dependences between them (e.g., EW–MN and LW–MX relationships), since our purpose was to assess how climate distinctively influenced seasonal growth and density data. A pro of analysing MN is that it offers indirect clues on wood phenology. For example, the shift in the strongest precipitation–MN association from May (Spanish and Russian sites) to June (Mongolian sites; Table S1) could be caused by a late peak of earlywood formation in Mongolian sites. This agrees with our knowledge of xylogenesis, with, e.g., maximum rates of tracheid production occurring from May to July (Antonova and Stasova 1993; Vaganov et al. 2006; Camarero et al. 2010).

In cold northern Siberian larch forests, width and density variables mainly respond to temperature changes during the short growing season (June–July) (Esper et al. 2010), and indirectly to winter precipitation which affects the length of snow melt season and soil temperatures (Vaganov et al. 1999; Kirdyanov et al. 2003). Our results also evidenced that MN was negatively related to spring precipitation in southern Siberia sites subjected to water shortage during that season (Fig. 4), suggesting that earlywood tracheid expansion is constrained by water availability. Low soil temperature can increase water viscosity leading to a high root hydraulic resistance, decreasing water flow from the soil to the tree and reducing stomatal conductance (Wan et al. 2001). Spring cold conditions could therefore constrain the radial enlargement of earlywood tracheids. Despite variables as EW and MN showed significant correlations with May–June temperatures, the associations between them and precipitation were stronger in absolute terms suggesting a secondary role of thermal factors (Fig. 4, Supporting

Information, Table S1). Partial correlation analyses and the aforementioned arguments on xylogenesis confirm that temperature does not influence EW and MN in the study sites as much as precipitation does.

In cool–arid inner Asian forests, wet conditions during the growing season enhance radial growth (Poulter et al. 2013). However, Mongolian forest-steppe ecotones are experiencing warmer and drier conditions since the 1940s, causing growth decline and the retreat of larch forests (Dulamsuren et al. 2010). Increased aridity and drought stress are major restrictions of tree growth and functioning in these biomes where larches show low shoot water potentials and high stomatal conductance (Dulamsuren et al. 2009). This agrees with our findings and emphasizes the key role of sufficient moisture availability for tree growth and wood formation.

In Mediterranean pine forests, wet conditions during the previous winter and the current spring are associated with improved earlywood production (De Luis et al. 2007; Martín-Benito et al. 2008; Pasho et al. 2012) and a decrease in minimum density (Olivar et al. 2015). Wet conditions favour tracheid division which results in low minimum density (Bouriaud et al. 2005), as we found in the low elevation Spanish sites (Fig. 5a; Table S1). Under more continental and colder conditions, cambial resumption is delayed and summer conditions become more relevant as drivers of Scots pine growth (Sánchez-Salguero et al. 2015). This explains why earlywood density may not be related to spring precipitation in some Mediterranean Scots pine stands (Olivar et al. 2015). In contrast, the positive association between summer temperatures and maximum density found in high-elevation Scots pine stands (PN site) (Table S1) is in agreement with other studies of cold biomes such as boreal and subalpine forests (e.g., Briffa et al. 1998). This relationship has previously been reported for pine species in Mediterranean environments (Olivar et al. 2015), and it is due to an improved thickening and lignification of latewood cell walls in response to warm summer and fall conditions (Gindl et al. 2000; Yasue et al. 2000).

Contrary to most previous studies at cold sites which found that summer temperatures and maximum latewood density are positively associated (e.g., Briffa et al. 2004), we found significant and negative correlations of MX with May-to-July temperatures (Fig. 4). We assume that such negative relationships are driven by water shortage caused by warm spring-to-summer temperatures. This is a typical climate effect observed in Mediterranean forests such as the Spanish study sites, but we also found such effect in other drought-prone sites from Russia (Scots pine CA site) and Mongolia

(Siberian larch M7 site). We interpret this negative association as a drought-related reduction in the thickening and lignifications rates of latewood tracheids. This is confirmed by the positive effect of wet June conditions on MX observed in most of those sites, whilst MX was mainly negatively related to July temperatures, because water deficit usually peaks during that month.

An increasing influence of climate warming on temporal coherence in ring width records (spatial synchrony) has been observed across some of the studied Eurasian regions (i.e., Iberian Peninsula and Siberia; Shestakova et al. 2016). It could be tested if this enhanced synchrony holds too for density records at similar sub-continental scales, since wood density seasonal components (MN and MX) respond to different climate variables, and wood density is a fundamental variable to estimate forest carbon uptake and woody biomass pools (Bouriaud et al. 2015). It is expected that growing-season precipitation would drive earlywood density and hydraulic conductivity (Pacheco et al. 2016). Consequently, we predict that warmer and drier conditions during the most active phase of the growing season would reduce earlywood production, but increase earlywood density. These predictions must be taken with caution because of the case-study-like nature of this investigation. It should be underlined that much more research is needed to prove the validity and generality of our findings. If they turn to be true for other cold–dry sites where other Pinaceae species dominate, this would imply that minimum wood density could be a valuable proxy of early season precipitation.

To conclude, minimum wood density of three conifer species (*L. sibirica*, *P. nigra*, and *P. sylvestris*) reflects changes in growing-season (spring) precipitation in cool– dry Eurasian regions. An increase in minimum wood density in response to dry spring conditions was observed in drought-prone sites from the forest-steppe and Mediterranean biomes. The associations between minimum wood density and precipitation were stronger than those observed with seasonal width variables. Future drier conditions during the growing season may increase minimum wood density and reduce radial growth of Eurasian conifer forests, negatively affecting their potential to fix and store carbon pools as stem wood.

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6. References

Antonova GF, Stasova VV (1993) Effects of environmental factors on wood formation in Scots pine stems. Trees 7:214–219

Block J, Magda VN, Vaganov EA (2004) Temporal and spatial variability of tree growth in mountain forest steppe in Central Asia. In: Trace, Proceedings of the Dendrosymposium, vol 2, pp 46–53. Utrecht, Netherlands, May the 1st–3rd, 2003

Bouche PS, Larter M, Domec JC, Burlett R, Gasson P, Jansen S, Delzon S (2014) A broad survey of hydraulic and mechanical safety in the xylem of conifers. J Exp Bot 65:4419–4431

Bouriaud O, Leban J, Bert D, Deleuze C (2005) Intra-annual variations in climate influence growth and wood density of Norway spruce. Tree Physiol 25:651–660

Bouriaud O, Teodosiu M, Kirdyanov AV, Wirth C (2015) Influence of wood density in tree-ring-based annual productivity assessments and its errors in Norway spruce. Biogeosciences 12:6205–6217

Briffa KR, Schweingruber FH, Jones PD, Osborn TJ, Shiyatov SG, Vaganov EA (1998) Reduced sensitivity of recent tree-growth to temperature at high northern latitudes. Nature 391:678–682

Briffa KR, Osborn TJ, Schweingruber FH (2004) Large-scale temperature inferences from tree rings: a review. Glob Planet Chang 40:11–26

Bunn AG (2008) A dendrochronology program library in R (dplR). Dendrochronologia 26:115–124

Bunn A, Korpela M, Biondi F, Campelo F, Me´rian P, Qeadan F, Zang C (2016) dplR: Dendrochronology Program Library in R. R package version 1.6.4. https://CRAN.R-project.org/package= dplR

Büntgen U, Frank D, Trouet V, Esper J (2010) Diverse climate sensitivity of Mediterranean tree-ring width and density. Trees 24:261–273

Camarero JJ, Olano JM, Parras A (2010) Plastic bimodal xylogenesis in conifers from continental Mediterranean climates. New Phytol 185:471–480

Camarero JJ, Rozas V, Olano JM (2014) Minimum wood density of *Juniperus thurifera* is a robust proxy of spring water availability in a continental Mediterranean climate. J Biogeogr 41:1105–1114

Camarero JJ, Gazol A, Tardif JC, Conciatori F (2015) Attributing forest responses to global-change drivers: limited evidence of a CO_2-fertilization effect in Iberian pine growth. J Biogeogr 42:2220–2233

Carlquist S (1975) Ecological strategies of xylem evolution. University of California Press, Berkeley

Chave J, Coomes D, Jansen S, Lewis SL, Swenson NG, Zanne AE (2009) Towards a worldwide wood economics spectrum. Ecol Lett 12:351–366

Cleaveland MK (1986) Climatic response of densitometric properties in semiarid site tree rings. Tree-Ring Bull 46:13–29

Cook ER, Kairiukstis LA (1990) Methods of dendrochronology: applications in the environmental science. Kluwer, Dordrecht

Dalla-Salda G, Martı´nez-Mier A, Cochard H, Rozenberg P (2009) Variation of wood density and hydraulic properties of Douglas- fir (*Pseudotsuga menziesii* (Mirb.) Franco) clones related to a heat and drought wave in France. For Ecol Manage 257:182–189

De Luis M, Gričar J, Čufar K, Raventós J (2007) Seasonal dynamics of wood formation in *Pinus halepensis* from dry and semi-arid ecosystems in Spain. IAWA J 28:389–404

Devi N, Hagedorn F, Moiseev P, Bugmann H, Shiyatov S, Mazepa V, Rigling A (2008) Expanding forests and changing growth forms of Siberian larch at the Polar Urals treeline during the 20th century. Glob Chang Biol 14:1581–1591

Domec JC, Warren JM, Meinzer FC, Lachenbruch B (2009) Safety factors for xylem failure by implosion and air-seeding within roots, trunks and branches of young and old conifer trees. IAWA J 30:101–120

Dulamsuren Ch, Hauck M, Bader M, Osokhjargal D, Oyungerel S, Nyambayar S, Runge M, Leuschner C (2009) Water relations and photosynthetic performance in *Larix sibirica* growing in the forest-steppe ecotone of northern Mongolia. Tree Physiol 29:99–110

Dulamsuren Ch, Hauck M, Leuschner C (2010) Recent drought stress leads to growth reductions in *Larix sibirica* in the western Khentey, Mongolia. Glob Chang Biol 16:3024–3035

Dylis NV (1961) Larch in Eastern Siberia and the Far East: variation and natural diversity (in russian). Akad. Nauk SSSR, Moscow

Eilmann B, Zweifel R, Buchmann N, Pannatier EG, Rigling A (2011) Drought alters timing, quantity, and quality of wood formation in Scots pine. J Exp Bot 62:2763–2771

Esper J, Frank D, Bu¨ntgen U, Verstege A, Hantemirov RM, Kirdyanov AV (2010) Trends and uncertainties in Siberian indicators of 20th century warming. Global Chang Biol 16:386–398

Fajardo A (2016) Wood density is a poor predictor of competitive ability among individuals of the same species. For Ecol Manage 372:217–225

Fritts HC (2001) Tree rings and climate. Caldwell, New York

Galván DJ, Büntgen U, Ginzler C, Grudd H, Gutiérrez E, Labuhn I, Camarero JJ (2015) Drought-induced weakening of growth temperature associations in high-elevation Iberian pines. Glob Planet Chang 124:95–106

Gimeno TE, Camarero JJ, Granda E, Pías B, Valladares F (2012) Enhanced growth of *Juniperus thurifera* under a warmer climate is explained by a positive carbon gain under cold and drought. Tree Physiol 32:326–336

Gindl W (2001) Cell-wall lignin content related to tracheid dimensions in drought-sensitive Austrian pine (*Pinus nigra*). IAWA J 22:113–120

Gindl W, Grabner M, Wimmer R (2000) The influence of temperature on latewood lignin content in treeline Norway spruce compared with maximum density and ring width. Trees 14:409–414

Hacke UG, Sperry JS (2001) Functional and ecological xylem anatomy. Perspect Plant Ecol Evol Syst 4:97–115

Hacke UG, Sperry JS, Pockman WT, Davis SD, McCulloh KA (2001) Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. Oecologia 126:457–461

Hacke UG, Lachenbruch B, Pittermann J, Mayr S, Domec JC, Schulte PJ (2015) The hydraulic architecture of conifers. In: Hacke UG (ed) Functional and ecological xylem anatomy. Springer, Berlin, Heidelberg, pp 39–75

Hargreaves GH, Samani ZA (1982) Estimating potential evapotranspiration. J Irrig Drain Eng 108:225–230

Harris I, Jones PD, Osborn TJ, Lister DH (2014) Updated high resolution grids of monthly climatic observations—the CRU TS3.10 Dataset. Int J Climatol 34:623–642

Jolliffe I (2002) Principal component analysis. Springer, New York

Jyske T, Hölttä T, Mäkinen H, Nöjd P, Lumme I, Spiecker H (2010) The effect of artificially induced drought on radial increment and wood properties of Norway spruce. Tree Physiol 30:103–115

Kirdyanov AV, Hughes MK, Vaganov EA, Schweingruber F, Silkin P (2003) The importance of early summer temperature and date of snow melt for tree growth in Siberian Subarctic. Trees 17:61–69

Kirdyanov AV, Vaganov EA, Hughes MK (2007) Separating the climatic signal from tree-ring width and maximum latewood density records. Trees 21:37–44

Knorre AA, Siegwolf RTW, Saurer M, Sidorova OV, Vaganov EA, Kirdyanov AV (2010) Twentieth century trends in tree ring stable isotopes (δ13C and δ18O) of *Larix sibirica* under dry conditions in the forest steppe in Siberia. J Geophys Res Biogeosci 115:G03002

Larjavaara M, Muller-Landau HC (2010) Rethinking the value of high wood density. Funct Ecol 24:701–705

Larson PR (1994) The vascular cambium: development and structure. Springer, New York

Lundgren C (2004) Microfibril angle and density patterns of fertilized and irrigated Norway spruce. Silva Fenn 38:107–117

Mäkinen H, Hynynen J (2014) Wood density and tracheid properties of Scots pine: responses to repeated fertilization and timing of the first commercial thinning. Forestry 87:437–447

Martín JA, Esteban LG, de Palacios P, Fernández FG (2010) Variation in wood anatomical traits of *Pinus sylvestris* L. between Spanish regions of provenance. Trees 24:1017–1028

Martín-Benito D, Cherubini P, Del Río M, Cañellas I (2008) Growth response to climate and drought in *Pinus nigra* Arn. trees of different crown classes. Trees 22:363–373

Martínez-Vilalta J, Cochard H, Mencuccini M, Sterck F, Herrero A, Korhonen J, Llorens P, Nikinmaa E, Nolè A, Poyatos R (2009) Hydraulic adjustment of Scots pine across Europe. New Phytol 184:353–364

Muller-Landau HC (2004) Interspecific and inter-site variation in wood specific gravity of tropical trees. Biotropica 36:20–32

Niklas KJ (1992) Plant biomechanics. University of Chicago Press, Chicago

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H (2013) vegan: Community Ecology Package. R package version 2.0-7. http://CRAN.R-project.org/package=vegan

Olivar J, Rathgeber C, Bravo F (2015) Climate change, tree-ring width and wood density of pines in Mediterranean environments. IAWA J 36:257–269

Pacheco A, Camarero JJ, Carrer M (2016) Linking wood anatomy and xylogenesis allows pinpointing of climate and drought influences on growth of coexisting conifers in continental Mediterranean climate. Tree Physiol 36:502–512

Pasho E, Camarero JJ, Vicente-Serrano SM (2012) Climatic impacts and drought control of radial growth and seasonal wood formation in *Pinus halepensis*. Trees 26:1875–1886

Pittermann J, Sperry JS, Wheeler JK, Hacke UG, Sikkema EH (2006) Mechanical reinforcement of tracheids compromises the hydraulic efficiency of conifer xylem. Plant Cell Env 29:1618–1628

Polge H (1978) Fifteen years of wood radiation densitometry. Wood Sci Technol 12:187–196

Poulter B, Pederson N, Liu H, Zhu Z, D'Arrigo R, Ciais P, Davi N, Frank D, Leland C, Myneni R, Piao S, Wang T (2013) Recent trends in inner Asian forest dynamics to temperature and precipitation indicate high sensitivity to climate change. Agric For Meteorol 178–179:31–45

R Development Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Richardson DM (1998) Ecology and biogeography of *Pinus*. Cambridge University Press, Cambridge

Ruiz Diaz Britez M, Sergent A-S, Martinez Meier A, Bréda N, Rozenberg P (2014) Wood density proxies of adaptive traits linked with resistance to drought in Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco). Trees 28:1289–1304

Sánchez-Salguero R, Camarero JJ, Hevia A, Madrigal-González J, Linares JC, Ballesteros-Canovas JA, Sánchez-Miranda A, Alfaro-Sánchez R, Sangüesa-Barreda S, Galván JD, Gutiérrez E, Génova M, Rigling A (2015) What drives growth of Scots pine in continental Mediterranean climates: drought, low temperaturas or both? Agric For Meteorol 206:151–162

Sangüesa-Barreda G, Camarero JJ, García-Martín A, Hernández R, de la Riva J (2014) Remote-sensing and tree-ring based characterization of forest defoliation and growth loss due to the Mediterranean pine processionary moth. For Ecol Manage 320:171–181

Shestakova TA, Guti´wrrez E, Kirdyanov AV, Camarero JJ, Génova M, Knorre AA, Linares JC, Resco de Dios V, Sánchez-Salguero R, Voltas J (2016) Forests synchronize their growth in contrasting Eurasian regions in response to climate warming. PNAS 113:662–667

Treter U (2000) Stand structure and growth patterns of the larch forests of Western Mongolia—a dendrochronological approach. Geowiss Abh Reihe A 205:60–66

Tuhkanen S (1980) Climatic parameters and indices in plant geography. Acta Phytogeogr Suec 67:1–110

Vaganov EA, Kirdyanov AV (2010) Dendrochronology of larch trees growing on Siberian permafrost. In: Osawa A, Zyryanova OA, Matsuura Y, Kajimoto T, Wein RW (eds) Permafrost ecosystems: Siberian Larch forests, ecological studies, vol 209. Springer, Heidelberg, pp 347–363

Vaganov EA, Hughes MK, Kirdyanov AV, Schweingruber FH, Silkin PP (1999) Influence of snowfall and melt timing on tree growth in subarctic Eurasia. Nature 400:149–151

Vaganov EA, Hughes MK, Shashkin AV (2006) Growth dynamics of conifer tree rings. Images of past and future environments. Springer, Berlin, Heidelberg

Vaganov EA, Schulze ED, Skomarkova MV, Knohl A, Brand WA, Roscher C (2009) Intra-annual variability of anatomical structure and δ13C values within tree rings of spruce and pine in alpine, temperate and boreal Europe. Oecologia 161:729–745

Velisevich SN, Kozlov DS (2006) Effects of temperature and precipitation on radial growth of Siberian Larch in ecotopes with optimal, insufficient, and excessive soil moistening. Russ J Ecol 37:241–246

Wan X, Zwiazek JJ, Lieffers VJ, Landhäusser SM (2001) Hydraulic conductance in aspen (*Populus tremuloides*) seedlings exposed to low root temperatures. Tree Physiol 21:691–696

Yasue K, Funada R, Kobayashi O, Ohtani J (2000) The effects of tracheid dimensions on variations in maximum density of *Picea glehnii* and relationships to climatic factors. Trees 14:223–229

Zhang SB, Ferry Silk JW, Zhang JL, Cao KF (2011) Spatial patterns of wood traits in China are controlled by phylogeny and the environment. Glob Ecol Biogeogr 20:241–250

Zobel BJ, van Buijtenen JP (1989) Wood variation. Its causes and control. Springer, Berlin

7. Supplementary Material

Table S1. Correlations (Pearson coefficients) calculated by relating monthly climatic variables (precipitation, mean temperature) and residual tree-ring chronologies (EW, earlywood width; LW, latewood width; MN, minimum density; MX, maximum density) considering the common 1950-2002 period. Data correspond to the nine study sites sampled at three countries (Spain, Russia and Mongolia) and corresponding to three conifer species (Ls, *Larix sibirica*; Pn, *Pinus nigra*; Ps, *Pinus sylvestris*). Correlations were calculated from September prior to tree-ring formation up to October (previous- and current-year months are abbreviated by lowercase and uppercase letters, respectively). Cells with grey background indicate correlations significant at the 0.05 level, and bold values indicate $P < 0.01$.

				Precipitation														Mean temperature													
				Previous year				Current year										Previous year				Current year									
Country	**Site**	**Species**	**Variable**	**sep**	**oct**	**nov**	**dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**sep**	**oct**	**nov**	**dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**
Russia	CA	Ps	EW	-0.12	0.22	0.04	0.06	-0.06	-0.05	0.16	-0.09	0.27	-0.02	-0.29	0.02	0.15	0.19	0.12	0.06	0.21	0.22	0.21	0.30	0.26	0.06	**-0.48**	**-0.53**	0.13	0.03	-0.08	-0.06
	EF	Ls		0.21	0.00	0.14	-0.23	**-0.50**	-0.03	-0.08	0.03	-0.04	0.12	-0.11	-0.17	0.05	-0.12	-0.28	-0.09	0.15	0.10	0.04	-0.11	0.13	0.06	-0.18	-0.20	0.05	0.27	-0.03	-0.01
	KH			0.33	0.03	0.14	-0.21	-0.35	0.06	-0.08	0.28	0.11	0.05	-0.20	-0.18	0.14	-0.15	-0.35	-0.05	0.07	-0.01	0.02	0.08	0.08	0.08	-0.29	-0.23	-0.02	0.07	-0.07	-0.10
Mongolia	M0	Ls		0.10	-0.10	-0.08	-0.05	-0.23	-0.01	-0.28	0.15	-0.06	**0.47**	-0.01	0.11	0.14	0.07	-0.25	-0.12	0.10	-0.04	0.07	-0.26	-0.13	-0.09	0.03	-0.16	**-0.43**	-0.12	-0.17	0.06
	M7			-0.01	0.01	-0.21	-0.03	-0.31	0.20	-0.09	0.29	0.23	**0.55**	0.13	0.05	0.15	-0.07	-0.12	0.04	0.08	0.02	0.15	0.11	0.01	-0.24	-0.19	-0.28	-0.31	-0.09	-0.06	0.19
Spain	PN	Ps		0.16	-0.24	-0.01	0.03	-0.29	0.04	-0.03	-0.16	0.05	0.12	0.10	-0.08	-0.11	0.06	-0.15	0.12	0.01	-0.33	-0.24	-0.05	-0.32	0.11	0.34	-0.01	-0.23	-0.18	0.01	-0.12
	LR			0.03	-0.22	0.12	0.18	-0.07	0.19	0.27	0.09	**0.56**	0.11	0.11	-0.26	-0.07	0.13	0.06	-0.13	0.09	0.16	0.06	0.14	-0.23	0.03	-0.14	-0.14	-0.21	0.12	0.02	-0.18
	LR	Pn		0.13	-0.19	0.13	0.20	0.12	0.01	0.04	0.03	**0.44**	0.30	0.10	-0.08	-0.12	0.08	-0.13	-0.08	0.10	0.20	0.07	0.35	0.01	0.13	-0.03	-0.11	-0.31	0.09	0.07	-0.14
	AC			0.32	-0.10	0.21	0.28	0.11	0.13	0.19	0.17	**0.57**	0.24	0.16	-0.02	-0.17	-0.06	-0.01	-0.06	0.00	0.05	0.01	0.17	-0.12	-0.07	-0.26	-0.12	-0.33	-0.03	0.10	-0.15
Russia	CA	Ps	LW	0.20	-0.08	0.30	-0.21	-0.03	-0.23	0.07	-0.22	0.40	0.01	-0.06	0.05	0.11	0.03	-0.09	-0.02	-0.09	-0.11	-0.02	-0.01	0.02	-0.08	**-0.44**	-0.35	-0.22	-0.09	-0.18	-0.05
	EF	Ls		0.20	0.00	0.14	-0.23	**-0.50**	-0.03	-0.08	0.02	-0.04	0.13	-0.09	-0.17	0.06	-0.11	-0.28	-0.09	0.17	0.09	0.04	-0.12	0.14	0.04	-0.19	-0.20	0.03	0.28	-0.03	-0.02
	KH			0.29	-0.04	-0.02	-0.08	-0.29	-0.02	-0.12	0.10	0.08	0.34	-0.02	-0.12	-0.01	-0.10	-0.25	-0.03	0.15	0.00	0.04	0.10	0.12	0.21	-0.26	-0.16	-0.10	-0.08	-0.01	0.06
Mongolia	M0	Ls		0.09	-0.02	-0.03	-0.08	-0.22	0.08	-0.10	0.18	-0.06	0.36	0.16	0.12	0.17	-0.07	-0.05	0.11	0.10	0.04	0.06	-0.02	-0.05	0.03	0.06	-0.25	-0.25	0.19	-0.16	0.20

