UNIVERSIDAD COMPLUTENSE DE MADRID

FACULTAD DE CIENCIAS BIOLÓGICAS
Departamento de Ecología

TESIS DOCTORAL

Copa y concierto: variabilidad de caracteres funcionales foliares en *Olea europaea*

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

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Madrid, 2015

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DEPARTAMENTO DE ECOLOGÍA

Copa y Concierto; variabilidad de caracteres funcionales foliares en *Olea europaea*

Memoria presentada por Adrián Gaspar Escribano Rocafort, licenciado en Ciencias Biológicas por la Universidad Complutense de Madrid, para optar al grado de Doctor. Dirigida por el Dr. Luis Balaguer Núñez, de la Universidad Complutense de Madrid, por el Dr. Juan A. Delgado Sáez, de la Universidad Complutense de Madrid y por el Dr. Adrián Escudero Alcántara, de la Universidad Rey Juan Carlos.

El doctorando *V°B° del director* *V°B° del director* *V°B° del director*

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La presente Tesis Doctoral ha sido financiada por una beca de Formación de Personal Investigador (FPI, BES-2010-032767) concedida por el Ministerio de Ciencia y Educación. El estudio presentado ha formado parte del proyecto nacional CGL2009-10392.

A mi abuela

"*All evolutionary biologists know that variation itself is nature's only irreducible essence... I had to place myself amidst the variation*"

- Stephen Jay Gould

"*Everything starts somewhere, although many physicists disagree*"

- Sir Terry Pratchett

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INTRODUCCIÓN GENERAL

1. Caracteres funcionales.

El concepto de carácter o rasgo funcional surge con la emergencia de la Ecología Funcional como disciplina ecológica y del estudio de grupos de caracteres morfológicos y/o fisiológicos a distintos niveles de organización, de las relaciones empíricas entre los mismos y de su relación con el ambiente (Calow 1987, Keddy 1992). Debido a la dificultad de identificar funcionalidad *per se*, y con el objetivo de sintetizar y categorizar los rasgos basándose en su importancia relativa a nivel de organismo, se define rasgo funcional como cualquier característica morfológica, fisiológica o fenológica, de la célula al individuo, con un impacto indirecto en la eficacia biológica a través de sus efectos sobre los componentes de la misma (crecimiento, reproducción y supervivencia) (Lavorel *et al*. 1997; Ackerly *et al*. 2000; Geber y Griffen 2003; Violle *et al*. 2007). Explícitamente, esta definición excluye todas aquellas características del ambiente y de niveles de organización superiores al individuo e identifica uno de los mecanismos (variabilidad de los caracteres funcionales) mediante el cual se producirían cambios en la distribución de los rasgos fenotípicos de la siguiente generación debido a su efecto sobre los componentes de la eficacia biológica (Futuyma 1998; 2009).

1.1 Variabilidad de los caracteres funcionales.

En las plantas vasculares, los caracteres funcionales constituyen una aproximación indirecta entre la eficacia biológica y el ambiente que experimentan, útiles para desentrañar cuestiones relevantes en varias áreas de la ecología (McGill *et al*. 2006; Ackerly *et al*. 2007). Los caracteres funcionales se han empleado a nivel de especie y comunidad para investigar el funcionamiento de ecosistemas, la diversidad funcional de comunidades y para esclarecer los efectos del ambiente entre las distintas especies o grupos de caracteres (Lavorel *et al*. 1997; Reich *et al*. 2003; Wright *et al.* 2004; Diaz *et al*. 2004, 2007; de Bello *et al*. 2010). A nivel de órgano, la investigación de rasgos funcionales (como las características estructurales y bioquímicas foliares) constituye una vía de estudio de la capacidad fotosintética a distintas escalas, de su impacto a nivel de organismo y de los procesos evolutivos asociados con la captura de luz (Smith *et al*. 2004; Smith &

Hughes 2009). El empleo de caracteres funcionales también supone un paso fundamental en la construcción de herramientas predictivas y como referentes frente al cambio climático (Sitch *et al*. 2003; Norberg *et al*. 2001; Savage, Webb & Norberg 2007; Gornish & Prather 2014).