	M7			0.07	0.04	-0.18	-0.07	-0.23	0.20	-0.08	0.27	0.23	**0.56**	0.34	0.11	0.12	-0.08	-0.15	-0.05	-0.06	0.05	0.05	0.08	0.13	-0.06	-0.16	-0.32	-0.31	-0.10	-0.07	0.13
Spain	PN	Ps		-0.14	-0.24	0.20	0.12	-0.06	-0.08	-0.08	0.02	0.08	0.23	0.24	0.36	0.16	-0.17	-0.24	0.17	0.26	-0.15	-0.04	0.06	0.03	**0.42**	0.26	-0.02	-0.17	-0.26	-0.11	-0.05
	LR			-0.17	-0.12	0.16	0.23	-0.01	-0.03	0.07	0.08	0.26	0.10	0.36	0.35	0.23	-0.23	-0.03	0.05	0.01	-0.17	0.05	-0.01	-0.02	0.27	-0.06	-0.11	-0.23	-0.37	-0.22	-0.14
	LR	Pn		-0.16	-0.21	0.21	0.24	0.12	-0.07	0.00	0.07	0.25	0.13	0.34	0.42	0.20	-0.22	-0.12	0.02	0.02	-0.12	0.04	0.07	0.09	0.26	-0.03	-0.09	-0.25	-0.31	-0.17	-0.14
	AC			0.07	-0.18	0.22	0.34	0.07	0.03	0.08	0.22	0.27	0.27	**0.43**	0.23	0.18	-0.19	0.07	0.02	0.04	-0.11	0.08	0.10	0.02	0.16	-0.05	-0.01	-0.17	-0.22	-0.08	-0.14

				Precipitation														Mean temperature													
				Previous year				Current year										Previous year				Current year									
Country	**Site**	**Species**	**Variable**	**sep**	**oct**	**nov**	**dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**sep**	**oct**	**nov**	**dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**
Russia	CA	Ps	MN	0.13	-0.17	0.05	-0.12	0.03	0.02	-0.10	0.05	**-0.58**	-0.16	-0.04	-0.08	-0.26	0.03	-0.16	-0.17	0.21	-0.10	-0.16	-0.13	0.09	0.11	**0.45**	0.21	0.17	-0.26	0.27	0.10
	EF	Ls		-0.18	0.08	-0.16	0.06	0.44	0.05	0.03	-0.18	**-0.47**	-0.16	-0.01	0.14	0.02	0.01	0.25	0.11	-0.12	-0.02	0.03	-0.01	-0.31	-0.11	0.17	0.30	0.11	-0.29	0.03	-0.02
	KH			-0.26	-0.06	-0.18	0.13	0.42	0.02	0.02	-0.35	**-0.40**	0.01	0.13	0.02	-0.09	0.17	0.33	-0.04	0.07	-0.07	0.06	0.11	0.15	-0.07	0.21	0.34	0.05	-0.09	0.05	0.00
Mongolia	M0	Ls		-0.04	0.11	0.01	0.28	0.25	-0.15	0.17	-0.08	-0.18	**-0.52**	0.06	-0.01	-0.16	-0.01	0.05	0.02	0.19	-0.10	-0.02	0.01	0.00	0.14	0.13	0.10	0.27	-0.03	-0.14	-0.37
	M7			-0.06	-0.20	-0.08	0.09	0.20	-0.29	0.00	-0.16	-0.11	**-0.57**	0.26	-0.04	-0.22	-0.10	-0.02	-0.03	-0.10	0.09	0.01	0.02	0.28	0.20	0.27	0.21	0.14	0.04	-0.01	-0.26
Spain	PN	Ps		-0.05	0.21	-0.17	-0.01	0.21	-0.17	-0.28	-0.05	-0.02	-0.36	-0.28	0.05	-0.07	0.08	-0.06	-0.13	-0.14	0.14	0.06	0.07	0.32	-0.04	-0.04	-0.03	-0.09	0.11	0.13	0.04
	LR			0.04	0.31	-0.17	-0.19	-0.03	-0.23	-0.36	-0.18	**-0.54**	-0.30	-0.14	0.12	-0.06	-0.01	-0.09	0.06	-0.11	-0.08	-0.14	0.01	0.26	-0.02	0.21	0.08	0.05	-0.05	0.19	0.10
	LR	Pn		-0.02	0.24	-0.11	-0.17	-0.08	-0.08	-0.22	-0.09	**-0.52**	-0.32	-0.15	0.03	0.03	-0.14	0.04	0.05	-0.17	-0.09	-0.10	-0.20	0.28	-0.11	0.13	0.18	0.17	-0.06	0.07	0.16
	AC			-0.21	0.15	-0.25	-0.28	-0.25	-0.02	-0.26	-0.21	**-0.65**	-0.25	-0.15	-0.08	0.18	0.05	0.02	0.01	-0.10	-0.08	-0.05	-0.12	0.16	-0.07	0.29	0.23	0.33	0.10	0.00	0.17
Russia	CA	Ps	MX	-0.02	0.07	-0.13	0.09	-0.04	0.04	-0.11	-0.11	0.17	0.01	0.02	0.05	0.19	-0.03	-0.15	-0.08	-0.16	0.07	-0.08	0.06	0.12	0.02	-0.19	**-0.43**	-0.10	-0.02	-0.07	-0.03
	EF	Ls		0.21	0.11	0.14	-0.10	-0.35	0.04	0.05	0.01	0.04	0.26	0.05	-0.07	0.03	-0.04	-0.19	-0.16	-0.07	-0.06	0.02	-0.07	0.16	0.16	-0.19	-0.26	-0.13	0.15	0.04	0.09
	KH			0.23	0.04	0.05	-0.08	-0.28	0.06	-0.06	0.18	0.18	0.29	-0.02	-0.02	0.05	-0.13	-0.19	-0.04	-0.02	-0.12	0.08	0.07	0.10	0.18	-0.32	-0.22	-0.32	-0.01	0.00	0.10
Mongolia	M0	Ls		0.00	-0.31	-0.03	0.02	-0.05	-0.05	-0.01	0.00	-0.20	0.11	-0.36	0.37	0.14	0.01	-0.26	-0.08	0.20	0.23	-0.13	-0.26	-0.19	0.05	0.07	-0.02	-0.03	0.11	-0.16	-0.02
	M7			0.16	-0.09	-0.12	0.00	-0.27	0.08	-0.11	0.02	0.12	**0.60**	0.08	0.15	0.15	-0.01	-0.12	-0.08	0.15	-0.03	0.09	-0.01	-0.06	-0.08	-0.11	-0.35	-0.37	-0.06	-0.17	0.32
Spain	PN	Ps		0.16	0.17	-0.14	-0.37	-0.13	-0.09	-0.21	-0.27	-0.22	0.19	**-0.49**	-0.31	-0.36	0.05	0.07	0.00	0.12	0.23	0.10	0.23	0.05	0.07	0.18	0.11	0.10	0.35	**0.39**	0.21
	LR			-0.07	-0.07	0.09	0.13	0.14	-0.11	0.03	0.04	0.22	0.32	0.15	0.22	-0.05	-0.06	-0.05	0.10	0.11	-0.04	0.15	0.06	-0.07	0.26	-0.11	-0.21	**-0.57**	-0.32	-0.10	-0.03
	LR	Pn		0.17	0.00	0.08	0.11	0.17	-0.25	-0.16	-0.11	0.17	**0.42**	-0.01	0.09	-0.15	0.06	-0.10	0.15	0.09	0.08	0.04	0.29	0.13	0.31	0.02	-0.09	**-0.51**	-0.13	0.20	0.09
	AC			0.25	-0.18	0.20	0.25	0.17	-0.02	0.18	0.22	0.32	0.37	0.19	0.18	-0.12	-0.07	0.08	0.11	0.06	-0.15	-0.04	0.10	-0.15	0.14	-0.13	-0.18	**-0.45**	-0.21	0.11	-0.20

Table S2. Partial correlations (Pearson coefficients) calculated by relating May and June precipitation and residual EW (earlywood width) and MN (minimum density) chronologies after controlling for the effects of mean temperature of those months. Correlations were calculated only for those sites with significant (*P* < 0.05) associations of EW and MN with May and June precipitation and temperature (see Table S1). The rest of explanations are as in Table S1.

Country	**Site**	**Species**	**Variable**	**Precipitation**		**Temperature**	
				May	**June**	**May**	**June**
Mongolia	M7	Ls	EW	–	**0.54**	–	-0.06
Spain	AC	Pn		**0.56**	–	-0.09	–
Russia	CA	Ps	MN	**-0.49**	–	0.14	–
	KH	Ls		**-0.30**	–	0.18	–
Mongolia	M7	Ls		–	**-0.38**	–	0.19
Spain	LR	Ps		**-0.50**	–	0.14	–
	AC	Pn		**-0.58**	-0.21	0.16	0.18

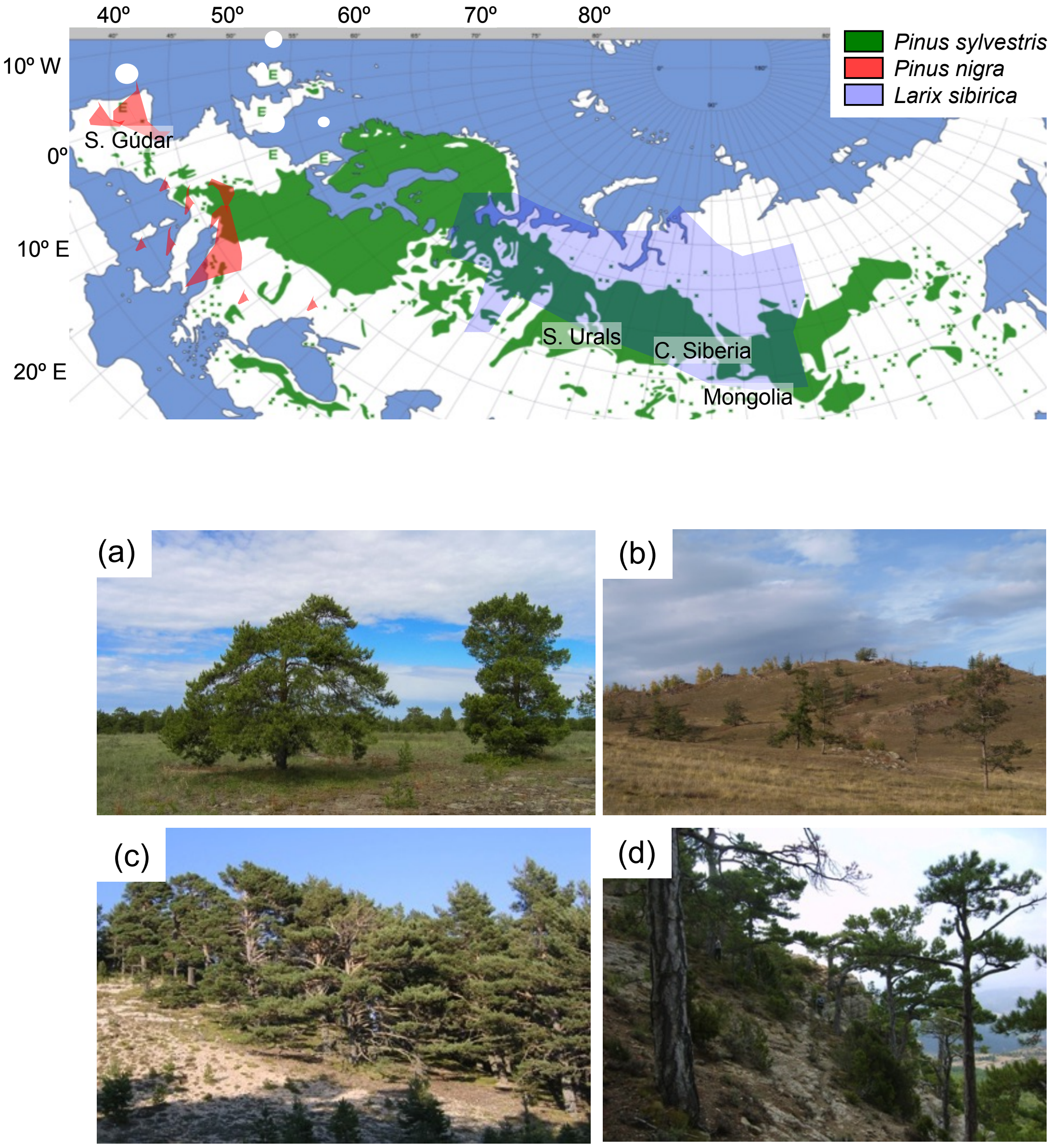

Figure S1. Map showing the location of the four study regions and the distribution of the three study tree species in Eurasia. Some images showing sampled forests: (a) Scots pine and (b) Siberian larch forest-steppe ecotones from southern Siberia; (c) Scots pine and (d) Black pine stands from eastern Spain (Sierra de Gúdar).

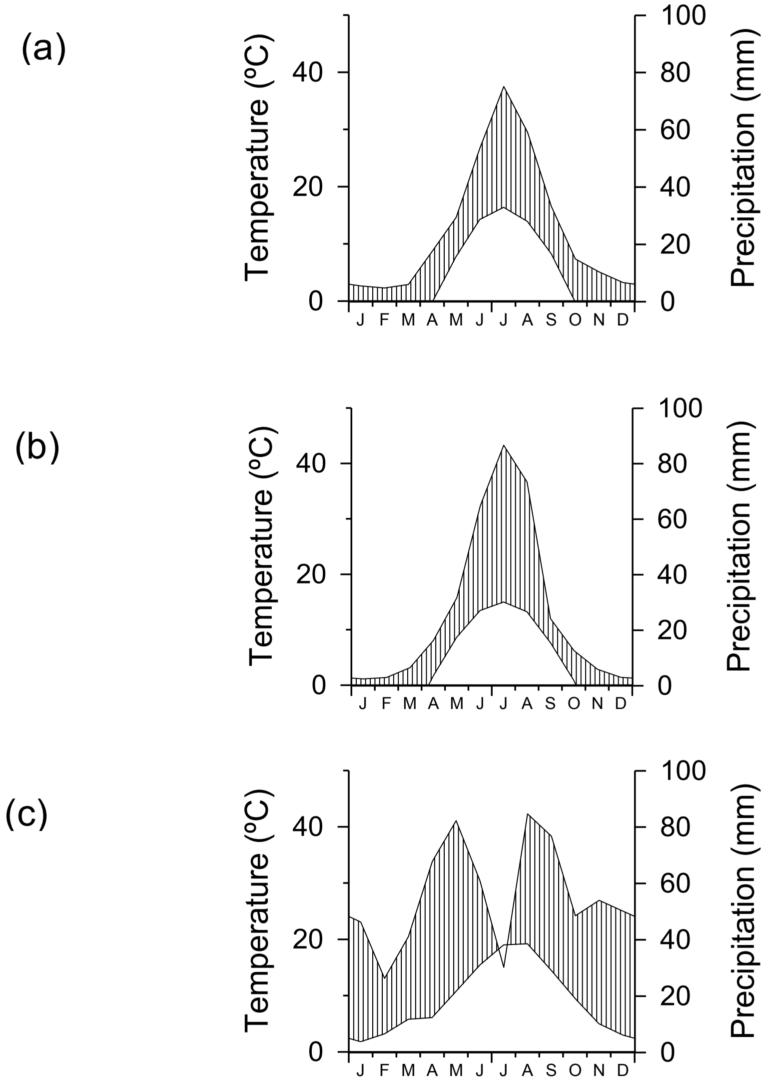

Figure S2. Climate diagrams of the three study regions: (a) Russia, (b) Mongolia and (c) Spain (see stations and data sources in Table 2).

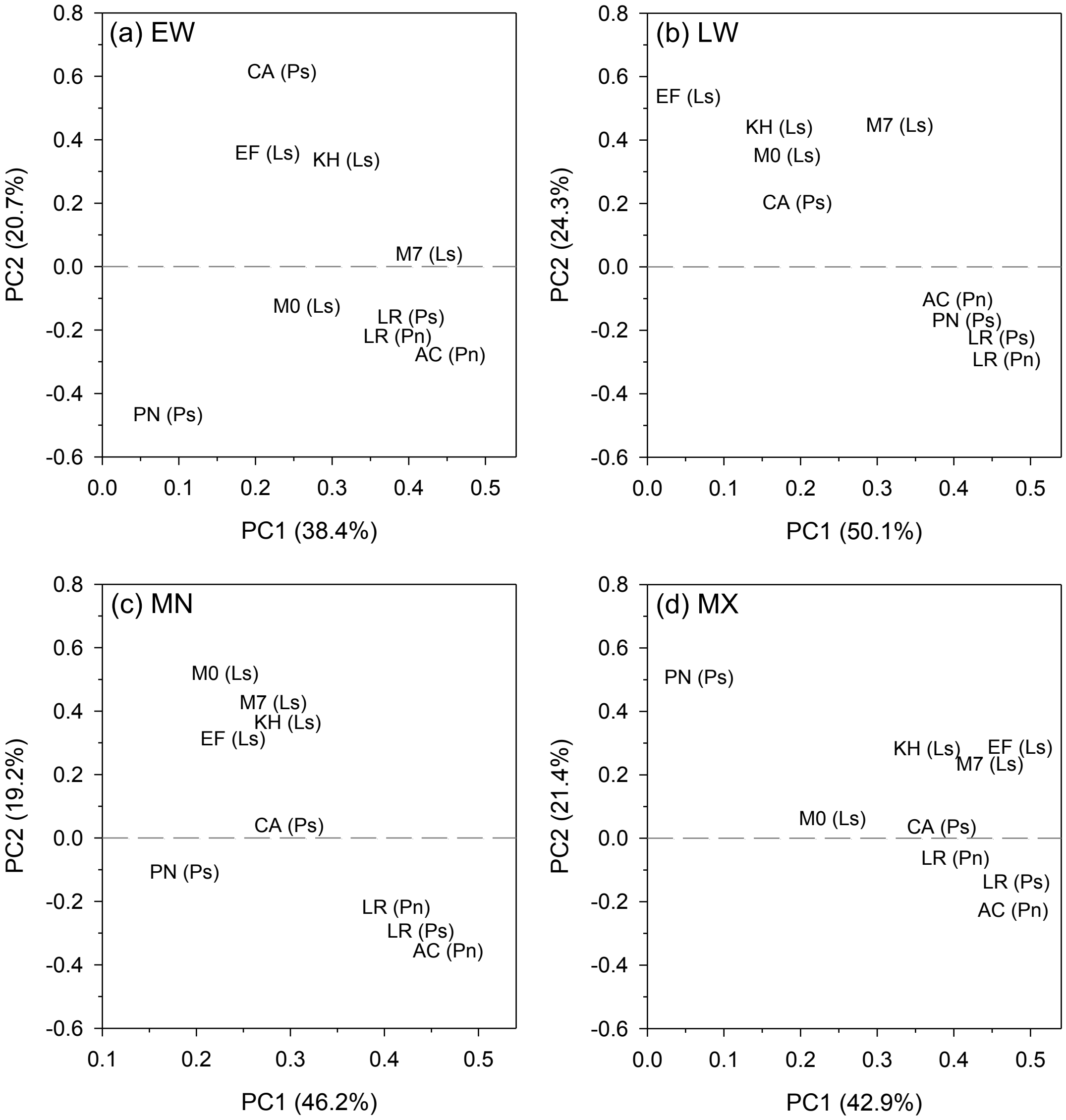

Figure S3. Scatter plots showing the loadings of the first (PC1) and second (PC2) principal components obtained by calculating Principal Component Analyses on the variance-covariance matrices of climate-growth or climate-density (seasonal width and density data) correlations. The graphs correspond to earlywood (EW, a) and latewood (LW, b) width indices and minimum (MN, c) and maximum (MX, d) wood densities. Correlations were calculated for the common period (1950-2002). The sites are abbreviated as in Table 1 followed by species' abbreviations between parentheses (Ls, *Larix sibirica*; Pn, *Pinus nigra*; Ps, *Pinus sylvestris*). The percentages of total variance accounted for by PC1 and PC2 are indicated in each graph.

CAPÍTULO 4

Divergent last century tree growth along an altitudinal gradient in a *Pinus sylvestris* dry-edge population

Foto: *Pinus sylvestris.* Laura Fernández-Pérez

Divergent last century tree growth along an altitudinal gradient in a *Pinus sylvestris* dry-edge population

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RESUMEN

El aumento de la temperatura asociado con el cambio climático podría tener una gran influencia sobre el crecimiento de los árboles de especies de bosques templados y la productividad forestal. Sin embargo, la dirección e intensidad de estos efectos en el límite seco del rango de especies aún no están claras, particularmente dada la interacción entre los factores ambientales locales y otros factores de cambio global, como el cambio en el uso del suelo y el aumento del CO_2 atmosférico y la deposición de nitrógeno. Si bien, algunos estudios recientes sugieren que el crecimiento de los árboles en climas templados fríos se ha acelerado durante las últimas décadas del siglo XX, otros sugieren una prevalencia de fenómenos de decaimiento, especialmente en las poblaciones en el límite seco. Utilizando inventarios forestales históricos, analizamos las tendencias de crecimiento de los árboles del siglo pasado (1930-2010) a lo largo de un gradiente altitudinal (1350-1900 m s.n.m.) en un bosque de *Pinus sylvestris* localizado en e límite latitudinal en la Península Ibérica. El crecimiento se estimó como el incremento decadal en volumen en árboles cortados de diferentes clases de tamaños de 1930 a 2010 (1930-1940, 1939-1949, 1949-1959, 1959-1968, 1989-1999, 2000-2010). Los resultados mostraron tendencias temporales opuestas de crecimiento dependiendo de la elevación. Mientras que el crecimiento de los árboles se ha acelerado en el extremo inferior del gradiente altitudinal (1350-1623 m s.n.m.), se ha desacelerado a elevaciones más altas (1624-1895 m s.n.m). El crecimiento disminuyó con la elevación, pero la magnitud aumentó con la edad del árbol. Estos resultados resaltan la importancia de las condiciones ambientales locales en las respuestas de crecimiento de las especies a los factores de cambio global y la dificultad de atribuir impactos a un solo controlador direccional.

Palabras clave: altitud, cambio global, Mediterraneo, Pino silvestre.

ABSTRACT

Temperature increase associated with climate change might strongly influence tree growth and forest productivity in temperate forest species. However, the direction and intensity of these effects at the dry edge of species range are still unclear, particularly given the interaction between local environment factors and other global change drivers such as land use change and increase in atmospheric CO_2 and nitrogen deposition. While some recent studies suggest that tree growth in cool temperate climates has accelerated during the last decades of the 20th century in temperate climates, others suggest a prevalence of decay phenomena especially in dry-edge populations. Using historical forest inventories, we analyzed last century tree growth trends (1930-2010) along an elevation gradient (1350-1900 m a.s.l.) in a dry edge *Pinus sylvestris* forest in Central Iberian Peninsula. Growth was estimated as decadal volume increments in harvested trees of different size classes from 1930 to 2010 (1930-1940, 1939-1949, 1949-1959, 1959-1968, 1989-1999, 2000-2010). The results showed opposite growth trends over time depending on elevation. While tree growth has accelerated in the low end of the altitudinal gradient (1350-1623 m a.s.l.), it slowed down at higher elevations (1624-1895 m a.s.l.). Growth decreased with elevation, but the magnitude of the reduction increased with tree age. These results highlight the importance of local environmental gradients in shaping species growth responses to global change drivers and the difficulty of attributing impacts to a single directional driver.

Key words: altitude, global change, high mountain, Mediterranean, Scots Pine.

1. INTRODUCTION

Global change drivers such as the increase in atmospheric CO_2 concentrations (C_a) or nitrogen (N) deposition might affect forest productivity in temperate forests (Boisvenue and Running 2006; Lindner et al. 2010; Smith 2011; Frank et al. 2015). A higher C_a and soil N may enhance net photosynthesis but have variable effects on stomatal conductance. If photosynthesis increases more rapidly than stomatal conductance, it can increase plant water use efficiency (Morison 1985; Norby et al. 1999; Ainsworth and Rogers 2007). Experiments using CO_2 enriched environments support this hypothesis and show that the increment of photosynthesis and water use efficiency in tree species in response to higher C_a is mainly due to increased carboxylation (Gunderson et al. 1993; Norby et al. 2005). Tree ring studies, models and inventory data indicate that tree growth has increased in boreal and temperate forest ecosystems during the last decades in parallel to increments in the atmospheric C_a and N deposition (Bolker et al. 1995; Koutavas, 2008; Lamarche et al. 1984), providing indirect support to the C_a and N "fertilizing" effects. In conjunction with a rise in C_a, the frequency of drought spells and vapor pressure deficit have also increased during the last decades in many regions (Resco De Dios et al. 2007; Wallace et al. 2007). Some studies question the "fertilization" effect of C_a increase and N deposition on tree growth, particularly for water-limited environments (Körner and Miglietta 1994; Camarero et al. 2015) as water stress may override it. It is therefore unclear whether "fertilization" effects can compensate or exceed the costs of increased aridity in drought-prone ecosystems (e.g. Keenan et al. 2010). Moreover, this balance can also be critically modulated by other local factors such as competition, evapotranspiration rate or forest management (see for example, Vayreda et al. 2012; Madrigal-González et al. 2015).

In water-limited Mediterranean ecosystems, previous findings have not shown clear tree growth trends in response to global change drivers. While some studies have reported a neutral (Peñuelas et al. 2011) or even a negative relation between tree growth and rising C_a (Linares and Camarero 2012), other studies have reported positive effects (Koutavas 2008; Madrigal-González et al. 2015). Differences among studies could be due to local environmental conditions -which offset the effects of C_a and N deposition on tree growth- and/or to species individual functional responses, which may show different responsiveness along resource and environmental gradients (Granda et al. 2014). Mountain Mediterranean forests represent natural ecotones dominated by Eurosiberian tree species at the edge between the semi-arid and cool temperate biomes. That is why mountain forests in the Mediterranean are considered highly vulnerable to global change (e.g. García-Valdés et al. 2013). During the last decades, the intensity of droughts has increased, reducing tree productivity in drought-prone environments (Solomon et al, 2007). In the Mediterranean basin, the future climatic scenarios predict a temperature increase of approx. 0.4-0.6 ºC relative to the present temperature (Moreno 2005). Tree populations living at the dry edge of species distribution are often vulnerable to rapid climate changes, and particularly to intense drought spells (Keller et al. 1997). Accordingly, recent studies conducted in dry-edge populations provide evidence of a reduction in tree growth and other demographic parameters caused by drought along local environmental gradients (e.g. Vilà-Cabrera et al. 2014). Further evidence, however, is needed to understand how global change impacts these ecosystems and, in particular, how these impacts are modulated by both endogenous (such as tree age) and exogenous (such as local environmental gradients) factors. This knowledge is critical in order to unveil the mechanisms underlying forest resistance and resilience to the impact of global change and to develop efficient adaptation measures for population persistence.

Pinus sylvestris L. (Scots Pine) is a widely distributed tree species in the northern hemisphere having its dry edge of distribution in mountain areas of southern Europe (Richardson and Rundel, 1998; Martínez del Castillo et al. 2016). Several studies have focused on the abiotic determinants of *P. sylvestris* tree growth across its distribution range (Grace and Norton 1990; Rigling et al. 2002). Particularly, drought is a key factor driving growth in *P. sylvestris* in Mediterranean-climate mountains (Camarero et al. 2015), albeit low winter temperatures can also play an important role (Sánchez-Salguero et al. 2015a). Global change drivers (i.e. warming winters and C_a and N fertilization) might have increased *P. sylvestris* growth throughout the last century, yet this hypothesis has little support in Mediterranean mountains (Camarero et al. 2015).

In this study, we analyze last century tree growth trends in an extensive Mediterranean *P. sylvestris* forest located in the Central System range in the Iberian Peninsula where the species is at the dry edge of its distribution. Specifically, we examined whether tree growth has accelerated over time as expected from increased C_a, and N "fertilization" effects and milder winters, or, on the contrary, whether tree growth has decreased due to increased aridity. *Pinus sylvestris* forests in the Central System range are under a typical mountain Mediterranean climate within an altitudinal gradient that ranges from 1300 to 2100 m.a.s.l. (Martínez-García 1999). We examined tree growth responses along an altitudinal gradient as a surrogate of environmental variation and throughout the period 1930-2010 which encompasses several global change drivers correlated with time.

2. METHODS

2.1 Study area

Tree growth was recorded in a 3891 ha forest (named MUP 198 "Pinar de Navafría") dominated by *P. sylvestris* and located in the municipality of Navafría (Lat 41º03'17" N; Long 3º49'21" W) in the Sierra de Guadarrama, Central System Range, Spain (Figure 1). In this area, *P. sylvestris* is bound to a narrow altitudinal strip ranging from 1300 to 2100 m.a.s.l. (Martínez-García 1999). Climate is continental Mediterranean with important altitudinal variations in temperature and rainfall. Winters are cold and humid and the summers are warm and dry. At the lowest part of the altitudinal gradient (village of Navafría), the annual, minimum and maximum mean temperatures are 10.7, 4.4 and 17 ºC, respectively. Total annual rainfall is 611 mm and no apparent change for rainfall is observed over time (ESM1). Similar to other mountains, rainfall increases while temperature decreases with elevation (Vaganov et al. 2006; Sánchez-Salguero et al. 2015a). In Puerto of Navacerrada (1894 m.a.s.l.), annual rainfall is 1324 mm and the mean annual temperature is 6.4 ºC. At this high-altitude site, a slight decrease in annual rainfall is recognized while in Segovia city (~1000 m.a.s.l.), the precipitation has remained constant throughout the years (ESM1). In contrast, the temperature has increased in the last 50 years in both locations.

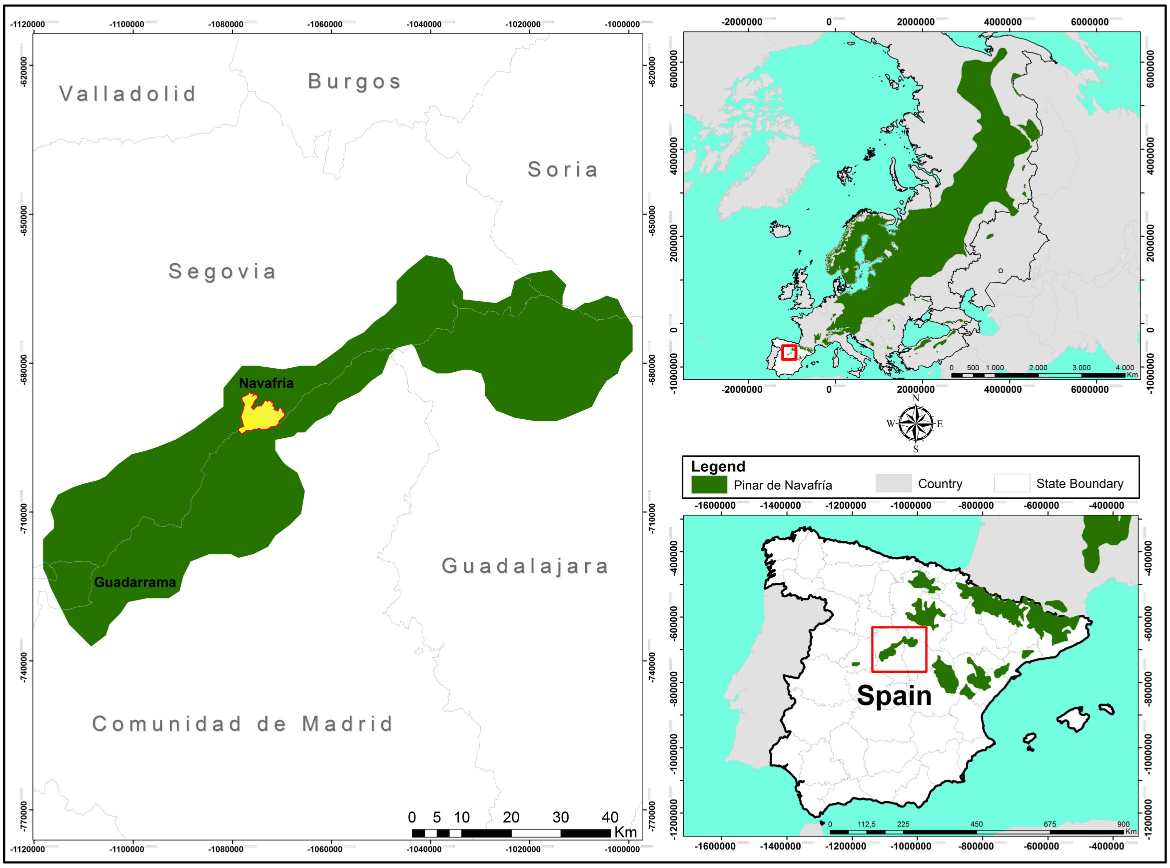

Fig. 1. Distribution of *Pinus sylvestris* in Europe and Spain (right) and location of the forest Pinar de Navafría in the Central System range (left).

2.2 Tree size and growth measurements

Scots Pine forest management in Sierra de Guadarrama has been subjected to management plans since 1890, mainly oriented towards sustainable timber production. The "Pinar de Navafría" forest began to be managed in 1885 using a shelterwood thinning framework to maximize natural regeneration and the production of high quality timber (Diaz and Prieto 1999). For administrative purposes, the forest was divided into nine sections with identical management plans. Each section in turn was subdivided into five subsections, thus resulting in a total of 45 forest units. Rotation averages 120 years and the regeneration period spans 20 years. Periodic thinning is conducted every 10 years depending on the stage of the rotation period and the stand is managed in order to achieve even-aged stands for the long term.

lForest management prescriptions have been audited almost every ten years, when trees of different diameter classes were selected and harvested for estimating dasometric tree variables. Specifically, these variables were tree age and stem volume, which have been recorded since 1890, thus providing an invaluable source of information to assess the evolution of tree structure and growth throughout the last century. All data were recorded in a regional historical archive. Tree growth data in particular are available for decadal periods in 1930-1940, 1939-1949, 1949-1959, 1959-1968, 1989-1999, 2000-2010. In the first record (1930-1940), the present and definitive stands were defined, preventing the use of the data from the first management plan (1885-1895). Slope aspect, area, altitude and soil type were recorded for each forest unit. For each fallen tree the diameter at breast height after removing the bark (dbh, cm), height (m) and age (number of rings) was measured. Then the trunk was transversally cut into small cylindrical segments to measure the volume without bark assuming the segments to be cylinders. The total volume of the trunk (V) was calculated as the sum of the volume of each trunk segment. The volume of each tree 10 years before (V') was calculated in a similar way by subtracting the growth of the last 10 tree-ring width. Absolute growth was estimated as the difference between trunk volumes (V-V'). Annual growth rate was estimated for each tree as the absolute growth divided by number of years. The age of the studied trees ranged between 30 and 235 years old.

2.3 Data analysis

We used the platform R to perform the statistical analyses (R 3.1.2, R, Development Core Team, 2008). We used Linear Mixed Models (LMM) of the nlme package (Bates et al, 2015) to analyze tree growth as a function of the three order interaction altitude × calendar years × tree age to evaluate whether global-change trends of tree growth are dependent on tree age and altitude since 1930. To avoid collinearity between the variables, we firstly analyzed a correlation among variables using Pearson correlation with the Corrplot function in the Hmisc package. Selected predictor variables were all uncorrelated and thus multicollinearity is expected to be negligible in the LMM (Table 1). Importantly, tree density depicted a somewhat linear increment throughout the studied period (Figure 2) so we decided to fit a first model with only this variable to remove its effects prior to analyzing long-term linear trends using the calendar years. To do that, we used the lme4 statistical package in the R environment (Bates et al, 2015). The model was developed as follows:

$$\mathrm{Y_{ijt}} = \beta_0 + \beta_1\,\mathrm{Density} + b_1 + \varepsilon_{\mathrm{ijt}} \text{ ....Equation 1}$$

where Y_{ijt} represents annual growth (untransformed). The fixed parameters are $\beta_0 - \beta_1$, while b_1 is random effect $(b_1 \sim \mathrm{N}\,(0, \tau_1^2)$, and finally ε_{ijt} represents the error of the model $\varepsilon_{ijt} \sim \mathrm{N}\,(0, \tau_1^2)$.

We thereafter developed the final model using the residuals of the previous model as the dependent variable and the third order interaction Altitude × Calendar year × tree age as the fixed effects term. The biological meaning of this third-order interaction is that depending on the tree age, and altitude, differential global change trends associated with rising C_a, N or temperature should be recognized in tree growth. If the set of contemporary trees develops differently in different years, it will indicate changes in growth and site conditions. The model with the most appropriate structure was as follows:

$$Y_{ijt} = \beta_0 + \beta_1 \text{ Age} \times \beta_2 \text{ Altitude} \times \beta_3 \text{ Year} + b_1 + \varepsilon_{ijt} \ldots\ldots\ldots\ldots \text{Equation 2}$$

where Y_{ijt} represents jth annual growth (untransformed) on the ith individual (untransformed) on a tth specific year. The fixed parameters are β_0 - β_4, while b_1 is random effect $(b_1 \sim \mathrm{N}\,(0, \tau_1^2)$, and finally ε_{ijt} shows the error $\varepsilon_{ijt} \sim \mathrm{N}\,(0, \tau_1^2)$.

Table 1. Correlations among independent variables.

Variables	Year	Altitude	Stand Density	Trunk diameter	Tree Age
Year	1	-	-	-	-
Altitude	-0.04	1	-	-	-
Stand density	0.39	-0.37	1	-	-
Trunk diameter	-0.06	-0.01	-0.04	1	-
Tree age	-0.02	0.07	-0.04	0.70	1

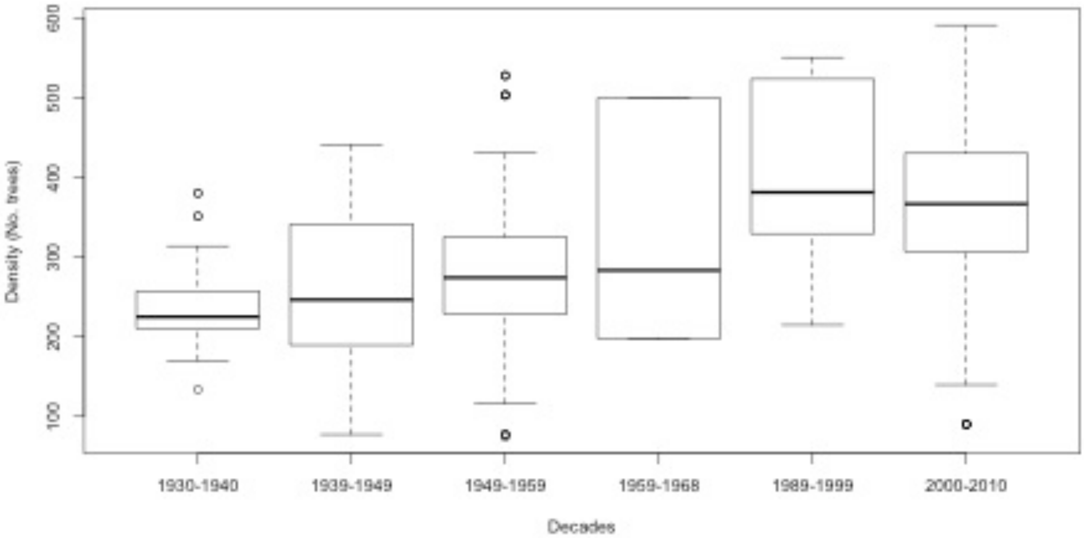

Fig. 2. Tree density increase in the last decades in Navafría, Sierra the Guadarrama, Spain.

3. Results

The effect of the interactions Age × Altitude and Year × Altitude were both significant (Table 2). However, the third-order interaction (Age × Altitude × Year) was not supported in the LMM. At low altitudes (1350-1623 m.a.s.l.), tree growth increased over time, while at high altitudes (1624-1892 m.a.s.l.) growth slowed down (Fig. 3 and Table 2). Growth change across the altitudinal gradient was age–dependent; young trees showed overall low growth with no differences along the altitudinal gradient. As trees became older, they grew more slowly at high altitudes than at the low altitude sites, where growth was high (Fig. 4). The correlation among the model parameters was negligible (ESM3).

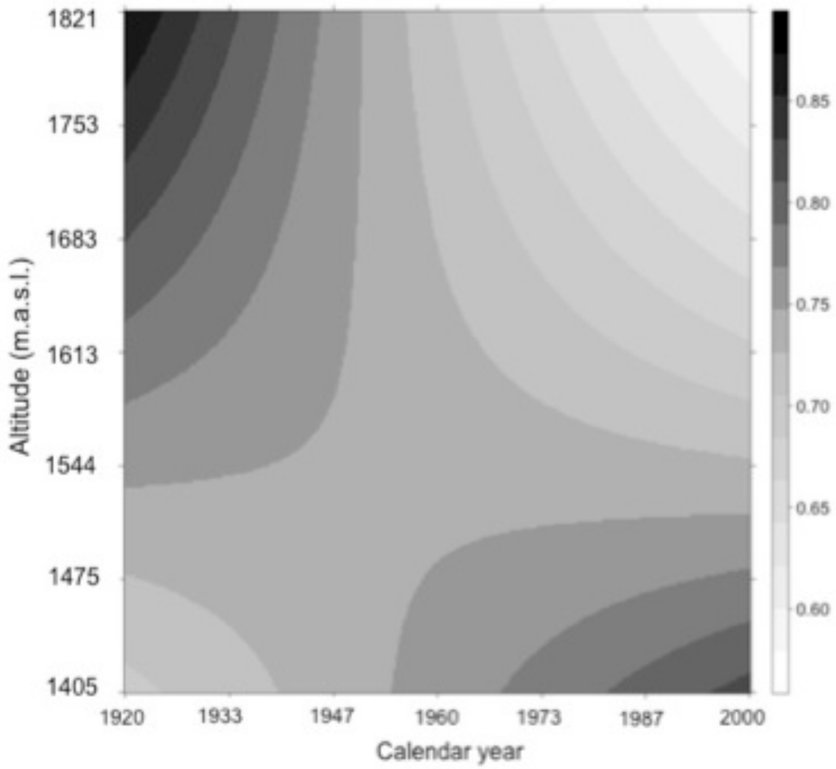

Fig. 3. Contour plots showing tree growth responses to pair-wise interactions over the years and the altitude.

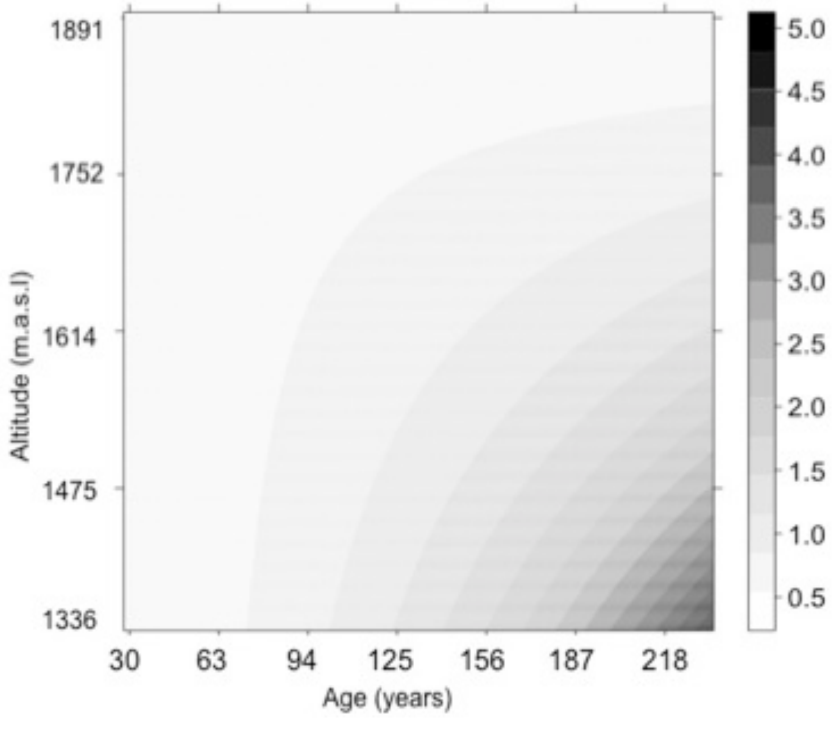

Fig. 4. Contour plots showing tree growth responses to pair-wise interactions over the tree age and the altitude gradient.

Table 2. Parameters estimated for the triple interaction model including associated standard error, t statistic and p value.

Model parameters	Parameter value	Std.Error	t-value	P value
Intercept	-0.147	0.024	-6.06	<0.0001
Age	0.156	0.024	6.37	<0.0001
Altitude	-0.115	0.025	-4.63	<0.0001
Year	-0.046	0.024	-1.88	0.060
Age × Altitude	-0.100	0.026	-3.91	0.000
Age × Year	-0.037	0.025	-1.46	0.144
Altitude × Year	-0.073	0.026	-2.82	0.005
Age × Altitude × Year	-0.047	0.030	-1.55	0.122

4. Discussion

Our results evidence divergent last century *P. sylvestris* growth patterns along the altitudinal gradient: while growth has increased notably over time at low altitudes, growth at high altitudes has significantly decreased. This result is somehow opposite to global expectations which emerged from trends reported in cool temperate and boreal forests, and which point to net positive effects of climate warming and CO_2 enrichment in forests in which low temperature is the predominant environmental constraint. Our results also showed that tree age modulated these responses along the altitudinal gradient. Specifically, growth divergence was more evident along the altitudinal gradient for older trees. Growth reduction with altitude is commonly seen in many mountains of the world (Körner, 1998). Temperature decrease reduces plant metabolism and shortens the growing season (Körner 2003; Larcher, 2005), which reduces tree growth. A low growth rate and the lack of altitudinal gradient effects for young trees suggest that they experience similar stress level along the entire gradient. Growth at low altitudes might be constrained by competition and drought stress (Castro et al, 2004), while at high altitudes cold stress might be the main stressor for young trees.

Mean temperature has increased throughout the 20th century but especially in the last 20 years (Beniston 2003; Pauli et al. 2003; García-Romero et al. 2010). While some studies have reported growth acceleration at high elevation locations (Salzer et al. 2009; Pretzsch et al. 2014), others have shown no change or even a negative response (Briffa et al. 2004; Wilmking et al. 2004). In the Sierra de Guadarrama, the mean annual and winter temperatures have increased by 19% (5.75 ºC to 6.87 ºC) and 127% (-1.38 ºC to 0.38 ºC), respectively in the second half of the 20th century (Muñoz and García, 2004). The increase in N deposition and atmospheric C_a from the industrial era has also been proposed to enhance tree growth in some boreal conifers (Bolker et al, 1995; Cannell et al. 1998; Emmett et al. 1998). Field experiments of N enrichment point to modest increase in forest growth (Bolker et al. 1995; Cannell et al. 1998; Emmett et al, 1998). Specifically, for *P. sylvestris* boreal stands, N enrichment increased growth by 12% (Nissinen and Hari, 1998). In contrast, others studies showed no effect of N deposition (Reynolds et al. 1998). N deposition in the Sierra of Guadarrama is relatively high (García-Gómez et al. 2014) due to its proximity to a big city (~ 70 km from the metropolitan area of Madrid). It is possible that the temperature, N deposition and C_a increase over time have stimulated tree growth at low altitudes due to enhanced water use efficiency (Saurer et al. 2004). Paradoxically, at high altitudes where the cold is a major limitation for plant performance (Sánchez-Salguero et al. 2015a), growth has decreased over time (Figure 3), suggesting lack of "fertilization" and warming temperature benefits on tree growth. This suggests that the benefits of these global change drivers have been overtaken out by unaccounted local factors operating at high altitudes. Consistent with our findings, a poor relation between tree growth and main global change drivers has been reported for other Mediterranean pine forests (Camarero et al. 2015), providing further support to the idea that other local or regional environmental factors can mask the positive effects of warming and C_a and N deposition "fertilization" effects.