1.2 Variabilidad intraespecífica.

Una de las premisas adoptadas en la teoría ecológica implica que la variabilidad en la expresión de caracteres funcionales es despreciable entre individuos conespecíficos comparada con la variabilidad existente entre especies (Keddy 1992; Garnier *et al*. 2001; Roche *et al*. 2004). Sin embargo, la evidencia acumulada destaca la variabilidad intraespecífica en el funcionamiento de los ecosistemas y en los procesos de ensamblaje y dinámica de comunidades, especialmente en aquellas dominadas por una o pocas especies (Booth & Grime 2003; Boege & Dirzo 2004; Crutsinger *et al*. 2006; Whitlock *et al*. 2007; Lecerf & Chauvet 2008; De Frenne *et al*. 2011; Siefert 2012; Violle *et al*. 2012; Kraft *et al*. 2014; Niinemets 2014). Bolnick *et al*. (2011) identifican hasta seis mecanismos teóricos mediante los cuales la variabilidad en los caracteres funcionales pueden alterar la dinámica ecológica (Tabla 1). Estos mecanismos se dividen en los que tienen implicaciones ecológicas directas independientemente de la heredabilidad de los caracteres y los que tienen un efecto indirecto debido a dinámicas eco-evolutivas producto de la variación genética. La naturaleza de estos mecanismos y su presencia ubicua manifiesta el impacto que puede tener la variabilidad funcional dentro de una misma especie a escala ecológica (Bolnick *et al*. 2011).

Tabla 1. Mecanismos descritos en Bolnik *et al* . (2011) mediante los cuales la variabilidad intraespecífica puede alterar la dinámica y estructura de comunidades.

Implicación ecológica directa	***Producto de:***
- Inequalidad de Jensen	Las interacciones ecológicas guardan dependencias no lineales con una variable.
- Incremento del grado de interacción	La variabilidad de los caracteres funcionales altera la fuerza o número de interacciones de las especies.
- Efecto cartera de inversion	La densidad de los distintos fenotipos muestra una covariación negativa en el tiempo.
Implicación ecológica indirecta	
- Subsidio fenotípico	El intercambio genético entre fenotipos o durante el desarrollo permite desacoplar la tasa de crecimiento de los fenotipos de su *fitness* individual.
- Dinámicas eco-evolutivas	La heredabilidad de la variación de los caracteres funcionales posibilita una evolución acelerada.
- Efecto de muestreo ecológico y evolutivo	Las poblaciones pequeñas están expuestas a muestreo estocástico de la distribución de caracteres.

1.3 Escalas de la variabilidad intraespecífica.

La variabilidad funcional intraespecifica (VF) es la expresión diferencial en los valores de los caracteres funcionales de los individuos de una especie dada. Surge de la diversidad genética y de la respuesta fenotípica a distintas escalas espaciales (ver Albert *et al*. 2011). La jerarquía de la VF se descompone en tres escalas anidadas; variabilidad funcional entre poblaciones (VFpop), variabilidad entre individuos de una población (VF_{BI}) y variabilidad dentro de los individuos (por ejemplo entre hojas de un mismo individuo; VF_{WI}). La magnitud y distribución de la VF en las distintas escalas proporcionan información sobre la respuesta individual frente al ambiente y la potencial diversidad de respuestas funcionales entre individuos. La VF es producto de la heterogeneidad ambiental en el tiempo y en el espacio que experimentan los individuos de una especie durante el transcurso de su vida (Coleman *et al*. 1994) y de la capacidad de los distintos genotipos de expresar distintos fenotipos (Fisher 1930; Hughes *et al*. 2008). La interacción de ambos mecanismos no permite diferenciar entre ambas fuentes de variación (Scheiner & Lyman 1991; Coleman *et al.* 1994; Grassein *et al.* 2010). En un contexto evolutivo la VF es esencial para los procesos de selección natural y especiación la cual debería cuantificarse tanto en términos de curvas-respuesta entre caracteres funcionales y el ambiente (que habitualmente no pueden extrapolarse de relaciones interespecíficas) como abarcando la interacción entre las diferentes escalas en las que se estructura (Ryser & Eek 2000; Sugiyama 2003; Albert *et al.* 2010a).

La validación de modelos predictivos y la creación de nuevos modelos funcionales requieren datos empíricos que recojan la magnitud y estructura de la VF en las distintas escalas. Sin esta información no es posible obtener la resolución necesaria para predecir el ensamblaje y respuesta de las poblaciones y comunidades. A pesar de reconocer la existencia de variación intraespecífica en la expresión de caracteres funcionales y sus implicaciones a escala ecológica, existen pocos trabajos y modelos que intenten caracterizar la magnitud y estructura de la variabilidad funcional intraespecífica en especies arbóreas. Esto resulta sorprendente dado que es bien sabido que la variabilidad de los caracteres está organizada en múltiples escalas, incluso dentro de los individuos, y no debería

obviarse (Bolnick *et al*. 2003; Herrera 2009; Albert *et al*. 2010; Jung *et al*. 2010; Bolnick *et al*. 2011).