Several local factors might explain the patterns described in our study. First, soils at high altitude may have lower water holding capacity and fertility than sites at the low end of the gradient (Pachepsky et al. 2001; Charan et al. 2013). Temperature increase over time involves higher evapotranspiration that, along with a slight reduction in precipitation at the high elevations (see ESM1) may result in increasing drought stress (Allen and Breshears 1998; Wilmking et al. 2004). A growth reduction has been reported in *P. sylvestris* populations located at high altitudes, mainly associated with moisture conditions (Lebourgeois et al. 2010), suggesting that in Mediterranean mountains Scots Pine populations at high elevations may in fact be more vulnerable to drought stress than lower populations (but see Sánchez-Salguero et al. 2015a). Second, water availability rather than low temperature is the main limiting factor for tree growth at low- and mid-altitude sites in Mediterranean mountains (Castro et al. 2004; Sánchez-Salguero et al. 2015b). *Pinus sylvestris* populations at low altitudes may be locally adapted and more drought tolerant than high altitude populations. Both altitudinal and latitudinal drought tolerance differences among *P. sylvestris* populations have been reported (Fournier et al. 2006; Matías et al. 2014). Ecotypic differences in drought tolerance of tree species can be found even at short spatial scales (see Kubiske and Abrams 1992). Moreover, many plant species show ecotypic variations along altitudinal gradients with high altitude ecotypes growing slower than low altitude ecotypes due to inherent differences in metabolism, phenology and stress tolerance (von-Wuehlisch et al. 1995; Reich et al. 1996; Vitasse et al. 2009; Vitasse et al. 2013). Thus, the negative effects of low temperatures with altitude can interact synergistically with ecotype differences to reduce tree growth (Matías et al. 2014; Matías et al. 2017). Finally, a third explanation is that *P. sylvestris* populations at high altitudes are more sensitive to late spring frosts. Spring frosts might damage seedling emergence and hinder tree growth and survival (Fisichelli et al. 2014). Cold hardening and dehardening in many conifers is partially controlled by temperature (Bigras et al. 2001) and mild winter spells can reduce cold hardening due to reduction in the needle concentration of the cryoprotectant soluble sugars (Ögren et al. 1997; Morin et al. 2007). It is possible that the increase in winter temperature may have advanced cold dehardening, making aboveground organs more vulnerable to spring frosts, whose frequency has remained stable as mean surface temperature has increased (IPCC 2014). Increased damage caused by spring frosts due to premature dehardening has been observed for conifers (Ögren et al. 1997; Man et al. 2009) and for broadleaf trees (Hufkens et al. 2012).

5. Conclusions

Throughout the last 80 years a rear-edge *P. sylvestris* forest has shown divergent growth patterns along an altitudinal gradient. Tree growth at low altitudes has increased in the second half of the 20th century, while at high altitudes it has slightly decreased. This suggests that environmental conditions have become more adverse for growth at high altitudes and have improved at low altitudes. The positive effects of temperature, atmospheric C_a and N deposition increase on tree growth at low altitudes may have been greater than the potential negative effects of increased summer aridity. This study highlights the importance of local environmental factors, which may interact with global scale environmental factors, to drive forest responses suggesting the need to incorporate local environmental variation and ontogeny in models projecting likely forest responses to global change.

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7. References

Ainsworth E, Rogers A (2007) The response of photosynthesis and stomatal conductance to rising [CO_2]: Mechanisms and environmental interactions. Plant, Cell Environ 30:258–270 doi: 10.1111/j.1365-3040.2007.01641.x

Allen C, Breshears D (1998) Drought-induced shift of a forest-woodland ecotone: Rapid landscape response to climate variation. Proced Nat Acad Sci 95:14839–14842 doi: 10.1073/pnas.95.25.14839

Bates D, Maechler M, Bolker B, Walker S (2015) Lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7.

Beniston M (2003) Climatic change in mountain regions: a review of possible impacts. Clim Chang 59:5-31 doi: 10.1023/A:1024458411589.

Bigras FJ, Ryyppo A, Lindström A, Stattin E (2001) Cold acclimation and deacclimation of shoots and roots of conifer seedlings, in: Bigras FJ, Colombo SJ (eds.) Conifer cold hardiness. Kluwer Academic Publishers, Dordrecht - Boston, pp. 57–88.

Boisvenue C, Running S (2006) Impacts of climate change on natural forest productivity - Evidence since the middle of the 20th century. Glob Chang Biol 12:862–882 doi: 10.1111/j.1365-2486.2006.01134.x

Bolker B, Pacala S, Bazzaz F, Canhams CD, Levin SA (1995) Species diversity and ecosystem response to carbon dioxide fertilization: conclusions from a temperate forest model. Glob Chang Biol 1:373–381.

Briffa K, Osborn T, Schweingruber F (2004) Large-scale temperature inferences from tree rings : A review. Glob Plant Ch 40:11–26 doi: 10.1016/S0921-8181(03)00095-X

Camarero J, Gazol A, Tardif J, Conciatori F (2015) Attributing forest responses to global-change drivers: Limited evidence of a CO_2-fertilization effect in Iberian pine growth. J Biogeogr 42:2220–2233 doi: 10.1111/jbi.12590

Cannell M, Thornley J, Mobbs D, Friend A (1998) UK conifer forests may be growing faster in response to increased N deposition, atmospheric CO_2 and temperature. Forestry 71:277–296. doi: 10.1093/forestry/71.4.277

Castro J, Zamora R, Hódar JA, Gómez JM (2004) Alleviation of summer drought boosts establishment success of *Pinus sylvestris* in a Mediterranean mountain: an experimental approach, Plant Ecol 181:191-202.

Charan G, Bharti VK, Jadhav SE, Kumar S, Acharya S, Kumar P, Gogoi D, Srivastava RB (2013) Altitudinal variations in soil physico-chemical properties at cold desert high altitude. J. Soil Sci. Plant Nutr 13, 267–277 doi:10.4067/s0718-95162012005000016

Emmett B, Boxman D, Bredemeier M, Gundersen P, Kjønaas OJ, Modan F, Schleppi P, Tietema A, Wright RF (1998) Predicting the effects of atmospheric nitrogen deposition in conifer stands: Evidence from the NITREX ecosystem-scale experiments. Ecosystems 1:352–360. doi: 10.1007/s100219900029

Fisichelli N, Vor T, Ammer C (2014) Broadleaf seedling responses to warmer temperatures "chilled" by late frost that favors conifers. Eur J Forest Res 133:587-596 doi: 10.1007/s10342-014-0786-6

Fournier N, Rigling A, Dobbertin M, Gugerli F (2006) Faible différenciation génétique, à partir d'amplification aléatoire d'ADN polymorphe (RAPD), entre les types de pin sylvestre (*Pinus sylvestris* L.) d'altitude et de plaine dans les Alpes à climat continental. Ann For Sci 63:431–439 doi: 10.1051/forest

Frank D, Reichstein M, Bahn M, Thonicke K, Frank D, Mahecha MD, Smith P, Van der Velde M, Vicca S, Babst F, Beer C, Buchman N, Canadell JG, Ciais P, Cramer W, Ibrom A, Miglietta F, Poulter B, Rammig A, Seneviratne SI, Walz A, Wattenbach M, zavala MA, Zscheischler J (2015) Effects of climate extremes on the terrestrial carbon cycle: concepts, processes and potential future impacts. Glob Chang Biol 21:2861–2880 doi: 10.1111/gcb.12916

García-Gómez H, Garrido J, Vivanco M, Lassaletta L, Rábago I, Ávila A, Tsyro S, Sánchez G, González OA, González-Fernández I, Alonso R (2014) Nitrogen deposition in Spain: Modeled patterns and threatened habitats within the Natura 2000 network. Sci Total Environ 485–486:450–460 doi: 10.1016/j.scitotenv.2014.03.112

García-Romero A, Muñoz J, Andrés N, Palacios D (2010) Relationship between climate change and vegetation distribution in the Mediterranean mountains: Manzanares Head valley, Sierra de Guadarrama (Central Spain). Clim Change 100:645–666 doi: 10.1007/s10584-009-9727-7

García-Valdés R, Zavala M, Araújo M, Purves D (2013) Chasing a moving target: Projecting climate change-induced shifts in non-equilibrial tree species distributions. J Ecol 101:441–453 doi: 10.1111/1365-2745.12049

Grace J, Norton D (1990) Climate and growth of *Pinus sylvestris* at its upper altitudinal limit in Scotland: evidence from tree growth-rings. J Ecol 78:601–610 doi: 10.2307/2260887

Granda E, Rossatto D, Camarero J, Voltas J, Valladares F (2014) Growth and carbon isotopes of Mediterranean trees reveal contrasting responses to increased carbon dioxide and drought. Oecologia 174:307–317 doi: 10.1007/s00442-013-2742-4

Gunderson C, Norby R, Wullschleger S (1993) Foliar gas exchange responses of two deciduous hardwoods during 3 years of growth in elevated CO_2: no loss of photosynthetic enhancement. Plant, Cell Environ 16:797–807 doi: 10.1111/j.1365-3040.1993.tb00501.x

Hufkens K, Friedl MA, Keenan TF, Sonnentag O, Bailey A, O'Keefe J, Richardson AD (2012) Ecological impacts of a widespread frost event following early spring leaf-out. Glob Chang Biol 18(7): 2365-2377 doi: 10.1111/j.1365-2486.2012.02712.x

IPCC (2014) Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.

Keenan T, Sabaté S, Gracia C (2010) The importance of mesophyll conductance in regulating forest ecosystem productivity during drought periods. Glob Chang Biol 16:1019–1034 doi: 10.1111/j.1365-2486.2009.02017.x

Keller T, Guiot J, Tessier L (1997) Climatic effect of atmospheric CO_2 doubling on radial tree growth in south eastern France. J Biogeogr 24:857–864 doi: 10.1046/j.1365-2699.1997.00140.x

Körner C, Miglietta F (1994) Long term effects of naturally elevated CO_2 on mediterranean grassland and forest trees. Oecologia 99:343–351 doi: 10.1007/BF00627748

Körner C (1998) A re-assessment of high elevation treeline positions and their explanation. Oecologia 115:445–459 doi: 10.1007/s004420050540

Körner C (2003) Ecological impacts of atmospheric CO_2 enrichment on terrestrial ecosystems. Philos Trans R Soc London 361:2023–2041 doi: 10.1098/rsta.2003.1241

Koutavas A (2008) Late 20th century growth acceleration in greek firs (*Abies cephalonica*) from Cephalonia Island, Greece: A CO_2 fertilization effect? Dendrochronologia 26:13–19 doi: 10.1016/j.dendro.2007.06.001

Kubiske M, Abrams M (1992) Photosynthesis, water relations, and leaf morphology of xeric versus mesic *Quercus rubra* ecotypes in central Pennsylvania in relation to moisture stress. Can J For Res 22:1402–1407.

Lamarche V, Graybill D, Fritts H, Rose M (1984) Increasing atmospheric carbon dioxide: tree ring evidence for growth enhancement in natural vegetation. Science (80-) 225:1019–1021 doi: 10.1126/science.225.4666.1019

Larcher W (2005) Climatic constraints drive the evolution of low temperature resistance in woody plants. J Agric Meteorol 61(4):189-202.

Lebourgeois F, Rathgeber CBK, Ulrich E (2010) Sensitivity of French temperate coniferous forests to climate variability and extreme events (*Abies alba*, *Picea abies* and *Pinus sylvestris*). J Veg Sci 21:364-376 doi: 10.1111/j.1654-1103.2009.01148.x

Linares J, Camarero J (2012) From pattern to process: linking intrinsic water-use efficiency to drought-induced forest decline. Glob Chang Biol 18:1000–1015 doi: 10.1111/j.1365-2486.2011.02566.x

Lindner M, Maroschek M, Netherer S, Kremer A, Barbati A, Grcia-Gonzalo J, Seidl R, Delzon S, Corona P, Kolström M, Lexer JM, Marchetti M (2010) Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. For Ecol Manage 259:698–709 doi: 10.1016/j.foreco.2009.09.023

Madrigal-González J, Hantson S, Yue C, Poulter B, Ciais P, Zavala MA (2015) Long-term wood production in water-limited forests: evaluating potential CO_2 fertilization along with historical confounding factors. Ecosystems 18:1043–1055 doi: 10.1007/s10021-015-9882-9

Man R, Kayahara GJ, Dang QL, Rice A (2009) A case of severe frost damage prior to budbreak in young conifers in Northestearn Ontario: consequence of climate change? Fores Chron, 85(3): 453:462 doi: 10.5558/tfc85453-3.

Martínez-García F (1999) Los bosques de *Pinus sylvestris* L. del Sistema Central Español. Distribución, historia, composición florística y tipología. PhD dissertation, INIA. Departamento de Biología Vegetal I, Facultad de Ciencias Biológicas, Universidad Complutense de Madrid.

Martinez del Castillo E, Longares L, Gričar J, Prislan P, Gil-Peregrín E, Čufar K, de Luis M (2016) Living on the edge: contrasted wood-formation dynamics in *Fagus sylvatica* and

Pinus sylvestris under mediterranean conditions. Front Plant Sci 7:1–11 doi: 10.3389/fpls.2016.00370

Matías L, González-Díaz P, Jump AS (2014) Larger investment in roots in southern range-edge populations of Scots pine is associated with increased growth and seedling resistance to extreme drought in response to simulated climate change. Environ Exp Bot 105, 32–38 doi:10.1016/j.envexpbot.2014.04.003

Matías L, Linares JC, Sánchez-Miranda Á, Jump AS (2017) Contrasting growth forecasts across the geographical range of Scots pine due to altitudinal and latitudinal differences in climatic sensitivity. Glob Chang Biol 23, 4106–4116 doi:10.1111/gcb.13627

Moreno JM (2005) Principales conclusiones de la evaluación preliminar de los impactos en España por efecto del cambio climático. Ministerio de Medio Ambiente, Madrid

Morin X, Améglio T, Ahas R, Kurz-Besson C, Lanta V, Lebourgeois F, Miglietta F, Chuine I (2007) Variation in cold hardiness and carbohydrate concentration from dormancy induction to bud burst among provenances of three European oak species. Tree Physiol 27:817–825 doi: 10.1093/treephys/27.6.817

Morison J (1985) Sensitivity of stomata and water use efficiency to high CO_2. Plant, Cell Environ 8:467–474 doi: 10.1111/j.1365-3040.1985.tb01682.x

Muñoz J, García R (2004) Modificaciones climáticas y evolución de la cubierta vegetal en las áreas culminantes de la Sierra de Guadarrama (Sistema Central español) durante la segunda mitad del XX: las altas cuencas del Ventisquero de La Condesa y de Valdemartín. Cuad Investig Geog 30:117–146.

Nissinen A, Hari P (1998) Effects of nitrogen deposition on tree growth and soil nutrients in boreal Scots pine stands, in: Environ Pollut. 102: 61–68 doi:10.1016/S0269-7491(98)80016

Norby R, Wullschleger S, Gunderson C, Johnson DW, Ceulemans R (1999) Tree responses to rising CO_2 in field experiments: Implications for the future forest. Plant, Cell Environ 22:683–714 doi: 10.1046/j.1365-3040.1999.00391.x

Norby R, DeLucia E, Gielen B, Calfapietra C, Giardina CP, King JS, Ledford J, McCarthy HR, Moore DJP, Ceulemans R, De Angelis P, Finzi AC, Karnosky DF, Kubiske ME, Lukac M, Pregitzer KS, Scarascia-Mugnozza GE, Schlesinger WH, Oren R (2005) Forest response to elevated CO_2 is conserved across a broad range of productivity. Proc Natl Acad Sci 102:18052–18056 doi: 10.1073/pnas.0509478102

Ögren E, Nilsson T, Sundblad LG (1997) Relationship between respiratory depletion of sugars and loss of cold hardiness in coniferous seedlings over-wintering at raised temperatures: Indications of different sensitivities of spruce and pine. Plant, Cell Environ. 20:247–253.

Pachepsky YA, Timlin DJ, Rawls WJ (2001) Soil and water management and conservation. Soil Sci Soc Am J 65:1787-1795.

Pauli H, Gottfried M, Grabherr G (2003) Effects of climate change on the alpine and nival vegetation of the Alps. J Meteor Ecol 7:9–12 doi: 10.1093/conphys/cot001

Peñuelas J, Canadell J, Ogaya R (2011) Increased water-use efficiency during the 20th century did not translate into enhanced tree growth. Glob Ecol Biogeogr 20:597–608 doi: 10.1111/j.1466-8238.2010.00608.x

Pretzsch H, Biber P, Schütze G, Uhl E, Rötzer T(2014) Forest stand dynamics in Central Europe has accelerated since 1870. Nat Commun 1–10 doi: 10.1038/ncomms5967.

Reich P, Oleksyn J, Tjoelker M (1996) Needle respiration and nitrogen concentration in Scots Pine populations from a broad latitudinal range: A common garden test with field-grown trees. Funct Ecol 10:768–776. doi: 10.2307/2390512

Resco De Dios V, Fischer C, Colinas C (2007) Climate change effects on mediterranean forests and preventive measures. New For 33:29–40 doi: 10.1007/s11056-006-9011-x

Reynolds B, Wilson E, Emmett B (1998) Evaluating critical loads of nutrient nitrogen and acidity for terrestrial systems using ecosystem-scale experiments (NITREX). For Ecol Manage 101:81–94. doi: 10.1016/S0378-1127(97)00127-8

Richardson D, Rundel PW (1998) Ecology and Biogeography of *Pinus*: an introduction. In: Ecology and Biogeography of *Pinus*. Cambridge Press. pp 3-46

Rigling A, Bräker O, Schneiter G, Schweingruber F (2002) Intra-annual tree-ring parameters indicating differences in drought stress of *Pinus sylvestris* forests within the Erico-Pinion in the Valais (Switzerland). Plant Ecol 163:105–121 doi: 10.1023/A:1020355407821

Salzer M, Hughes M, Bunn A, Kipfmueller K (2009) Recent unprecedented tree-ring growth in bristlecone pine at the highest elevations and possible causes. Proc Nat Acad Sci U S A 106:20348–53 doi: 10.1073/pnas.0903029106

Sánchez-Salguero R, Camarero J, Hevia A, Madrigal-González J, Linares JC, Ballesteros-Canovas JA, Sánchez-Miranda A, Alfaro-Sánchez R, Sangüesa-Barreda G, Diego GA, Gutiérrez E, Génova M, Rigling A (2015a) What drives growth of Scots pine in continental Mediterranean climates: Drought, low temperatures or both? Agric For Meteorol 206:151–162 doi: 10.1016/j.agrformet.2015.03.004

Sánchez-Salguero R, Linares J, Camarero JJ, Madrigal-González J, Hevia A, Sánchez-Miranda A, Ballesteros-Canovas JA, Alfaro-Sánchez R, García-Cervigón AI, Bigler C, Rigling A (2015b) Disentangling the effects of competition and climate on individual tree growth: A retrospective and dynamic approach in Scots pine. For Ecol Manage 358:12–25. doi: 10.1016/j.foreco.2015.08.034

Saurer M, Siegwolf R, Schweingruber F (2004) Carbon isotope discrimination indicates improving water-use efficiency of trees in northern Eurasia over the last 100 years. Glob Chang Biol doi: 10:2109-2120.

Smith M (2011) An ecological perspective on extreme climatic events: A synthetic definition and framework to guide future research. J Ecol 99:656–663 doi: 10.1111/j.1365-2745.2011.01798.x

Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) (2007) Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.

Vaganov E, Hughes M, Shashkin A (2006) Growth dynamics of conifer tree rings. Image of Past and Future environments. Springer, Berlin

Vayreda J, Martinez-Vilalta J, Gracia M, Retana J (2012) Recent climate changes interact with stand structure and management to determine changes in tree carbon stocks in Spanish forests. Glob Chang Biol 18:1028–1041 doi: 10.1111/j.1365-2486.2011.02606.x

Vilà-Cabrera A, Martínez-Vilalta J, Retana J (2014) Variation in reproduction and growth in declining Scots pine populations. Perspect Plant Ecol Evol Syst 16:111–120 doi: 10.1016/j.ppees.2014.02.005

Vitasse Y, Delzon S, Bresson C, Michalet R, Kremer A (2009) Altitudinal differentiation in growth and phenology among populations of temperate-zone tree species growing in a common garden. Can J For Res 39:1259–1269 doi: 10.1139/X09-054

Vitasse Y, Hoch G, Randin C, Lenz A, Kollas C, Scheepens JF, Körner C (2013) Elevational adaptation and plasticity in seedling phenology of temperate deciduous tree species. Oecologia 171:663–678 doi: 10.1007/s00442-012-2580-9

von-Wuehlisch G, Krusche D, Muhs HJ (1995) Variation in temperature sum requirement for flushing of beech provenances. Silvae Genet. 44, 343–346.

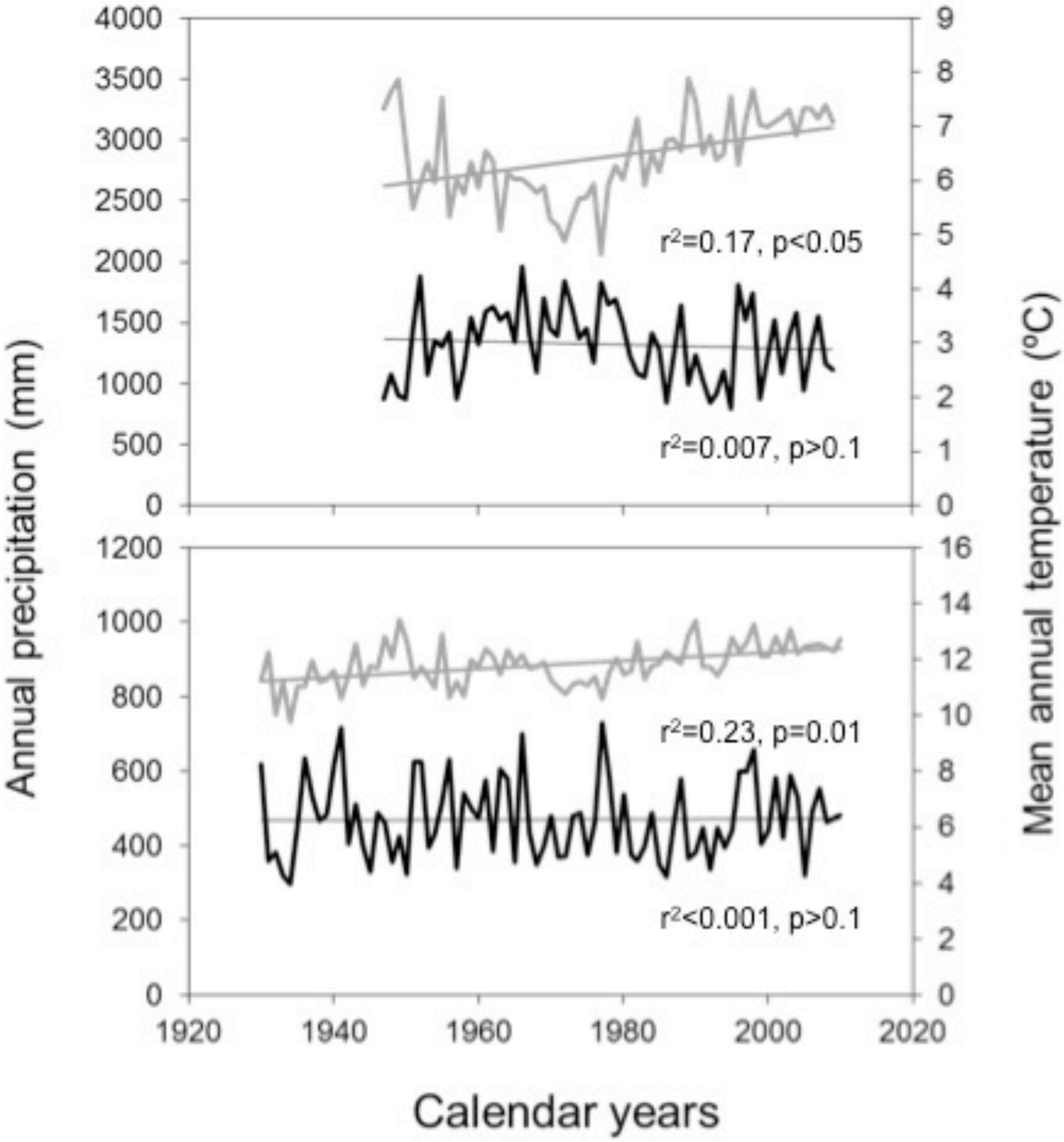
Wallace Z, Lovett G, Hart J, Machona B (2007) Effects of nitrogen saturation on tree growth and death in a mixed-oak forest. For Ecol Manage 243:210–218 doi: 10.1016/j.foreco.2007.02.015

Wilmking M, Juday G, Barber V, Zald H (2004) Recent climate warming forces contrasting growth responses of white spruce at treeline in Alaska through temperature thresholds.