1.4 Variabilidad funcional a escala de individuo.

Entre las distintas formas de crecimiento de las plantas terrestres es esperable que los árboles (forma de crecimiento convergente en múltiples familias) muestren elevada VF_{WI} (Hulshof & Swenson 2010; Messier *et al*. 2010). De hecho, los individuos longevos de porte arbóreo responden a a la heterogeneidad ambiental a través de la expresión de variedad de fenotipos, por ejemplo, las hojas se producen bajo condiciones concretas y coexisten en respuesta a esas condiciones (Bradshaw 1965; Sultan 1987; Petit & Hampe 2006) a y la prevalencia de un conjunto determinado de caracteres (síndrome fenotípico) que afectan a la eficacia biológica de todo el individuo (Marks & Lechowicz 2006; Wilson & Nussey 2010). La expresión del síndrome fenotípico refleja las relaciones de los caracteres funcionales con las distintas limitaciones tanto de la planta (Díaz & Cabido 1997) como las impuestas por factores ambientales a un grupo de caracteres (Wright *et al.* 2004; Webb *et al.* 2010).

La variabilidad en la expresión de caracteres morfológicos y estructurales de las unidades que forman la copa puede tener consecuencias profundas en la captura de luz (Howell *et al*. 2002; Falster & Westoby 2003) y a su vez ser fruto de filtros ambientales relacionados con la irradiación, temperatura, precipitación a distintas escalas espacio-temporales. De hecho, la configuración espacial de las hojas en el dosel influencia la función de toda la planta dado que participa en la capacidad fotosintética, la transpiración, el balance energético y la relación con las plantas vecinas (Hikosaka & Hirose 1997; Pearcy *et al*. 2005). La capacidad de ajustar la expresión del síndrome foliar en función de las limitaciones ambientales a escala local (Jurik, Chabot & Chabot 1979; Howell *et al.* 2002; Falster & Westoby 2003; Pearcy *et al.* 2005; Sack *et al*. 2006; Valladares & Niinemets 2008) permite alterar las limitaciones que surgen en la captación y uso de la luz. En su condición de organismos sésiles, la captura de luz en árboles está determinada tanto por patrones de radiación solar a escala local, variables climáticas (nubosidad, transmisividad de la atmósfera), gradientes ambientales en el interior de la copa,

como por la forma, estructura y disposición de los elementos que la componen. La copa de un árbol no está formada únicamente por hojas y tanto la estructura y disposición de ramas como el tipo de crecimiento son determinantes en la captura de luz (Fisher 1986; Farnsworth & Niklas 1995; Niinemets 2010). No obstante, en plantas de porte arbóreo, relativamente aisladas de estructuras o árboles vecinos, la eficiencia de la captura de luz es altamente dependiente del número, forma, tamaño, disposición, distribución y dispersión de las estructuras foliares (Niklas 1989; Ackerly & Bazzaz, 1995; Farnsworth & Niklas 1995; Planchais & Sinoquet 1998; Farque *et al*. 2001; Falster & Westoby 2003; Pugnaire & Valladares 2007). A su vez, las hojas, se ven influenciadas por la propia estructura y forma de la copa y por el gradiente lumínico que generan (Wang & Jarvis 1990; Sinoquet *et al*. 2001). Alterar la respuesta al ambiente lumínico y modificar sus propiedades puede conferir ventajas como aumentar el control de los recursos disponibles (Bellingham & Sparrow 2000) y alterar interacciones bióticas como la competencia por la luz (Hikosaka & Hirose 1997).

Uno de los mayores inconvenientes para abordar el estudio de la VF, a través de múltiples escalas espaciales y temporales en este tipo de caracteres, es la complejidad que supone caracterizar y medir un dosel arbóreo. La naturaleza modular de las plantas y la interacción con el gradiente ambiental por parte de los elementos que componen el dosel convierten la caracterización de esta VF en un reto. Las plantas son organismos que poseen una elevada conectividad y capacidad de respuesta producto de su organización jerárquica, lo que requiere una interpretación holística de la magnitud y estructura de la variabilidad en diversos grupos de caracteres funcionales (tanto de la parte aérea como subterránea) relacionada con los distintos componentes de la eficacia biológica. Finalmente, las relaciones entre caracteres funcionales foliares pueden verse alteradas por asociaciones con otros motores de este tipo de respuesta a otras escalas como por ejemplo las establecidas con los nutrientes del suelo (Wright *et al*. 2001; Niinemets & Kull 2003). Dicha interpretación excede los objetivos de la presente tesis que abordan el significado funcional, la magnitud y la estructura de la variabilidad de un conjunto de caracteres foliares (estructurales y morfológicos) relacionados con la captura de luz y la relación con el ambiente a escala global. La