Glob Chang Biol 10:1724–1736. doi: 10.1111/j.1365-2486.2004.00826.x

8. Supplementary Material

ESM1. Evolution of the annual rainfall (black lines) and mean temperature (grey line) through time in the Puerto de Navacerrada (upper panel) and Segovia city (bottom panel) located at 1894 m.a.s.l. and 1002 m.a.s.l. respectively (Data from AEMET, www.aemet.es).

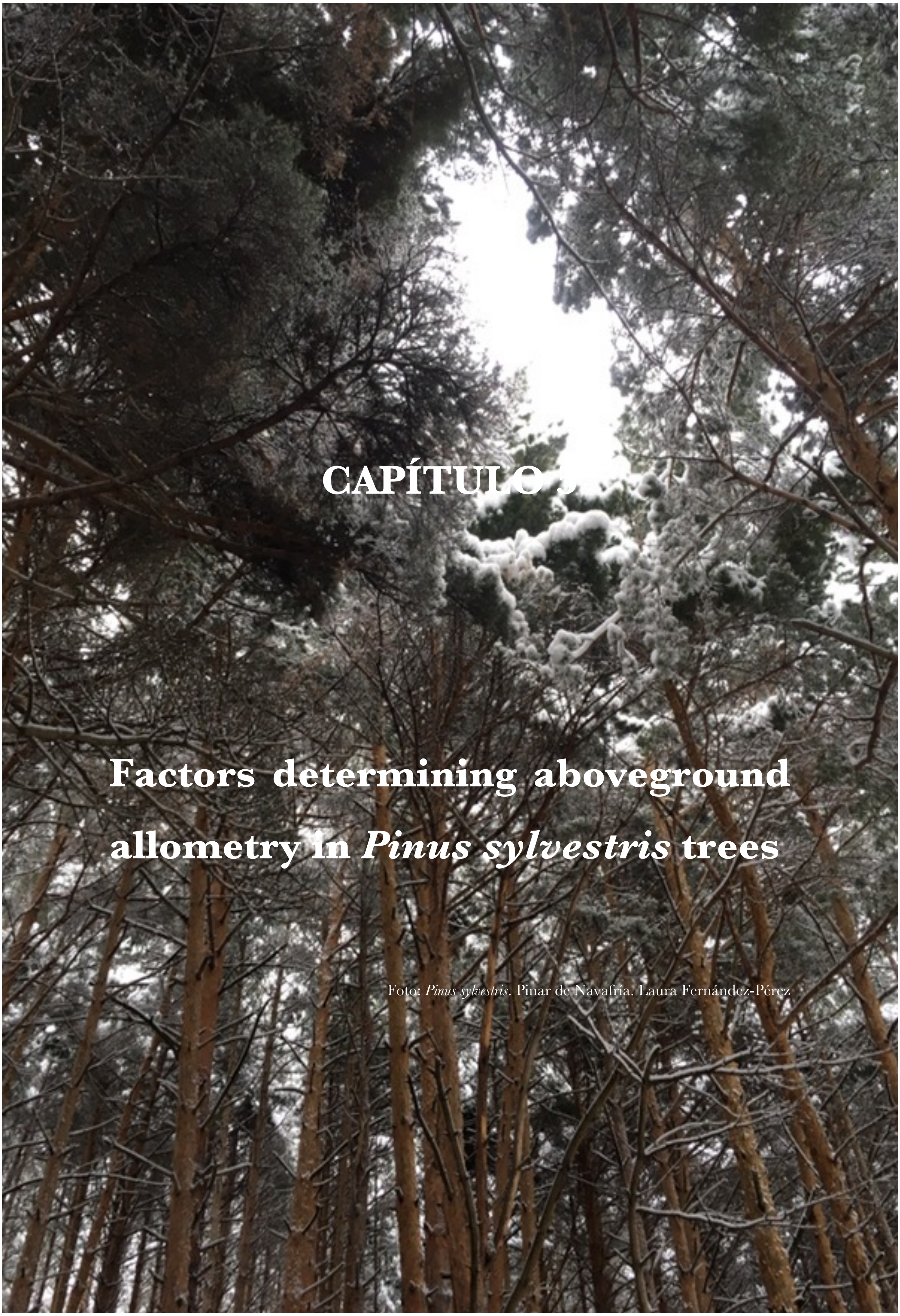


ESM2. Altitude of the sections located in Navafría.

		Elevation (m.a.s.l.)		
Section	**Area (ha)**	**Min**	**Max**	**Mean**
I	1051	1320	2010	1620
II	904	1270	1990	1554
III	758	1330	2070	1700

ESM3. Parameters correlation between variables.

	Intercept	Age	Altitude	Year	Age × Altitude	Age × Year	Altitude × Year
Age	0						
Altitude	-0.003	0.083					
Year	-0.003	-0.018	0.048				
Age × Altitude	0.074	0.067	0.075	-0.004			
Age × Year	-0.021	0.104	-0.02	0.012	0.059		
Altitude × Year	0.046	-0.01	-0.125	0.01	0.154	0.085	
Age × Altitude × Year	-0.008	0.067	0.145	0.087	0.204	0.038	0.161



CAPÍTULO 5

Factors determining aboveground allometry in *Pinus sylvestris* trees

Foto: *Pinus sylvestris*. Pinar de Navafría. Laura Fernández-Pérez

Factors determining aboveground allometry *Pinus sylvestris* trees

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RESUMEN

Los órganos de las plantas mantienen proporciones en su tamaño denominadas relaciones alométricas. Dichas relaciones reflejan la coordinación en la asignación de recursos entre diferentes órganos con el fin de mantener la estabilidad estructural y funcional de la planta. Las relaciones alométricas son el resultado de la interacción de las características intrínsecas de los individuos (genotipo) con el medio ambiente. En este artículo investigamos las variaciones en el espacio y el tiempo de las relaciones alométricas de la parte aérea en árboles de *Pinus sylvestris* L. (pino silvestre) en un bosque ubicado en el Sistema Central en la Península Ibérica. En concreto, se ha medido el Índice de esbeltez (altura/diámetro del tronco -dbh-) y el volumen tronco/volumen copa. Independientemente de la densidad de la masa, el índice de esbeltez ha cambiado a lo largo del tiempo pero la variación ha dependido de la altitud en la que crecen los individuos y su edad. Los árboles adultos se han vuelto más esbeltos a lo largo del tiempo en las zonas más bajas y menos altos en las zonas más elevadas. En cambio, los ejemplares jóvenes han seguido el patrón contrario. La relación entre el volumen del tronco y el volumen de copa de árbol de *P. sylvestris* disminuyó con la edad de los árboles y la altitud. Este estudio indica que el cambio en las condiciones ambientales tanto en altitud como los ocurridos en los últimos 70 años modulan la forma de los árboles y que dichos cambios son independientes de la densidad de la masa.

Palabras clave: Altura del árbol, diámetro normal, edad, gradiente altitudinal, relación alométrica, volumen de la copa.

ABSTRACT

Plants organs maintain proportions in their size named allometric relationships. Such relations show the coordination in resource allocation between different organs in order to maintain the structural and functional stability of the plant. Allometric relationships are the result of the interaction of intrinsic characteristics of individuals (genotype) with the environment. In this paper we investigate variation in aboveground allometric relationships in space and time of *Pinus sylvestris* L. (Scots pine) trees in a forest located in the Central System in the Iberian Peninsula. Specifically, the slenderness index (trunk height/diameter -dbh-) and trunk volume/crown volume have been measured. Independently of mass density, the slenderness index has changed over time but this variation has depended on the altitude at which individuals grow and their age. The older trees have become more slender throughout time in low altitudes and less slender at high elevations. Instead, young trees have been follow the opposite pattern. The relationship between the trunk volume and the crown volume of *P. sylvestris* decreased with tree age and with altitude, although the fact that the increase was only found among older trees but not among the younger trees. This study indicates that the change in environmental conditions, both in altitude and in the last 70 years modulate tree morphology and such changes are independent of the density of the mass.

Key words: Age, altitudinal gradient, allometric relationship, diameter at breast height, height, tree crown.

INTRODUCTION

Plant organs keep size proportions termed allometric relations, which reflect coordination in resource allocation to different plant organs that allow maintaining their structural and functional stability. Allometric relationships result of the interaction of the intrinsic characteristics of individuals with the environment (Grams and Andersen, 2007). Competitive environment plays a significant role in plant performance and population dynamics (King, 1996). Plants compete primarily for light, nutrients and water (Begon et al, 2005), and changes in these resources drive photosynthesis, growth and carbon allocation (Hubbell et al, 1999; Lemaire and Millard 1999; Potvin and Dutilleul 2009).

The relation between tree height and tree trunk diameter (tree slenderness) is related with resource allocation and affects forests structure (Enquist et al, 1998; Poorter et al, 2012). Tree slenderness changes along biotic and abiotic gradients; for instance tee slenderness is frequently correlated with climatic variables (King et al, 2006; López-Serrano et al, 2005). Plant height growth is promoted in high light competition scenarios to maximize carbon profit (Berntson and Wayne, 2000; Moles et al, 2009). This involves increased allocation of resources to stem elongation and to foliage at the expense of stem diameter (Takenaka, 2000; Suzuki, 2002; Vanninen and Makela, 2005). This results in crown asymmetry and lower relative projection area (Young and Hubbell, 1991).

Coordination between the water supply capacity and the transpiring demand of the tree can be expressed by the relationship between transversal area of trunk xylem (or more precisely the sapwood) (A_X) and the area of foliage (A_L) supported by tree trunk. This ratio is also termed the Huber value (Huber, 1928). High values of A_X /A_L indicate that the tree has high capacity to supply water to foliage. Large quantity of nutrients, non-structural carbohydrates and water are stored in the wood (Harmon et al. 1986;

Kozlowski 1992). Consequently, high A_X/A_L values can indicate high resource storage capacity, which can help trees to resist and recover prolonged periods of stress.

Phenotypic variation linked to plastic responses in tree allometry is expected due to spatio-temporal variation in environmental factors (Casper et al, 2003; De Kroon et al, 2005). In drought-prone environments, trees become shorter with proportionally thicker stems relative to crown foliage (Martínez-Vilalta et al, 2004; Pockman and Sperry, 2000). Under the forecasted increasing aridity conditions (e.g. longer and/or more intense droughts and extreme temperatures) in southern Europe due to Climate Change, plasticity in allometric adjustments might be key for tree fitness, particularly for rear-edge tree populations. For instance, higher investment in stems and roots in response to drought at the expense of foliage can increase the capacity to store water, nutrients and carbohydrates (Ludovici et al, 2002; Woodruff and Meinzer, 2011), which can enhance the capacity to resist drought and recovery after extreme stress events (Pinheiro et al, 2001; Chaves et al 2002). At the same time, the increase in atmospheric CO2 and nitrogen (N) deposition could increase the photosynthesis and carbon allocation as a "fertilization process". Increase in soil N increases allocation to leaves and shoots at the expense of roots (Hermans et al, 2006). It is unclear if the increased in CO2 concentration and the N depositions in recent decades due to Global Change (IPCC, 2014) could modify tree allometric relationships. The way in which allometric relationships change along temporal gradients irrespective of ontogeny has received less attention (Oleskyn et al, 1992).

In this paper, we investigate spatial and temporal trends in aboveground allometry in *Pinus sylvestris* L. (Scots pine) trees in a forest located in the Central System range in the Iberian Peninsula where the species is at the dry edge of its distribution. Scots pine is the most widely distributed pine in the north hemisphere, ranging from Scotland along Atlantic to Pacific coast to eastern Siberia, with relict populations in the

Mediterranean Basin (Richardson and Rundel, 1998). It reaches elevations from sea level to 2600 m a.s.l., occupying many types soils. This wide range of its distribution could be due to its highly plasticity and its genetic diversity, as observed by different authors, resulting in morphological differences among populations across the altitudinal gradient (Benito-Garzón et al, 2011; Rehfeldt et al, 2002; Savolainen et al, 2004). Specifically, we investigated to aboveground allometric relationships along an altitudinal gradient (1350-1890) where water and temperature conditions strongly vary. Moreover, we also studied changes among 1930 to 2010, which encompasses several global change drivers correlated with time. The studied allometric relationships were tree slenderness and the relationship between trunk volume and the branch volume of the crown (crown volume). We used the trunk volume/crown volume as a proxy of the ratio between transversal area of the xylem and total leaf area supported by a stem or a trunk (A_X/A_L). We hypothesize that tree slenderness and trunk/crown volume are driven by environmental factors, and have changed through time in response to Global Change drivers.

2. METHODS

2.1 Study area

Tree growth was recorded in a 3891 ha forest (named MUP 198 “Pinar de Navafría”) dominated by *P. sylvestris* and located in the municipality of Navafría (Lat 41º03'17" N; Long 3º49'21" W) in the Sierra de Guadarrama, Central System range, Spain (Figure 1). In this area, *P. sylvestris* is bound to a narrow altitudinal strip ranging from 1300 to 2100 m a.s.l. (Martínez-García, 1999). Climate is continental Mediterranean with important altitudinal variations in temperature and rainfall. Winters are cold and humid and the summers are warm and dry. At the lowest part of the altitudinal gradient (village of

Navafría), the annual, minimum and maximum mean temperature are 10.7, 4.4 and 17 ºC, respectively. Total annual rainfall is 611 mm and no apparent change has been observed through time for rainfall. Similar to other mountains, rainfall increases while temperature decreases with elevation (Rolland et al, 1998; Sánchez-Salguero et al, 2015; Tardif et al, 2003; Vaganov et al, 2006). In Puerto of Navacerrada (1894 m a.s.l.), annual rainfall is 1324 mm and mean annual temperature is 6.4 ºC. At this high-altitude site, a slight decrease in annual rainfall is recognized while in Segovia city (~1000 m a.s.l.), the precipitation has remained constant throughout the years. In contrast, the temperature has increased in the last 50 years in both locations.

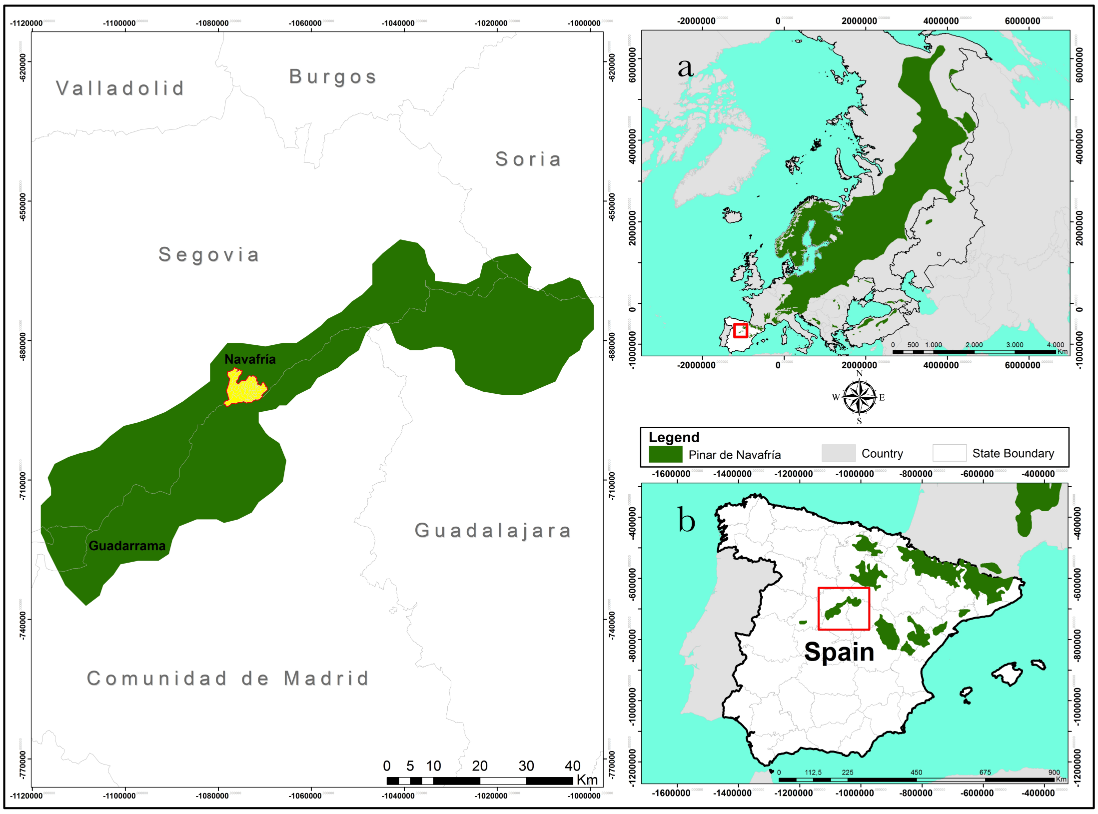

Fig. 1. Distribution of *Pinus sylvestris* in a) Europe and b) Spain and location of the forest Pinar de Navafría (yellow) in the Central System range (green).

2.2 Morphological measurements

Scots pine forest management in Sierra de Guadarrama is subjected to management plans since 1890, mainly oriented towards sustainable timber production. The "Pinar de Navafría" forest began to be managed in 1885 using a shelterwood thinning method, to maximize natural regeneration and the production of high quality timber (Diaz and Prieto, 1999). For administrative purposes, the forest was divided into nine sections with identical management plans. Each section in turn was subdivided into five subsections, thus resulting in a total of 45 forest units. Rotation averages 120 years and the regeneration period spans 20 years. Periodic thinning is conducted every 10 years depending on the stage of the rotation period and the stand is managed to achieve even aged stands in the long term.

Forest management prescriptions have been audited almost every ten years, where trees of different diameter classes were selected and harvested for estimating tree dasometric variables since 1890, thus providing an invaluable source of information to assess the evolution of tree structure and growth throughout the last century. All data were recorded in a regional historical archive. Tree morphological data in particular are available for decadal periods in 1930-1940, 1939-1949, 1949-1959, 1959-1968, 1989-1999, 2000-2010. In the first record (1930-1940), the present and permanent stands were defined, which make no comparable the stands of the first management plan (1885-1895). Slope aspect (%), area (ha), altitude (m a.s.l.) (SM1) and soil types were recorded for each forest unit. For each fallen tree, the variables were tree age, the diameter at breast height after removing the bark (dbh, cm), height (m), crown volume (m^3), and age (No. rings) were measured. Crown volume is the volume of the main branches of the crown. The age of the studied trees ranged between 30 and 235 years old. The examined relationships were the slenderness index (Height/dbh) and the trunk to crown volume ratio. This latter relationship is a proxy of the relationship A_X/A_L that indicates the capacity of stems or trunks to transport water and store resources per unit of supported foliage. We calculated the trunk volume (cm^3) using the cone formula: 1/3 × (π × r^2) x h, where r is the dbh/2 and h is tree height.

2.3 Data analysis

We performed the statistical analyses in R 3.1.2, (R Core Team, 2008). We fitted Linear Mixed Models (LMM) of the nlme package (Bates et al, 2015) to analyze slenderness index and dbh/crown volume (standardized variables). We checked collinearity was not a problem through Pearson correlation for the main effects (SM1). As explicative variables, we included the three order interaction: altitude × time × tree age (and the related two-order interactions and the main effects) to evaluate whether global-change trends of tree morphology are dependent on tree age and altitude since 1930. The biological meaning of this third-order interaction is that depending on the tree age, and altitude, differential global change trends associated to rising CO_2, N or temperature should be recognized in tree morphology. If the set of contemporary trees develops differently in different years indicates changes in tree morphology and site conditions. We used multivariate modelling (various combinations of variables) and model comparison by using the Akaike information criterion corrected (AICc). We first fitted the maximal model, containing all the explanatory variables and all the possible interactions. For model comparison and averaging we used the dredge function within the "MuMIn" package (Bartón, 2018). Importantly, tree density depicted a somewhat linear increment throughout the studied period (Pearson correlation: 0.52). To control for the density effect, we developed a model that included the dependent variable (Slenderness index or Trunk/crown volume) and tree density and we used the residuals for the successive models. The model with the most appropriate structure for the Slenderness index was as follows:

$$Y_{ijt}=\beta_0 +\beta_1 \text{ Altitude} \times \beta_2 \text{ Time}+ \beta_3 \text{ Age } b_1 + \varepsilon_{ijt}\text{.........Equation 1}$$

where Y_{ijt} is the *j*th slenderness response on the *i*th individual (untransformed) on a *t*th specific year. The fixed parameters are $\beta_0 - \beta_3$, while b_1 is random effect $(b_1 \sim \mathrm{N}\ (0, \tau_1^2)$**,** and finally ε_{ijt} shows the error $\varepsilon_{ijt} \sim \mathrm{N}\ (0, \tau_1^2)$.

The most appropriate model for the relationship between trunk/crown volume was as follows:

$$Y_{ij}=\beta_0+ \beta_1 \text{ Age} + \beta_2 \text{ Time} + b_1+ \varepsilon_{ij}\text{......................Equation 2}$$

where Y_{ijt} is the *j*th relationship between trunk/crown volume response on the *i*th individual (untransformed) on a *t*th specific year. The fixed parameters are $\beta_0 - \beta_2$, while b_1 is random effect $(b_1 \sim \mathrm{N}\ (0, \tau_1^2)$, and finally ε_{ijt} shows the error $\varepsilon_{ijt} \sim \mathrm{N}\ (0, \tau_1^2)$.

3. RESULTS

3.1 Slenderness index

Slenderness index of *P. sylvestris* trees has changed throughout time and along the altitudinal gradient. The most parsimonious model included the third pair-wise interaction Age × Altitude × Time (Table 1). In the first decades of the 20th century, young trees were more slender at high altitudes compared to low altitudes (Fig. 2, upper panel). For adult trees, the slenderness index has reversed through time: in the first decades of the 20th century, trees were more slender at high altitudes, but in the last decades, trees are becoming slender at low altitudes (Fig. 2, lower panel). The pseudo-

R^2 marginal, which is the part of the variance explained by only the fixed effects, suggested that 16% of the variance can be attributed to the effects of tree age and the triple interaction. Accordingly, 32% of the variance could be attributed to the random term plus the fixed effects, i.e. variability among forest stands (Table 2).

Table 1. Summary of the model ranking using Akaike information criterion corrected (AICc) to test the variables affecting Slenderness index and the relationship between Trunk/Crown volume. ΔAIC is the delta weight (difference between the AICc for a given model and the best fitting model), *k* is the number of estimated parameters and w_1 is the model selection probability (Akaike weights). The models are shown with ΔAIC <15.

Model ranking	Main effects	AICc	*k*	ΔAIC	w_1
	Slenderness index				
1	Altitude+Age+Time+Alt×Age+Alt×Time+Age×Time+Alt×Age×Time	4758.4	10	0	0.979
2	Altitude+Age+Time+Alt×Time	4767.7	7	9.36	0.009
3	Altitude+Age+Time+Alt×Age+Alt×Time	4768.9	8	10.54	0.005
4	Altitude+Age+Time+Alt×Time+Age×Time	4769.1	8	10.67	0.005
5	Altitude+Age+Time+Alt×Age+Alt×Time+Age×Time	4770.3	9	11.89	0.003
	Tree Trunk volume/ Crowm volume				
1	Altitude + Time	2909.1	5	0	0.445
2	Altitude+Time+Alt×Time	2909.8	6	0.73	0.308
3	Altitude+Age+Time	2912.2	6	3.13	0.093
4	Altitude+Age+Time+Alt×Time	2913.2	7	4.09	0.057
5	Time	2913.9	4	4.79	0.041
6	Altitude+Age+Time+Alt×Age	2915.5	7	6.36	0.018
7	Altitude+Age+Time+Age×Time	2916.6	7	7.53	0.01
8	Altitude+Age+Time+Alt×Age+Alt×Time	2916.8	8	7.71	0.009
9	Age + Time	2917.5	5	8.43	0.007
10	Altitude+Age+Time+Alt×Time+Age×Time	2917.6	8	8.49	0.006
11	Altitude+Age+Time+Alt×Age+Age×Time	2919.9	8	10.76	0.002
12	Altitude+Age+Time+Alt×Age+Alt×Time+Age×Time	2921.2	9	12.12	0.001
13	Age+Time+Age×Time	2921.9	6	12.81	0.001
14	Altitude	2923	4	13.93	0

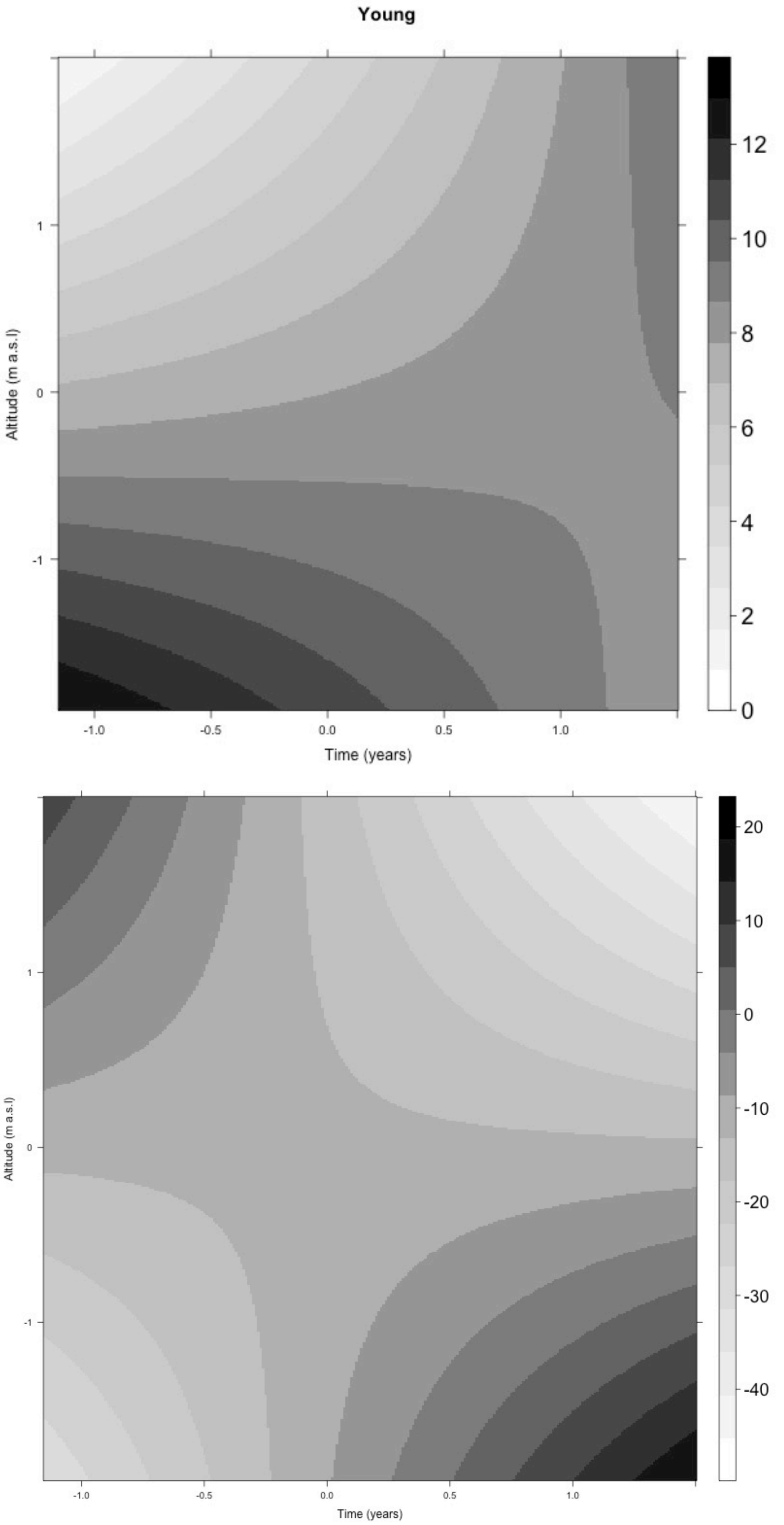

Fig. 2. Contour plot showing the variation of Slenderness index of *P. sylvestris* trees with pair-wise interaction (altitude × time) (Upper panel figure: Young trees; lower panel: Adult trees)

Table 2. Parameters estimated for the Slenderness index and the relationship between Trunk / Crown volume, including associated standard error, t statistic and p value. Variance components associated with the random term are shown at the bottom. All variables were standardized in all models to avoid problems with convergence in parameter estimate procedure. Marginal (pseudo-R^2m) and Conditional (pseudo-R^2c) R-squares.

Slederness index	Value	Std.Error	DF	t-value	p-value	Pseudo-R^2m
Fixed						
(Intercept)	-0.0905	0.5305	594	-0.1705	0.8647	0.1645
Altitude	-1.6280	0.5089	594	-3.1990	0.0015	
Age	0.2851	0.5035	594	0.5663	0.5714	
Time	-3.9828	0.5032	594	-7.9145	0.0000	
Altitude × Time	-3.1668	0.5253	594	-6.0291	0.0000	
Altitude × Age	-0.0010	0.5124	594	-0.0020	0.9984	
Age × Time	-0.1636	0.5217	594	-0.3137	0.7539	
Altitude × Age × Time	-2.2308	0.6093	594	-3.6614	0.0003	
Random	Intercept	Residual				Pseudo-R^2c
Stand	0.3462	12.1831				0.1652
Trunk/crown volume	Value	Std.Error	DF	t-value	p-value	Pseudo-R^2m
Fixed						0.04463
(Intercept)	-0.0021	0.1210	580	-0.0172	0.9863	
Altitude	-0.3605	0.1178	580	-3.0605	0.0023	
Time	-0.5130	0.1184	580	-4.3337	0.0000	
Random	Intercept	Residual				Pseudo-R^2c
Stand	<0.05	2.8479				0.04496

3.2 Relationship between Trunk / Crown volume

The relationship between trunk and tree crown volume of *P. sylvestris* is altitude and time-dependant (Table 2, Fig. 3). The relationship between trunk and tree crown volume decreased with the altitude (Fig. 3). Also, this relationship has change throughout time (Fig. 4). In recent decades, the relationship between trunk and crown volume has decreased. The most parsimonious model did not include the pair-wise interaction between the Altitude × Time, while the triple interaction was neglible (Table 2).

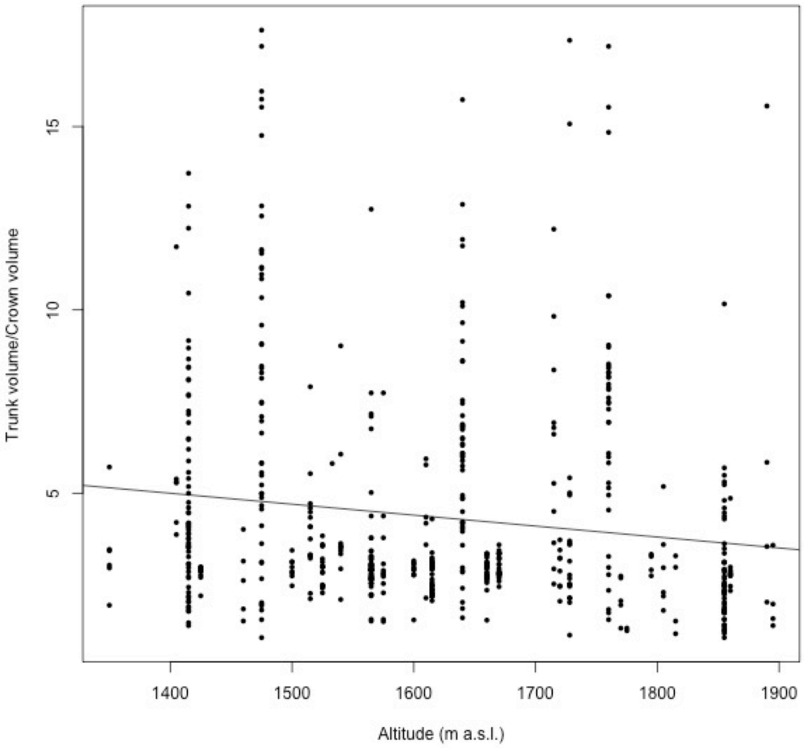

Fig. 3. Relationship between trunk/crown volume in the altitudinal gradient.

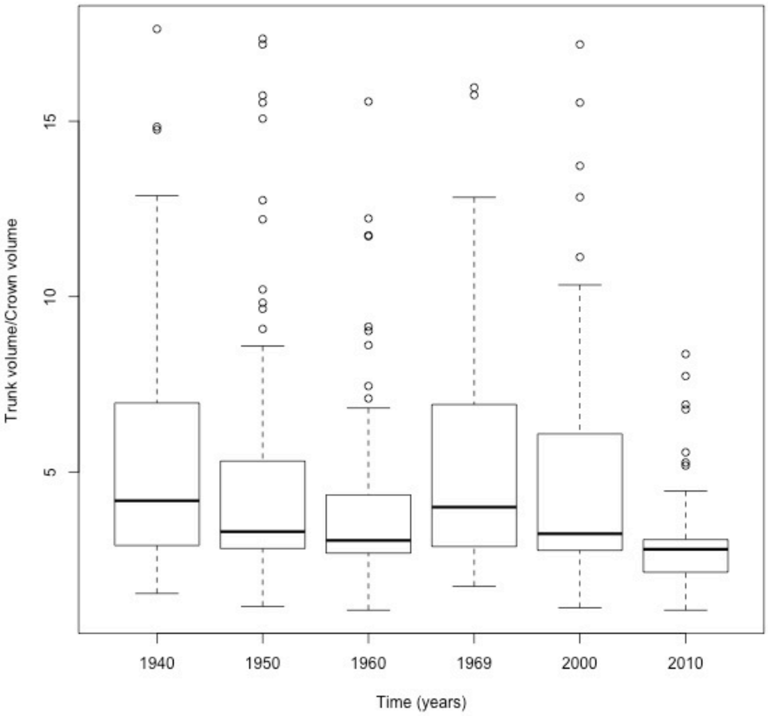

Fig. 4. Relationship between trunk/crown volume throughout time.

4. DISCUSSION

4.1 Slenderness index

We observed that in the beginning of the century, young trees were more slender at low altitudes than at high elevations (Fig. 2, upper panel). It has been observed a reduction in basal area and tree height with increasing altitude (Petit et al, 2011; Thomas, 2011; De Bello et al, 2012). However, for Iberian pine species, it has reported taller trees at low elevations (Candel-Pérez et al, 2012; Sánchez-Salguero et al, 2015, but see Homeier et al, 2010), mainly due to water stress. Drought constitutes an important constraint on Scots pine growth under Mediterranean climate. However, young trees are becoming slender in the entire altitudinal gradient (Fig 2, upper panel).

In contrast, for older trees, we observed that trees were slender at high altitudes. It has been reported that elevation increased tree slenderness (Holeksa et al, 2007; Homeier et al, 2010), due to biological and physiological processes linked to temperature and moisture availability (Oleksyn et al, 1998). In contrast, in the last decades of the 20th century, we observed that trees are more slender at low elevations (Fig. 2, low panel) This indicates that in the first decades of the 20th century, the environmental conditions were more adverse at higher altitudes, mainly by cold, which is the mainly stressful factor at high altitudes, Woodward and Williams, 1987). Mean air temperature decreases by 0.6 ºC per 100 m elevation (Paulsen et al, 2000). Also, it has been pointed out that the global temperatures were lower at the beginning of the century (IPCC, 2014), and at high altitudes could be even colder. In contrast, in recent decades, mean temperatures have increased (Beniston 2003; Pauli et al, 2003). This could induce the extension of growing season driven by warmer temperatures (Lucht et al, 2002; Piao et al, 2007) and improve the conditions at high elevations (Andreu et al, 2007; Salzer et al, 2009), as we observe for young trees (Fig.2, upper panel), but negative affect for the older trees. For Iberian pine species, it has reported taller trees at low elevations (Candel-Pérez et al, 2012; Sánchez-Salguero et al, 2015, but see Homeier et al, 2010), mainly due to water stress. Drought constitutes an important constraint on Scots pine growth under Mediterranean climate. Furthermore, the increased of temperatures in recent decades is exacerbating the impacts of droughts, reducing the productivity in drought-prone environments (Solomon et al, 2007). In Mediterranean ecosystems, trees at low altitudes experienced drier conditions than trees located high altitudes. It has been observed a negative correlation between drought and slenderness index (Worrall et al, 2010; Nepstad et al, 2015). Drought conditions were hypothesized to increased tree slenderness (Frey et al, 2004), because the decrease in water availability may reduce the length of the trees growing period, due to a drought-induced declining in C uptake (McDowell et al, 2010). Also, taller trees could have more difficulties to transport water (Woodruff et al, 2008) and may have a greater penetration by the sunlight and wind who promote a higher rates of evapotranspiration (Koike et al, 2001). However, studies including the interaction of temporal and altitudinal gradients in response to slenderness are very scarce. More investigations are needed to clarify this tree change.

4.2 Trunk and Crown volume

Our results showed that the relationship between the trunk and crown volume has decreased in the altitudinal gradient and time. There are many studies on A_X/A_L ratios of forest stands (Margolis et al, 1995), but little is know in long-term change and altitudinal gradient in functional properties associated with A_X/A_L.

We observed a decreased in relationship between trunk/crown volume in the altitudinal gradient. Contrary results have been reported for different species: it has been observed a reduction in A_X/A_L in Mediterranean species associated with the increase in precipitation (Villar-Salvador et al, 1997). This response could be due to the reduction in foliar area to prevent xylem embolism (Tyree and Dixon, 1986). Contrary to Villar-Salvador and coworkers (1997), it has been observed an increase in A_X/A_L with increase in vapour pressure deficit in *Pinus sylvestris* (Mencuccini and Grace, 1995). Information about changes in A_S/A_L along altitudinal gradients is very scarce (but see Hernández-Calderón et al, 2014). More investigations are needed to clarify these responses.

We observed that the relationship between trunk/crown volume has decreased throughout time. The increase in temperature in recent decades (IPCC, 2014) could trigger high rates of evapotranspiration, which decreased on trunk/crown volume. Contrary to our observations, it has been reported that trees growing under warm and dry conditions have a higher A_X/A_L to overcome the risk of embolism (Delucia et al, 2000; but see Martínez-Vilalta et al, 2009). Moreover, larger trees reduced the foliar surface area because smaller leaves can result from greater limitation and lower leaf turgor in crowns of taller trees (Mencuccini and Grace, 1994; Woodruff et al, 2004;

Ambrose et al, 2009). Previous studies showed that trees increased trunk volume/crown volume with altitude where rainfall is higher and air evaporative demand is lower than in the low altitude locations. This means that high altitude trees may allocate more resources for constructing and supporting structural organs and carrying less leaf area with altitude. At high altitudes low temperature and higher wind could induce water stress during the cold season (Körner, 2007). Shifts in allocation to xylem tissues at the expense of foliage organs contributes to increase leaf-specific hydraulic conductivity. This allows improving water supply to foliage without important changes in conduit diameter, which could increase the risk of embolism. Increase in trunk volume relative foliage also increase the capacity for storing resources such as carbohydrates, which can be used by plants during prolonged periods of limited photosynthesis. According to the hydraulic limitation hypothesis (Ryan and Yoder, 1997), productivity decreased in aging trees.

5. CONCLUSIONS

Scots pine showed allometric changes in the size of aboveground allometric relationships across temporal and altitudinal gradients. The allometric relationship between height and dbh has change throughout the time and altitudinal gradients. This indicates that the change in climatic conditions in the different altitudes in Sierra de Guadarrama during the last 70 years have modified the resource allocation pattern of trees. Our results suggest that trees respond to the increase in transpiration rates determined by decrease in the relationships between the trunk volume and crown volume. The presence of a structural change in response to increased evaporative demand has important consequences for our understanding of the effects of water deficits on trees.

5. REFERENCES

Ambrose AR, Sillett SC, Dawson TE (2009) Effects of tree height on branch hydraulics, leaf structure and gas exchange in California redwoods. Plant Cell Environ 32:743–757.

Andreu L, Gutiérrez E, Macias M, Ribas M, Bosch O, Camarero JJ (2007) Climate increases regional tree-growth variability in Iberian pine forests. Glob. Change Biol. 13, 804–815.

Bartón K (2018) Multimodel inference. MuMIn package.

Bates D, Maechler M, Bolker B, Walker S (2015) Lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7.

Begon M, Townsend CR, Harper JL (2005) Ecology: from individuals to ecosystems.

Beniston M (2003) Climatic change in mountain regions: a review of possible impacts. Clim Chang 59:5-31.

Benito-Garzón M, Alía R, Robson T, Zavala M (2011). Intra-specific variability and plasticity influence potential tree species distributions under climate change. Glob. Ecol. Biogeogr. 20, 766–778.

Berntson G, Wayne P (2000) Characterizing the size dependence of resource acquisition within crowded plant populations. Ecology 81, 1072–1085.

Bond BJ (2000) Age-related changes in photosynthesis of woody plants. Trends Plant Sci 5:349–353.

Candel-Pérez D, Linares JC, Viñegla B, Lucas-Borja ME (2012) Assessing climate-growth relationships under contrasting stands of co-ocurring Iberian pines along an altitudinal gradient. Forest Ecology and Management. 274:48-57.h

Casper B, Jochen S, Jackson R (2003) Defining a Plant's belowground zone of influence A. Ecology 84, 2313–2321.

Chaves MM, Pereira JS, Maroco J, Rodrigues ML, Rocardo CPP, Osório ML, Carvalho I, Fria T, Pinheiro C (2002) How plants cope with water stress in the field. Photosynthesis and growth. Annals of Botany. 89:907-916.

De Bello F, Lavorel S, Lavergne S, Albert CH, Boulangeat I, Mazel F, Thuiller W (2012) Hierarchical effects of environmental filters on the functional structure of plant com- munities: a case study in the French Alps. Ecography 35: 1–10. Diaz,

De Kroon H, Huber H, Stuefer JF, Van Groenendael JM (2005) A modular concept of phenotypic plasticity in plants. New Phytol. 166, 73–82.

DeLucia EH, Maherali H, Carey EV (2000) Climate-driven changes in biomass allocation in pines. Global Change Biology. 6:587-593.

Diaz B, Prieto R (1999) Modelos de planificacion forestal basados en la programacion lineal. Aplicacion al monte “Pinar de Navafria” (Segovia). Inv Agrar. Sist. Recur. For. 8, 63–92.

Dye PJ, Olbrich BW, Poulter AG (1991) The influence of growth rings in *Pinus patula* on heat pulse velocity and sap flow measurement. J. Exp. Bot. 42:867–870.

Enquist B, Brown J, West G (1998) Allometric scaling of plant energetics and population density. Nature 395, 163–165.

Frey BR, Lieffers VJ, Hogg EHT, Landhäusser SM (2004) Predicting landscape patterns of aspen dieback: mechanisms and knowledge gaps. Can. J. Forest Res. 34, 1379–1390.

Grams T, Andersen C (2007) Competition for resources in trees: physiological versus morphology plasticity. Prog. Bot. 68, 356–381.

Harmon ME, Franklin JF, Swanson FJ, Sollins P, Gregory SV, Lattin JD, Anderson NH, Cline SP, Aumen NG, Sedell JR, Lienkaemper GW, Cromarck K, Cummins KW (1986). Ecology of coarse woody debris in temperate ecosystems. Adv. Ecol. Res., 15, 133–302.

Hermans C, Hammond JP, White PJ, Verbruggen N (2006) How do plants respond to nutrient shortage by biomass allocation? Trends in Plant Science 11: 610–617.

Hernández-Calderón E, Méndez-Alonzo R, Martínez-Cruz J, González-Rodríguez A, Oyama K (2014) Altitudinal changes in tree leaf and stem functional diversity in a semi-tropical mountain. Journal of Vegetation Science. 1-12.

Holeska J, Saniga M, Szwagrzyk J, Dziedzic T, Ferenc S, Wodka M (2007) Altitudinal variability of stand structure and regeneration in the subalpine spruce forest of the Pol'ana biosphere reserve, Central Slovakia. European Journal of forest Research. 126:303-313.

Homeier J, Brecke SW, Günter S, Rollenbeck RT, Leuschner C (2010) Tree diversity, forest structure and productivity along altitudinal and topographical gradients in a species-rich ecuadorian montain rain forest. Biotropica 42(2):140-148.

Hubbell S, Foster R, O’Brien S, Harms K, Condit R, Wechsler B, Wrigth S, Loo de Lao S (1999) Light-gap disturbances, recruitment limitation, and tree diversity in a Neotropical Forest. Science, 80(283): 554–557.

Huber B (1928) Weitere quantitative Untersuchungen überdas Wasserleitungssystem der Pflanzen. Jahrab. Wiss. Bot. 67:877-959.

IPCC, 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, IPCC.

King D (1996) Allometry and life history of tropical trees. J. Trop. Ecol. 12, 25–44.

King DA, Davies SJ, Tan S, Noor NSM (2006) The role of wood density and stem support costs in the growth and mortality of tropical trees. J. Ecol. 94, 670–680.

Koike T, Kitao M, Yutaka M, Mori S, Lei TT (2001) Leaf morphology and photosynthetic adjustments among deciduous broad-leaved trees within the vertical canopy profile. Tree Physiology 21: 951–958.

Körner C (2007) The use of "altitude" in ecological research. Trends in Ecology and Evolution. 22(11):569-574.

Kozlowski TT (1992). Carbohydrate sources and sinks in woody plants. Bot. Rev., 58, 107–122.

Lemaire G, Millard P (1999) An ecophysiological approach to modelling resource fluxes in competing plants. J. Exp. Bot. 50, 15–28.

López-Serrano FR, García-Morote A, Andrés-Abellán M, Tendero A, Del Cerro A, (2005) Site and weather effects in allometries: A simple approach to climate change effect on pines. Forest Ecology and Management. 215, 251–270.

Lucht W, Prentice IC, Myneni RB et al. (2002) Climatic control of the high-latitude vegetation greening trend and Pinatubo effect. Science, 296, 1687–1689

Ludovici KH, Allen HL, Albaugh TJ, Dougherty PM (2002) The influence of nutrient and water availability on carbohydrate storage in loblolly pine. Forest Ecology and Management, 159, 261–270.

Margolis H, Oren R, Whitehead D, Kaufmann MR (1995) Leaf area dynamics of conifer forests. In Ecophysiology of Coniferous Forests. Eds. W.K. Smith and T.M. Hinckley, Academic Press, San Diego, pp 181–223.

Martínez-García F (1999) Los bosques de *Pinus sylvestris* L. del Sistema Central Español. Distribución, historia, composición florística y tipología. Tesis Doctoral. Univesridad Complutense de Madrid.

Martínez-Vilalta J, Sala A, Piñol J (2004). The hydraulic architecture of Pinaceae – a review. Plant Ecol. 171, 3–13.

Martínez-Vilalta J, Cochard H, Mencuccini M, Sterck F, Herrero A, Korhonen JKJ, Llorens P, Nikinmaa E, Nolè A, Poyatos R, Ripullone F, Sass-Klaassen U, Zweifel R (2009) Hydraulic adjustments of Scots pine across Europe. New Phytologist.

184:353-364.

McDowell N, Allen CD, Marshall L (2010) Growth, carbon isotope discrimination, and climate-induced mortality across a *Pinus ponderosa* elevation transect. Global Change Biol. 16, 399–415.

Mencuccini M, Grace J (1995) Climate influences the leaf area:sapwood area ratio in Scots pine. Tree Physiology, 15,1-10.

Moles A, Warton D, Warman L, Swenson N, Laffan S, Zanne A, Pitman A, Hemmings F, Leishman M (2009). Global patterns in plant height. J. Ecol. 97, 923–932.

Nepstad DC, Tohver IM, Ray D, Moutinho P, Cardinot G (2015) Mortality of large trees and lianas following experimental drought in an amazon forest. Ecology. 88(9):2259-2269.

Oleksyn J, Chalupka W, Tjoelker MG, Reich PB (1992) Geographic origin of *Pinus sylvestris* populations influences the effects of air pollution on flowering and growth. Water, Air and Soil Pollution. 62:201-212.

Oleksyn J, Tjoelker MG, Reich PB (1998) Adaptation to changing environment in Scots pine populations across a latitudinal gradient. Silva Fennica 32(2):129-140.

Pauli H, Gottfried M, Grabherr G (2003) Effects of climate change on the alpine and nival vegetation of the Alps. J Meteor Ecol 7:9–12.

Paulsen J, Weber UM, Körner C (2000) Tree growth near treeline: abrupt or graduela reduction with altitude? Artic, Antartic and Alpine Research. 32(1):14-20.

Petit G, Anfodillo T, Carraro V, Grani F, Carrer M (2011) Hydraulic constraints limit height growth in trees at high altitude. NewPhytologist 189: 241–252.

Piao SL, Friedlingstein P, Ciais P, Viovy N, Demarty J (2007) Growing season extension and its impact on terrestrial carbon cycle in the Northern Hemisphere over the past 2 decades. Global Biogeochemical Cycles, 21,49–53.

Pinheiro C, Chaves MM, Ricardo CPP (2001) Alterations in carbon and nitrogen metabolism induced by water deficit in the stem and leaves of *Lupinus albus* L. Journal of Experimental Botany. 52:1063-1070.

Pockman W, Sperry J (2000). Vulnerability to xylem cavitation and the distribution of Sonoran Desert vegetation. Am. J. Bot. 87, 1287–1299.

Poorter L, Lianes E, Moreno-de las Heras M, Zavala M (2012) Architecture of Iberian canopy tree species in relation to wood density, shade tolerance and climate. Plant Ecol. 213, 707–722.

Potvin C, Dutilleul P (2009) Neighborhood effects and size-asymmetric competition in a tree plantation varying in diversity 90, 321–327.

Rehfeldt G, Tchebakova N, Parfenova Y, Wykoff W, Kuzmina N, Milyutin L (2002) Intraspecific responses to climate in *Pinus sylvestris*. Glob. Chang. Biol. 8, 912–929.

Richardson D, Rundel PW (1998) Ecology and Biogeography of *Pinus*: an introduction. In: Ecology and Biogeography of *Pinus*. Cambridge Press. pp 3-46.

Rolland C, Petitcolas V, Michalet R (1998) Changes in radial tree growth for *Picea abies*, *Larix decidua*, *Pinus cembra* and *Pinus uncinata* near the alpine timberline since 1750. Trees 13, 40–53.

Ryan MG, Yoder BJ (1997) Hydraulic limits to tree height and tree growth. What keeps trees from growing beyond a certain height? Bioscience 47:235–242.

Salzer M, Hughes M, Bunn A, Kipfmueller K (2009) Recent unprecedented tree-ring growth in bristlecone pine at the highest elevations and possible causes. Proc Nat Acad Sci U S A 106:20348–53.

Sánchez-Salguero R, Camarero J, Hevia A, Madrigal-González J, Linares J, Ballesteros-Cánovas J, Sánchez-Miranda A, Alfaro-Sánchez R, Sangüesa-Barreda G, Galván J, Gutiérrez E, Génova M, Rigling A (2015) What drives growth of Scots pine in continental Mediterranean climates: Drought, low temperatures or both? Agric. For. Meteorol. 206, 151–162.

Savolainen O, Bokma F, Repo T (2004) Genetic variation in cessation of growth and frost hardiness and consequences for adaptation of *Pinus sylvestris* to climatic changes. For. Ecol. Manage. 197, 79–89.

Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) (2007) Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.

Suzuki A (2002) Influence of shoot architectural position on shoot growth and branching patterns in *Cleyera japonica.* Tree Physiol. 22, 885–90.

Takenaka A (2000) Shoot growth responses to light microenvironment and correlative inhibition in tree seedlings under a forest canopy. Tree Physiol. 20, 987–991.

Tardif J, Camarero J, Ribas M, Gutiérrez E (2003) Spatiotemporal variability in tree growth in the Central Pyrenees : climatic and site influences. Ecol. Monogr. 73, 241–257.

Thomas SC (2011) Genetic vs. phenotypic responses of trees to altitude. Tree Physiology 31: 1161–1163.

Vaganov E, Hughes M, Shashkin A (2006) Growth dynamics of conifer tree rings. Image of Past and Future environments.

Vanninen P, Makela A (2005) Carbon budget for Scots pine trees: effects of size, competition and site fertility on growth allocation and production. Tree Physiol. 25, 17–30.

Woodruff DR, Bond BJ, Meinzer FC (2004) Does turgor limit growth in tall trees? Plant Cell Environ 27:229–236.

Woodruff DR, Meinzer FC, Lachenbruch B (2008) Height-related trends in leaf xylem anatomy and shoot hydraulic characteristics in a tall conifer: safety versus efficiency in water transport. New Phytologist. 180:90-99.

Woodruff D, Meinzer F (2011) Water stress, shoot growth and storage of non-structural carbohydrates along a tree height gradient in a tall conifer. Plant, Cell and Environment. 34(11):1920-1930.

Woodward F, Williams B (1987) Climate and plant distribution at global and local scales. Vegetatio 69:189–197.

Worrall JJ, MArchetti SB, Egeland L, Mask RA, Eager T, Howell B (2010) Effects and etiology of sudden aspen decline in southwestern Colorado, USA. Forest Ecology and Management. 260:638-648.

Young T, Hubbell S (1991) Crown asymmetry , treefalls , and repeat disturbance of broad-leaved forest gaps. Ecology 72, 1464–1471.

7. SUPPLEMENTARY MATERIAL.

SM1. Correlations among independent variables.

Slenderness index	Altitude	Age	Calendar year
Altitude			
Age	0.075		
Calendar year	0.037	-0.024	
Altitude × Calendar year	-0.157	-0.036	-0.002

SM2. Correlations among independent variables (Relationship between crown volume and DBH).

	Altitude	Calendar year	Age	Density	Altitude × Calendar year	Altitude × Age	Calendar year × Age
Altitude							
Calendar year	0.013						
Age	-0.147	0.003					
Density	0.144	-0.304	-0.023				
Altitude × Calendar year	0.081	0.183	-0.082	-0.03			
Altitude × Age	-0.794	-0.05	0.08	-0.005	-0.074		
Calendar year × Age	-0.079	-0.731	0.023	-0.016	-0.169	0.081	
Altitude ×Calendar year × Age	-0.074	-0.145	0.09	0.041	-0.835	-0.018	0.109

SM2. Twenty century structural parameters for low altitudes (1350-1615 m a.s.l.) and high altitudes (1640-1895 m a.s.l.). Given are means ± SE.

	Low altitudes	**High altitudes**
Calendar year	**Slenderness index**	
1940	37.091±11.64	38.95±11.62
1950	35.948±13.54	38.066±13.65
1960	39.126±14.17	35.0795±11.10
1969	30.415±11.39	35.07±9.91
2000	31.997±12.64	32.42±9.02
2010	46.209±17.22	36.116±8.80
	dbh/crown volume	
1940	0.557±0.36	0.617±0.36
1950	0.434±0.2	0.547±0.36
1960	0.403±0.2	0.474±0.32
1969	0.688±0.36	0.388±0.15
2000	0.657±0.33	0.45±0.19
2010	0.29±0.19	0.312±0.17

CAPÍTULO 6

DISCUSIÓN GENERAL

Las especies de pino presentan importantes diferencias en las respuestas funcionales al frío y al estrés hídrico (Sakai, 1983; Valladares y Sánchez-Gómez, 2006; Climent et al, 2009), lo que les ha permitido colonizar una amplia variedad de ambientes. El experimento llevado a cabo en esta Tesis (Capítulo 2) muestra que los juveniles de las seis especies de pinos nativas de Europa presentan importantes diferencias en su capacidad para sobrevivir a las bajas temperaturas. Estas diferencias son consistentes con su distribución en la Península Ibérica y en Europa, lo que sugiere que la distribución de los pinos está, en parte, determinada por las bajas temperaturas. En cambio, hemos observado que el estrés hídrico moderado en nuestro estudio no parece ser un condicionante tan limitante como las heladas. No obstante, el estrés hídrico prolongado e intenso tanto edáfico como atmosférico afecta más negativamente a los pinos de montaña, especialmente Pinus uncinata y P. sylvestris que los pinos típicamente mediterráneos (Matías et al 2017; Salazar-Tortosa et al. 2018). Por tanto, nuestros datos y los recientemente publicados apuntan a que las diferencias entre especies a las heladas y la sequía explican en buena medida la distribución de las poblaciones naturales en la Península Ibérica y en el resto de Europa.

La sensibilidad a las heladas limitan la capacidad de colonización de los pinos más típicamente mediterráneos (*P. halepensis* y *P. pinea*) hacia latitudes y altitudes más elevadas. Las tolerancia a las heladas, medida como la capacidad de sobrevivir de los juveniles, de las especies más mediterráneas fue menor que las especies de montaña en el sur de Europa o a menores altitudes o latitudes más elevadas (*P. nigra*, *P. sylvestris* y *P. uncinata*). *P. uncinata* y sobre todo *Pinus sylvestris* y alcanzan mayores latitudes y constituyen el límite latitudinal y altitudinal del bosque en muchas montañas del sur de Europa (Camarero and Gutiérrez 1999, Strimbeck and Schaberg 2009). Aunque *P. nigra* a menudo ocupa bandas altitudinales por debajo de *P. sylvestris* y *P. uncinata*, hemos visto que tienen una mayor tolerancia a las heladas (experimento de supervivencia, Capítulo 2), que dichas especies. Este resultado sugiere que la distribución altitudinal de *P. nigra* podría estar controlado por otros factores como la sequía estival, muy común en las montañas del sur de Europa. En concreto, experimentalmente, se ha demostrado que *P.*

nigra tiene una mayor capacidad de tolerar el estrés hídrico que *P. sylvestris* y *P. uncinata* (Matías et al, 2017). En concreto, también es posible que en zonas más elevadas donde la precipitación es mayor y la aridez estival menos intensa, *P. sylvestris* sea menos competitivo que *P. sylvestris*, lo que dificultaría su colonización en zonas más elevadas. Esta idea debería ser corroborada experimental en el futuro.

Los resultados experimentales (Capítulo 2) sugieren que el estrés hídrico modula de distinta manera la resistencia a las heladas en las especies de pinos. En *P. sylvestris* y *P. pinea*, el estrés hídrico reduce el daño por las heladas. A diferencia de estas especies, el estrés hídrico agudizó el daño por heladas en *P. uncinata*. Ello indica que esta especie es más vulnerable al estrés hídrico que el resto de ellas, tal como se han observado experimentalmente (Matías et al, 2017; Salazar-Tortosa et al, 2018). La congelación en los tejidos puede inducir a la deshidratación celular por dos mecanismos. El primero es la congelación de agua extracelular, en la cual, la expansión de los cristales de hielo provocan que el agua celular se difunda a través de la membrana plasmática al hielo extracelular, ocasionando que la célula se contraiga y se deshidrate. El segundo es la congelación intercelular, ya que se destruyen las membranas bioplasmáticas debido a que los cristales de hielo se expanden muy rápido en el protoplasto (Sakai and Larcher 1987). Se ha observado este mismo patrón para especies caducifolias como *Rhododendron catawbiense* (Peng et al. 2008) y *Larrea tridentata* (Medeiros et al, 2011). Sin embargo, existen pocos estudios para las especies perennifolias y coníferas (Ewers et al. 2003, Cavender-Bares et al. 2005). Para las demás especies, *P. halepensis*, *P. pinaster* y *P. nigra*, la interacción entre el estrés hídrico y las heladas no fue significativa (Capítulo 2, Figura 2). Esto sugiere que existen mecanismos alternativos para soportar el estrés hídrico y las heladas, como pueden ser el aumento en la producción de hormonas como el ácido absícico (Mäntylä et al, 1995; Pagter et al, 2008; De Diego et al, 2013), una mayor plasticidad de la pared celular, como lo observado para *Eucaliptus globulus* (Coopman et al. 2008), aumentar el grosor de la membrana plasmática acumulando lípidos, proteínas (Pearce 2001) o lignina (Jyske et al, 2010). Es muy probable que el estrés hídrico sea un determinante en la distribución de los pinos ibéricos, tal como están evidenciando algunos resultados del equipo de investigación en el que he realizado mi tesis doctoral están evidenciando (Martínez et al. 2014; Matías et 2017; Salazar-Tortosa et al. 2018a; Salazar-Tortosa et al en revisión).

Las diferencias en la tolerancia a las heladas entre las especies de pinos no se explican por diferencias en la vulnerabilidad a la formación de embolias (Capítulo 2). La formación de embolias causadas por las heladas está directamente relacionada con el diámetro de los conductos de xilema y de su potencial hídrico (Davis et al. 1999, Pitterman and Sperry 2003, Sperry, 1995). Los conductos xilemáticos de las coníferas está formado exclusivamente por traqueidas, las cuales tienen un diámetro significativamente menor que los vasos conductores de muchas angiospermas (Pitterman and Sperry 2003). Por ello, bajo el mismo potencial hídrico, la vulnerabilidad a las embolias por heladas en una conífera es más baja comparada con plantas con conductos más grandes (Cavender-Bares and Holbrook 2001, Cavender-Bares 2005). En el capítulo 2 se observa que las diferencias de diámetro de las traqueidas entre especies fue pequeña y osciló entre 6.6 y 10.6 mm. Para que una embolia causada por una helada pueda formarse en conductos tan estrechos, el potencial hídrico en el xilema debe ser inferior a -3.0 MPa. Sin embargo, los potenciales hídricos más bajos a los que estuvieron sometidas las plantas fueron -2.0 MPa. El reducido diámetro del lumen de las traqueidas permite que los pinos no se embolicen después de una helada y en condiciones de sequía (Pittermann and Sperry 2006). Además la sequía provoca que las traqueidas tengan lúmenes más estrechos y por tanto una reducida conductividad hidráulica que se traduce en una mayor densidad de la madera (Capítulo 3) por lo que la vulnerabilidad a la formación de embolias es muy baja en las coníferas. Las raíces (Toca et al, 2017) y las semillas (Gil Martín, 2014) de los pinos ibéricos también presentan diferencias de tolerancia al frío en la misma línea que las observadas para el follaje. Todos estos resultados indican quelas presiones selectivas que han conducidos a diferencias de tolerancia al frío han operado sobre distintas partes de la planta y etapas del ciclo de vida.

En contra de lo esperado y de trabajos previos (Charrier et al. 2013), no hemos encontrado mayor SS en las especies de pinos más tolerantes a las heladas. Por el contrario, los pinos que habitan en localidades de inviernos fríos y que presentaron una elevada tolerancia a las heladas, mostraron una menor concentración de SS que los pinos de zonas de inviernos suaves o frescos (Capítulo 2, Tabla 2). La acumulación de SS durante el invierno se ha relacionado con un incremento en la fluidez y estabilidad de las membranas celulares con el fin de mitigar los daños causados por la congelación de los tejidos en las plantas (Sakai and Larcher 1987, Uemura and Steponkus 2003).

Durante el proceso de aclimatación al frío de las plantas perennes en los biomas templados y fríos, la concentración de SS en los tejidos se incrementa durante el otoño hasta un máximo en mitad del invierno coincidiendo con el periodo de mayor tolerancia a las heladas (Ögren et al, 1997.) Posteriormente, los SS disminuyen durante la primavera en paralelo con la pérdida de tolerancia a las heladas (Chomba et al. 1993, Cavender-Bares 2005, Tjoelker et al. 2008). Además, el crecimiento y la formación de los distintos órganos en las plantas son desencadenados por hormonas y sustentados por el suministro de azúcares (Mehouachi et al, 1996). Los azúcares en las plantas sirven como fuentes de carbono y energía, protectores de estreses ambientales, señales moleculares y osmosis (Lipavská y Konrádová, 2004). La mayor parte de los estudios que han demostrado una relación entre la tolerancia a la helada y SS son estudios a escala interespecífica (Hinesley et al. 1992, Ögren et al. 1997, Charrier et al. 2013). Sin embargo, los estudios que relacionan la tolerancia a las heladas y la SS en el mismo género son muy escasos. Entre especies de *Quercus*, no se observaron diferencias significativas en el contenido de azúcares solubles en hojas entre las especies (Cavender-Bares et al, 2005). Es posible que las plantas más adaptadas a las heladas presenten una mayor tasa de respiración, lo que reduce la concentración de carbohidratos, como lo reportado para algunas coníferas (Mariko and Koizumi 1993; Ögren et al, 1997). Para la Sierra de Guadarrama se ha observado que las temperaturas medias anuales e invernales han aumentado un 19% (5.75 ºC a 6.87 ºC) y un 127% (-1.38 ºC a 0.38 ºC) respectivamente (Muñoz y García, 2004) durante la segunda mitad del siglo XX. El aumento de las temperaturas por efecto del cambio global, particularmente las de invierno, puede reducir la tolerancia a las heladas, ya que la respiración neta aumenta en respuesta del aumento de las temperaturas, lo que puede estar ocurriendo con el aumento de las temperaturas invernales reportadas en el capítulo 3.

Las poblaciones de *P. sylvestris* en altitudes elevadas son más sensibles a las heladas de finales de primavera (Gu et al, 2008; Fisichelli et al, 2014). Las heladas de primavera pueden inhibir la emergencia de las plántulas y obstaculizar el crecimiento y la supervivencia de los árboles (Fisichelli et al, 2014). El endurecimiento y el desendurecimiento en muchas coníferas está parcialmente controlado por la temperatura (Bigras et al, 2001) y los inviernos suaves pueden reducir el endurecimiento debido a la reducción de la concentración de azúcares solubles crioprotectores (Ögren et al, 1997; Morin et al. 2007). Es posible que el aumento de la temperatura invernal haya provocado que los órganos superiores sean más vulnerables a las heladas primaverales, cuya frecuencia se ha mantenido estable a medida que ha aumentado la temperatura media de la superficie (IPCC, 2014).

La temperatura media mundial se ha incrementado durante el ultimo siglo, especialmente en los últimos 20 años (Beniston 2003; Pauli et al. 2003; García-Romero et al, 2010). Algunos estudios muestran un efecto positivo en el crecimiento de los árboles debido al aumento de la temperatura y la extensión en la temporada de crecimiento (Salzer et al, 2009; Pretzsch et al, 2014). Sin embargo otros no han encontrado ningún efecto o incluso respuestas negativas (Briffa et al. 2004; Wilmking et al. 2004). El aumento de la temperatura implica una mayor evapotranspiración que, junto con una ligera reducción de la precipitación en las elevaciones altas, puede aumentar el estrés hídrico (Allen y Breshears 1998; Wilmking et al, 2004). Aunado a que los suelos a gran altura pueden tener una capacidad menor en la retención de agua y fertilidad que los sitios en el extremo inferior del gradiente (Pachepsky y otros, 2001; Charan et al, 2013). Para *P. sylvestris*, los resultados del Capítulo 3 muestran un crecimiento diferencial en el gradiente altitudinal durante el ultimo siglo. En las zonas bajas, el crecimiento de los árboles se ha incrementado, mientras que a mayores altitudes ha disminuido significativamente (Capítulo 3, Figura 3).

A bajas altitudes los árboles están sujetos a mayor estrés hídrico y a una mayor competencia (Castro et al, 2004), mientras que a altitudes mayores, el frío es el mayor factor de estrés. Los árboles situados a bajas altitudes se encuentran más expuestos al estrés hídrico, por lo que las heladas tardías no afectarían el crecimiento de los árboles, de acuerdo con los resultados obtenidos del capítulo 2. En el capítulo 3 observamos que, efectivamente los árboles situados a bajas altitudes han aumentado su crecimiento en las últimas décadas. Esto podría explicarse debido a que las poblaciones de *P. sylvestris* localizadas a bajas altitudes se han adaptado a las condiciones ambientales locales y son más tolerantes a la sequía que las poblaciones localizadas a mayores altitudes y latitudes. Esto se corrobora con lo reportado por Fournier et al, (2006) y Matías et al, (2014). Las diferencias ecotípicas en la tolerancia a la sequía de las especies arbóreas se pueden encontrar incluso a escalas espaciales pequeñas (ver Kubiske y Abrams, 1992), como lo observado en el Capítulo 5. Además, muchas especies muestran variaciones ecotípicas a lo largo de gradientes altitudinales, en donde los ecotipos situados a mayores altitudes crecen más lentamente que los ecotipos de bajas altitudes, debido a diferencias inherentes en metabolismo, fenología y tolerancia al estrés (von-Wuehlisch et al, 1995; Reich et al, 1996; Vitasse et al, 2009; Vitasse et al, 2013). La diferencias ecotípicas entre las poblaciones puede determinar tanto las respuestas fisiológicas en la tolerancia a las heladas en el follaje (como lo observado para *P. pinaster*, Capítulo 2), como en el crecimiento y morfología de la planta (*P. sylvestris,* Capítulo 4 y 5). Se observó que *P. pinaster* muestra notables diferencias interpoblacionales en la tolerancia a las heladas, de forma que las procedencias de lugares más fríos y climas continentales presentan una mayor tolerancia a las heladas que las procedencias de lugares de inviernos suaves (Corcuera et al, 2011, Prada et al, 2013). Por lo tanto, los efectos negativos de las bajas temperaturas con la altitud pueden interactuar sinérgicamente con las diferencias de ecotipos para reducir el crecimiento de los árboles (Matías et al, 2014; Matías et al, 2017).

Se ha observado que el aumento en la deposición de N y el CO_2 atmosférico desde la era industrial puede mejorar el crecimiento en algunas coníferas boreales (Bolker et al, 1995, Cannell et al, 1998, Emmett et al, 1998). Los experimentos de campo de N enriquecimiento apuntan a un aumento modesto en el crecimiento del bosque (Bolker et al, 1995, Cannell et al, 1998, Emmett et al, 1998). Específicamente, para rodales boreales de *P. sylvestris*, el enriquecimiento de N aumentó el crecimiento en un 12% (Nissinen y Hari, 1998). Por el contrario, otros estudios no mostraron ningún efecto de la deposición de N (Reynolds et al, 1998). La deposición de N en la Sierra de Guadarrama es relativamente alta (García-Gómez et al, 2014) debido a su proximidad a una gran ciudad (~70 km del área metropolitana de Madrid). Es posible que el aumento de la temperatura, el CO_2 y la deposición de N hayan estimulado el crecimiento de los árboles a bajas altitudes debido a una mayor eficiencia en el uso del agua (Saurer et al, 2004). Paradójicamente, a mayores altitudes, donde el frío es una limitación importante para el rendimiento de la planta (Sánchez-Salguero et al, 2015a), el crecimiento ha disminuido con el tiempo (Capítulo 3, Figura 3), lo que sugiere un efecto bajo en la "fertilización" y en el aumento de la temperatura en el crecimiento de los árboles. Esto sugiere que los beneficios de factores de cambio global han sido superados por factores locales que operan a grandes altitudes. De acuerdo con nuestros resultados, existe una relación poco significativa entre el crecimiento de los arboles y los principales factores de cambio global, como lo reportado para otros bosques de pinos mediterráneos (Camarero et al, 2015), lo que apoya la idea de que otros factores ambientales locales o regionales pueden enmascarar los efectos positivos del calentamiento y los efectos de "fertilización" de deposición de CO_2 y N. En primer lugar, los suelos a gran altura pueden tener una capacidad menor en la retención de agua y fertilidad que los sitios en el extremo inferior del gradiente (Pachepsky y otros, 2001; Charan et al, 2013). Se ha informado una reducción del crecimiento en poblaciones de *P. sylvestris* ubicadas a mayores altitudes, principalmente asociadas con condiciones de humedad (Lebourgeois et al, 2010), lo que sugiere que en las montañas mediterráneas, las poblaciones de pino silvestre localizadas en lo alto del gradiente pueden ser más vulnerables al estrés por sequía que poblaciones situadas en las partes más bajas (pero ver Sánchez-Salguero et al, 2015a). En segundo lugar, la disponibilidad de agua es el principal factor limitante para el crecimiento y las relaciones alométricas de los árboles en sitios de baja y media altitud en las montañas mediterráneas (Castro et al, 2004; Sánchez-Salguero et al, 2015b). En el Capítulo 5 observamos que las relaciones de los árboles cambian dependiendo a la altitud a la que se encuentren. Esto puede ser debido a que la fotosíntesis disminuye tanto en invierno como en verano en regiones frías y secas, y el estrés hídrico en la temporada de crecimiento causado por un déficit de vapor atmosférico y una baja disponibilidad hídrica del suelo conducen a una reducción radial del crecimiento (Dulamsuren et al, 2009; Gimeno y et al, 2012), como observado para algunas coníferas en el capítulo 3. Los cambios en la precipitación durante la estación de crecimiento (primavera) pueden aumentar la densidad mínima de madera y reducir el crecimiento radial de los bosques de coníferas, afectando negativamente su potencial para fijar y almacenar reservas de carbono como madera del tallo.

Por último, en el desarrollo de esta tesis hemos observado que, además de factores abióticos, la edad es una variable muy importante ya que modula el crecimiento y las relaciones alométricas de los árboles (Capítulo 4 y Capítulo 5). Los resultados del capítulo 5 sugieren que la edad determina las relaciones alométricas de los árboles entre el tamaño del tronco y el volumen de las copas. Se ha observado que las plántulas son más sensibles a estreses ambientales, debido a que el sistema radicular es más pequeño, y por consiguiente, tienen un acceso limitado al agua (Clark, 1983). Por lo anterior, se esperaría que los árboles más jóvenes sean más susceptibles a cambios ambientales. Sin embargo, los árboles más viejos mostraron un evidente cambio a lo largo del gradiente altitudinal, mientras que los árboles más jóvenes experimentan las mismas relaciones alométricas en todo el gradiente (Capítulo 5, Fig. 2). Distinto autores han demostrado que los árboles jóvenes son menos susceptibles a los cambios ambientales que los árboles adultos (Ettl y Peterson, 1995; Carrer y Urbinati, 2004). Esto se debe al papel de la limitación hidráulica, ya que cuando un árbol esta cerca de alcanzar su altura máxima existe una mayor resistencia hidráulica, debido a la combinación de varios factores como la gravedad, la longitud y la complejidad de la trayectoria hidráulica a través del tallo y las ramas, así como una reducida asignación de recursos en las raíces (Ryan y Yoder, 1997). El aumento en la resistencia hidráulica también puede disminuir el suministro del agua para la transpiración, lo que induce al cierre estomático para evitar la cavitación en el xilema (Ryan et al, 1997, Ryan y Yoder 1997), que a su vez limita la conductancia estomática y el intercambio gaseoso (Yoder et al, 1994; Hubbard et al, 1999; Kolb y Stone 2000). Las limitaciones hidráulicas son más importantes en árboles más viejos. Por el contrario, los árboles más jóvenes son capaces de enfrentar condiciones adversas debido a que mantienen más tiempo los estomas abiertos, por lo que la fotosíntesis y la transpiración es mayor.

Investigaciones futuras

Muchos aspectos sobre la interacción del estrés hídrico y las heladas en las respuestas funcionales de los pinos ibéricos necesitan investigaciones futuras. Algunas cuestiones relacionadas con los resultados de mi tesis son:

1) ¿Qué mecanismos subyacen las respuestas fisiológicas a las heladas y el estrés hídrico? Se necesitan investigaciones posteriores para determinar los mecanismos fisiológicos adyacentes en la interacción entre la tolerancia a las heladas y el estrés hídrico para las especies nativas de pinos en la Península Ibérica (Capítulo 2). Se ha reportado que el ácido absícico es fundamental en la tolerancia a estreses abióticos (Mäntylä et al, 1995; Pagter et al, 2008; De Diego et al, 2013), por lo que determinar las concentraciones a escalas inter e intraespecíficas podrá ayudar a esclarecer su papel como determinante en la distribución de las especies. Además, la determinación de los azúcares solubles en las distintas poblaciones y estaciones del año resulta necesario para conocer su papel funcional en respuesta al frío y al estrés hídrico. Nuestro experimento se llevó a cabo en plántulas, por lo que determinar si se mantienen las interacciones entre estreses abióticos resultaría interesante para determinar el papel de estos.

2) Debido a que *P. nigra* es la especie más tolerante a las heladas, desconocemos la razón por la que no coloniza latitudes mayores. ¿Cuáles son los mecanismos fisiológicos adyacentes que determinan la distribución de *P. nigra*? ¿Por qué es menos competitivo a mayores altitudes?

3. ¿Existen diferencias genéticas entre las poblaciones de los pinos para que los árboles presenten diferencias en el crecimiento? Como previamente se mencionó en el capítulo 4, podría haber diferencias genéticas entre las poblaciones de pinos que puedan explicar las diferencias en el crecimiento.

REFERENCIAS

Allen C, Breshears D (1998) Drought-induced shift of a forest-woodland ecotone: Rapid landscape response to climate variation. Proced Nat Acad Sci 95:14839–14842.

Beniston M (2003) Climatic change in mountain regions: a review of possible impacts. Clim Chang 59:5-31.

Bigras FJ, Ryyppo A, Lindström A, Stattin E (2001) Cold acclimation and deacclimation of shoots and roots of conifer seedlings, in: Bigras FJ, Colombo SJ (eds.) Conifer cold hardiness. Kluwer Academic Publishers, Dordrecht - Boston, pp. 57–88.

Bolker B, Pacala S, Bazzaz F, Canhams CD, Levin SA (1995) Species diversity and ecosystem response to carbon dioxide fertilization: conclusions from a temperate forest model. Glob Chang Biol 1:373–381.

Briffa K, Osborn T, Schweingruber F (2004) Large-scale temperature inferences from tree rings : A review. Glob Plant Ch 40:11–26.

Camarero J, Gutiérrez E (1999) Estructura, patrón espacial y regeneración de una población de *Pinus uncinata* Ram. en su límite occidental de distribución (Castillo de Vinuesa, Soria-La Rioja). Zubía 17:99–153.

Camarero J, Gazol A, Tardif J, Conciatori F (2015) Attributing forest responses to global-change drivers: Limited evidence of a CO_2-fertilization effect in Iberian pine growth. J Biogeogr 42:2220–2233.

Cannell M, Thornley J, Mobbs D, Friend A (1998) UK conifer forests may be growing faster in response to increased N deposition, atmospheric CO_2 and temperature. Forestry 71:277–296.

Carrer M, Urbinati C (2001). Spatial analyses of structural and tree ring related parameters in a timberline forest of Italian Alps. Journal of Vegetation Science. 12:643-652.

Castro J, Zamora R, Hódar JA, Gómez JM (2004) Alleviation of summer drought boosts establishment success of *Pinus sylvestris* in a Mediterranean mountain: an experimental approach, Plant Ecol 181:191-202.

Cavender-Bares J, Holbrook N (2001) Hydraulic properties and freezing-induced cavitation in sympatric evergreen and deciduous oaks with contrasting habitats. Plant, Cell Environ 24:1243–1256.

Cavender-Bares J (2005) Impacts of Freezing on Long Distance Transport in Woody Plants. In: Holbrook N, Zwieniecki M (eds) Vascular Transport in Plants. Elservier, pp 401–424.

Cavender-Bares J, Cortes P, Rambal S, Joffre R, Miles B, Rocheteau A (2005) Summer and winter sensitivity of leaves and xylem to minimum freezing temperatures: A comparison of co-occurring Mediterranean oaks that differ in leaf lifespan. New Phytol 168:597–612.

Charan G, Bharti VK, Jadhav SE, Kumar S, Acharya S, Kumar P, Gogoi D, Srivastava RB (2013) Altitudinal variations in soil physico-chemical properties at cold desert high altitude. J. Soil Sci. Plant Nutr 13, 267–277.

Charrier G, Cochard H, Ameglio T (2013) Evaluation of the impact of frost resistances on potential altitudinal limit of trees. Tree Physiol 33:891–902.

Chomba B, Guy R, Weger H (1993) Carbohydrate reserve accumulation and depletion in Engelmann spruce (Picea engelmannii Parry): effects of cold storage and pre-storage CO2 enrichment. Tree Physiol 13: 351–364.

Clark JR (1983) Age-related changes in trees. J Arboric 9:201–205

Climent J, Costa e Silva F, Chambel M, Pardos M, Almeida M (2009) Freezing injury in primary and secondary needles of Mediterranean pine species of contrasting ecological niches. Annals of Forest Science. 66, 407–407.

Coopman R, Jara J, Bravo L, Sáez K, Mella G, Escobar R (2008) Changes in morpho-physiological attributes of Eucalyptus globulus plants in response to different drought hardening treatments. Electron J Biotechnol 11:1–10.

Corcuera L, Gil-Pelegrin E, Notivol E (2011) Intraspecific variation in Pinus pinaster PSII photochemical efficiency in response to winter stress and freezing temperatures. PLoS One 6:1–12.

Davis S, Sperry J, Hacke U (1999) The relationship between xylem conduit diameter and cavitation caused by freezing. Am J Bot 86:1367–1372.

De Diego N, Ródríguez JL, Dodd IC, Pérez-Alfocea F, Moncaleán P, Lacuesta M (2013) Immunolocalization of IAA and ABA in roots and needles of radiata pine (*Pinus radiata*) during drought and rewatering. Tree Physiology. 33: 537-549.

Dulamsuren Ch, Hauck M, Bader M, Osokhjargal D, Oyungerel S, Nyambayar S, Runge M, Leuschner C (2009) Water relations and photosynthetic performance in *Larix sibirica* growing in the forest-steppe ecotone of northern Mongolia. Tree Physiol 29:99–110

Emmett B, Boxman D, Bredemeier M, Gundersen P, Kjønaas OJ, Modan F, Schleppi P, Tietema A, Wright RF (1998) Predicting the effects of atmospheric nitrogen deposition in conifer stands: Evidence from the NITREX ecosystem-scale experiments. Ecosystems 1:352–360.Ettl GJ, Peterson DL (1995) Extreme climate and variation in tree growth: individualistic response in subalpine fir (*Abies lasiocarpa*). Global change Biology. 1:231-241.

Ettl GF, Peterson DL (1995) Growth response of subalpine fir (*Abies lasiocarpa*) to climate in the Olympic Mountains, Washington, USA. Global Change Biology, 1:213-230.

Ewers F, Lawson M, Bowen T, Davis S (2003) Freeze/thaw stress in Ceanothus of southern California chaparral. Oecologia 136:213–219.

Fisichelli N, Vor T, Ammer C (2014) Broadleaf seedling responses to warmer temperaturas “chilled” by late forst that favors conifers. European Journal of Forest Research, 133:587-596.

Fournier N, Rigling A, Dobbertin M, Gugerli F (2006) Faible différenciation génétique, à partir d’amplification aléatoire d’ADN polymorphe (RAPD), entre les types de pin sylvestre (*Pinus sylvestris* L.) d’altitude et de plaine dans les Alpes à climat continental. Ann For Sci 63:431–439.

García-Gómez H, Garrido J, Vivanco M, Lassaletta L, Rábago I, Ávila A, Tsyro S, Sánchez G, González OA, González-Fernández I, Alonso R (2014) Nitrogen deposition in Spain: Modeled patterns and threatened habitats within the Natura 2000 network. Sci Total Environ 485–486:450–460.

García-Romero A, Muñoz J, Andrés N, Palacios D (2010) Relationship between climate change and vegetation distribution in the Mediterranean mountains:

Manzanares Head valley, Sierra de Guadarrama (Central Spain). Clim Change 100:645–666.

Gil Martín A (2014) Comparación de la resistencia a las heladas de las semillas de los pinos ibéricos. Trabajo de Fin de Grado. Universidad de Alcalá.

Gimeno TE, Camarero JJ, Granda E, Pías B, Valladares F (2012) Enhanced growth of *Juniperus thurifera* under a warmer climate is explained by a positive carbon gain under cold and drought. Tree Physiol 32:326–336

Gu L, Hanson PJ, Mac Post W et al (2008) The 2007 eastern US spring freeze: increased cold damage in a warming world? Bioscience 58:253–262.

Hinesley L, Pharr D, Snelling L, Funderburk S (1992) Foliar raffinose and sucrose in four conifer species: Relationship to seasonal temperature. J Am Soc Hortic Sci 117:852–855.

Hubbard RM, Bond BJ, Ryan MG (1999) Evidence that hydraulic conductance limits photosynthesis in old *Pinus ponderosa* trees. Tree Physiology 19:165-172.

IPCC (2014) Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.

Jyske T, Holtta T, Makinen H, Nojd P, Lumme I, Spiecker H (2010) The effect of artificially induced drought on radial increment and wood properties of Norway spruce. Tree Physiol 30(1):103–115

Kolb TE, Stone JE (2000). Differences in leaf gas exchange and water relations among species and tree sizes in an Arizona pine-oak forest. Tree Physiology 20:1-12.

Kubiske M, Abrams M (1992) Photosynthesis, water relations, and leaf morphology of xeric versus mesic *Quercus rubra* ecotypes in central Pennsylvania in relation to moisture stress. Can J For Res 22:1402–1407.

Lebourgeois F, Rathgeber CBK, Ulrich E (2010) Sensitivity of French temperate coniferous forests to climate variability and extreme events (*Abies alba*, *Picea abies* and *Pinus sylvestris*). J Veg Sci 21:364-376

Lipavská H, Konrádová H (2004) Somatic embryogenesis in conifers: the role of carbohydrate metabolism. In Vitro Celular and Developmental Biology Plant. 40(1):23-30.

Mäntylä E, Lang V, Palva E (1995) Role of Abscisic Acid in Drought-Induced Freezing Tolerance, Cold Acclimation, and Accumulation of LT178 and RAB18 Proteins in *Arabidopsis thaliana*. Plant Physiol 107:141–148.

Mariko S, Koizumi H (1993) Respiration for maintenance and growth in *Reynoutria japonica* ecotypes from different altitudes on Mt Fuji. Ecol Res 8:241–246.

Matías L, González-Díaz P, Jump AS (2014) Larger investment in roots in southern range-edge populations of Scots pine is associated with increased growth and seedling resistance to extreme drought in response to simulated climate change. Environ Exp Bot 105, 32–38.

Matías L, Castro J, Villar-Salvador P, Quero JJL, Jump ASA (2017) Differential impact of hotter drought on seedling performance of five ecologically distinct pine species. Plant Ecol:1–12.

Medeiros J, Pockman W (2011) Drought increases freezing tolerance of both leaves and xylem of Larrea tridentata. Plant Cell Environ 34:43–51.

Mehouachi J, Tadeo FR, Zaragoza S, Primo-Millo E, Talon M (1996) Effects of gibberellic acid and paclobutrazol on growth and carbohydrate accumulation in shoots and roots of citrus rootstock seedlings. J. Hort. Sci. 71:747–754; 1996.

Morin X, Améglio T, Ahas R, Kurz-Besson C, Lanta V, Lebourgeois F, Miglietta F, Chuine I (2007) Variation in cold hardiness and carbohydrate concentration from dormancy induction to bud burst among provenances of three European oak species. Tree Physiol 27: 817–825.

Muñoz J, García R (2004) Modificaciones climáticas y evolución de la cubierta vegetal en las áreas culminantes de la Sierra de Guadarrama (Sistema Central español) durante la segunda mitad del XX: las altas cuencas del Ventisquero de La Condesa y de Valdemartín. Cuad Investig Geog 30:117–146.

Nissinen A, Hari P (1998) Effects of nitrogen deposition on tree growth and soil nutrients in boreal Scots pine stands, in: Environ Pollut. 102: 61–68.

Ögren E, Nilsson T, Sundblad L (1997) Relationship between respiratory depletion of sugars and loss of cold hardiness in coniferous seedlings over-wintering at raised temperatures: indications of different sensitivities of spruce and pine. Plant Cell Environ 20:247–253.

Pachepsky YA, Timlin DJ, Rawls WJ (2001) Soil and water management and conservation. Soil Sci Soc Am J 65:1787-1795.

Pagter M, Jensen CR, Petersen KK, Liu F, Arora R (2008) Changes in carbohydrates, ABA and bark proteins during seasonal cold acclimation and deacclimation in *Hydrangea* species differing in cold hardiness. Physiologia Plantarum. 134:473-485.

Pauli H, Gottfried M, Grabherr G (2003) Effects of climate change on the alpine and nival vegetation of the Alps. J Meteor Ecol 7:9–12.

Pearce R (2001) Plant freezing and damage. Annals of Botany. 87: 417–424.

Peng Y, Arora R, Li G, Wang X, Fessehaie A (2008) Rhododendron catawbiense plasma membrane intrinsic proteins are aquaporins, and their over-expression compromises constitutive freezing tolerance and cold acclimation ability of transgenic Arabidopsis plants. Plant, Cell Environ 31:1275–1289.

Pittermann J, Sperry J (2003) Tracheid diameter is the key trait determining the extent of freezing-induced embolism in conifers. Tree Physiol 23:907–914.

Pittermann J, Sperry J (2006) Analysis of freeze-thaw embolism in conifers. The interaction between cavitation pressure and tracheid size. Plant Physiol 140:374–382.

Prada E, Toval G, Díaz R (2013) Influencia del método de preparació de muestras de *Pinus pinaster* en los ensayos de resistencia a heladas bajo condiciones controladas. 6º Congreso Forestal Español 1-8.

Pretzsch H, Biber P, Schütze G, Uhl E, Rötzer T(2014) Forest stand dynamics in Central Europe has accelerated since 1870. Nat Commun 1–10.

Reich P, Oleksyn J, Tjoelker M (1996) Needle respiration and nitrogen concentration in Scots Pine populations from a broad latitudinal range: A common garden test with field-grown trees. Funct Ecol 10:768–776.

Reynolds B, Wilson E, Emmett B (1998) Evaluating critical loads of nutrient nitrogen and acidity for terrestrial systems using ecosystem-scale experiments (NITREX). For Ecol Manage 101:81–94.

Ryan MG, Binkley D, Fownes JH (1997) Age- related decline in forest productivity: pattern and process. Advances in Ecological Research 27:213-262.

Ryan MG, Yoder BJ (1997). Hydraulic limits to tree height and tree growth. BioScience 47:235-242.

Sakai A (1983) Comparative study on freezing resistance of conifers with special reference to cold adaptation and its evolutive aspects. Canadian Journal of Botany. 61, 2323–2332.

Sakai A, Larcher W (1987) Frost Survival of Plants. Responses and Adaptation to freezing stress Billings WD, Galley F, Lange O, Olson J, Remmert H (eds). Springer-Verlag.

Salazar-Tortosa D, Castro J, Matías L, Viñegla-Pérez B, Villar-Salvador P, Querejeta I (2018) The "isohydric trap": a detrimental feedback between water shortage and nutrient acquisition drives differential response of European pines under climatic dryness (en revision Global Change Biology).

Salzer M, Hughes M, Bunn A, Kipfmueller K (2009) Recent unprecedented tree-ring growth in bristlecone pine at the highest elevations and possible causes. Proc Nat Acad Sci U S A 106:20348–53.

Sánchez-Salguero R, Camarero J, Hevia A, Madrigal-González J, Linares JC, Ballesteros-Canovas JA, Sánchez-Miranda A, Alfaro-Sánchez R, Sangüesa-Barreda G, Diego GA, Gutiérrez E, Génova M, Rigling A (2015a) What drives growth of Scots pine in continental Mediterranean climates: Drought, low temperatures or both? Agric For Meteorol 206:151–162

Sánchez-Salguero R, Linares J, Camarero JJ, Madrigal-González J, Hevia A, Sánchez-Miranda A, Ballesteros-Canovas JA, Alfaro-Sánchez R, García-Cervigón AI, Bigler C, Rigling A (2015b) Disentangling the effects of competition and climate on individual tree growth: A retrospective and dynamic approach in Scots pine. For Ecol Manage 358:12–25. doi: 10.1016/j.foreco.2015.08.034

Saurer M, Siegwolf R, Schweingruber F (2004) Carbon isotope discrimination indicates improving water-use efficiency of trees in northern Eurasia over the last 100 years. Glob Chang Biol.

Sperry JS (1995) Limitations on stem water transport and their consequences. In: Gartner B, ed. Plant Stems. London, UK: Academic Press, 105–124.

Strimbeck G, Schaberg P (2009) Going to extremes: low temperature tolerance and acclimation in temperate and boreal conifers. In: Plant cold hardiness: from the laboratory to the field. pp 226–239.

Tjoelker M, Oleksyn J, Reich P, Zytkowiak R (2008) Coupling of respiration, nitrogen, and sugars underlies convergent temperature acclimation in Pinus banksiana across wide-ranging sites and populations. Glob Chang Biol 14:782–797.

Toca A, Oliet JA, Villar-Salvador P, Maroto J, Jacobs DF (2017) Species ecology determines the role of nitrogen nutrition in the frost tolerance of pine seedlings. Tree Physiology. 1-13.

Uemura M, Steponkus P (2003) Modification of the intracellular sugar content alters the incidence of freeze-induced membrane lesions of protoplasts isolated from Arabidopsis thaliana leaves. Plant, Cell Environ 26:1083–1096.

Valladares F, Sánchez-Gómez D (2006) Ecophysiological traits associated with drought in Mediterranean tree seedlings: individual responses versus interspecific trend in eleven species. Plant biology. 8(5):688-697.

Vitasse Y, Delzon S, Bresson C, Michalet R, Kremer A (2009) Altitudinal differentiation in growth and phenology among populations of temperate-zone tree species growing in a common garden. Can J For Res 39:1259–1269.

Vitasse Y, Hoch G, Randin C, Lenz A, Kollas C, Scheepens JF, Körner C (2013) Elevational adaptation and plasticity in seedling phenology of temperate deciduous tree species. Oecologia 171:663–678.

von-Wuehlisch G, Krusche D, Muhs HJ (1995) Variation in temperature sum requirement for flushing of beech provenances. Silvae Genet. 44, 343–346.

Yoder BJ, Ryan MG, Waring RH, Schoettle AW, Kaufmann MR (1994) Evidence of reduced photosynthetic rates in old trees. Forest science. 40:513-527.

Wilmking M, Juday G, Barber V, Zald H (2004) Recent climate warming forces contrasting growth responses of white spruce at treeline in Alaska through temperature thresholds. Glob Chang Biol 10:1724–1736.

CAPÍTULO 7

CONCLUSIONES GENERALES

1) Los individuos jóvenes de las seis especies de pinos nativas de la Península ibérica presentan distinta capacidad para sobrevivir las heladas. Dichas diferencias coinciden con la distribución actual de las poblaciones naturales. Los pinos mediterráneos (*P. halepensis* y *P. pinea*), que viven en zonas de inviernos suaves, son las menos tolerantes a las heladas, mientras que las especies de montaña (*P. nigra*, *P. sylvestris* y *P. uncinata*) son las más tolerantes. *Pinus pinaster*, que ocupa zonas intermedias de frío presenta tolerancias a las heladas menores que los pinos de montaña.

2) Las diferencias de tolerancia a las heladas entre especies radican en diferencias de daño a las hojas pero no en la vulnerabilidad a la formación de embolias en el xilema.

3) El estrés hídrico moderado no afecta la formación de embolias en las especies de pino, pero incrementa la tolerancia del follaje a las heladas en *P. sylvestris* y *P. pinea*, la disminuye en *P. uncinata* y no tiene ningún efecto en *P. pinaster* y *P. halepensis*.

4) Las diferencias de concentración de azúcares solubles en el follaje entre especies no explica las diferencias interespecíficas de tolerancia a las heladas. Ello sugiere que hay otros mecanismos no relacionados con los carbohidratos solubles que determinan la tolerancia a las heladas.

5) En regiones frías con periódos secos como las montañas mediterráneas y otras zonas continentales del palneta, la densidad mínima de la madera temprana es un mejor indicador de episodios de sequía en primavera que la anchura de la madera temprana y tardía en los anillos de crecimiento.

6) El crecimiento corriente del tronco en los últimos 80 años de los individuos de *P. sylvestris* muestra tendencias opuestas a lo largo de un gradiente altitudinal en el Sistema Central. En los sitios a menor altitud, donde la aridez estival es más intensa, el crecimiento se ha incrementado, mientras que en las zonas más elevadas, con mayor

precipitación y frío, el crecimento ha disminuido. Estos resultados sugieren que el potencial beneficio de los motores de cambio global sobre el crecimiento pueden ser intensamente modulados por factores ambientales locales, lo que debe considerarse en las predicciones sobre el crecimiento y distribución de los árboles.

7) Las diferencia de crecimiento en los últimos 80 años de los árboles de *Pinus sylvestris* también dependieron de su edad. Las diferencias de crecimiento con la altitud en el Sistema Central fueron más evidentes en los árboles de más edad que en los jóvenes.

8) Las condiciones ambientales condicionan el patrón de asignación de biomasa entre las distintas partes del árbol y por tanto en su forma independienteente de la densidad del arbolado. Los individuos de *Pinus sylvestris* en el Sistema Central reducen la asignación de biomasa al tronco e incrementan la asignada a la construcción de la copa con la altitud. Esta mayor reparto de biomasa a favor de la copa también se ha incrementado a lo largo de los últimos 80 años.

9) La esbeltez de la parte áerea de los árboles de *Pinus sylvestris* en el Sistema Central se ha incrementado a lo largo de los últimos 80 años en las zonas a menor altitud, mientras que ha seguido el patrón opuesto en las zonas más elevadas. Esto implica que los motores de cambio climático no sólo han influido en el crecimiento del arbolado sino en su forma y que dichos cambios están fuertemente influidos por el ambiente local y la edad de los individuos.

“But in the end, it’s only a passing thing, this shadow.
Even darkness must pass”
J.R.R. Tolkien