expresión de la VF en caracteres foliares estructurales y morfológicos en respuesta al ambiente generado por la copa puede tener profundas consecuencias en la eficiencia fotosintética tanto a nivel de hoja como de individuo y, en última instancia, tener un efecto sobre los componentes de la eficacia biológica (Reich *et al*. 2003; Wilson & Nussey 2010). Trabajos en el campo de la genética cuantitativa reconocen la VF y las asociaciones entre grupos de caracteres a nivel de población como factores que pueden resultar clave en la adaptación local y en el potencial evolutivo de las especies (Kirkpatrick & Barton 1997; Gomulkiewicz & Houle 2009; Lavergne *et al*. 2010).

A pesar de la importancia de la partición de la VF a través de las distintas escalas espaciales y temporales y de sus implicaciones en la ecología de poblaciones y en las dinámicas evolutivas de las especies (Bolnick *et al*. 2003; Albert *et al*. 2011), existe una escasa caracterización de la VF y de la relación con las fuentes de variación ambientales en condiciones naturales, especialmente en especies de porte arbóreo. Sin embargo, la búsqueda de asociaciones entre rasgos funcionales, la cuantificación de su impacto y la relación con el ambiente constituyen un campo fructífero en diversas áreas de la ecología, que deberían realizarse en términos absolutos (magnitud) y relativos (estructura) para el conjunto de caracteres que definen el síndrome fenotípico (Ackerly *et al*. 2000, Geber & Griffen 2003; Reich *et al*. 2003; Albert *et al*. 2011).

2. OBJETIVOS.

El principal objetivo de la presente tesis es la caracterización detallada de los rasgos funcionales foliares relacionados con la forma, estructura y la captura potencial de luz a distintas escalas espaciales y temporales en el árbol perennifolio *Olea europaea* L., e investigar las implicaciones a nivel de individuo en cuanto a la intercepción de luz y su relación con gradientes ambientales sobre un extenso gradiente latitudinal a escala planetaria (40º N – 21ºS). Para abordar la magnitud de este objetivo principal se desarrollaron los siguientes objetivos específicos.

- Desarrollar una metodología capaz de capturar un número significativo de los ángulos foliares presentes en la copa de un árbol mediante los que estimar la exposición potencial a nivel de copa en un momento concreto de una localidad geográfica dada. Además, esta metodología debe incorporar una herramienta que supere las principales desventajas de los métodos frecuentemente utilizados en condiciones de campo. El capítulo I de la presente memoria se dedica a desarrollar una metodología capaz de abarcar este objetivo específico.

- Evaluar las implicaciones funcionales en cuanto a la captura de luz durante el período de máxima radiación en las distintas parte de la copa de *Olea europaea* L. en ambientes mediterráneos. Concretamente en el capítulo II se analizan las posibles relaciones de complementariedad espacial y temporal de los caracteres foliares y en los patrones de intercepción de luz a nivel de individuo en distintos momentos del día.

- Caracterizar la magnitud y estructura de la variabilidad de caracteres funcionales foliares relacionados con la captura de luz en *Olea europaea* L. a través de múltiples escalas espaciales a nivel planetario (análisis de la variabilidad entre las distintas poblaciones, dentro las poblaciones, entre individuos y dentro de los individuos). En el capítulo III se aborda esta caracterización en un extenso gradiente latitudinal que abarca ambos hemisferios y se establecen asociaciones con las principales variables

ambientales relacionadas con la estructura y forma de las superficies foliares.

- Estimar la presencia y magnitud de la variabilidad temporal en un conjunto de caracteres foliares relacionados con la captura de luz. Relación con los patrones de radiación diurno de dos situaciones estacionales de contraste (Invierno /Verano) en ambientes mediterráneos. En el capítulo IV se establece una aproximación de la magnitud y estructura de la variabilidad temporal de los caracteres foliares en *Olea europaea* L.

3. MATERIAL Y MÉTODOS.

En cada uno de los capítulos de esta memoria los cuales se presentan en todos los casos en formato de artículo científico, se desarrollan el material y los métodos empleados para la consecución de los objetivos. Sin embargo en este apartado se describe de manera resumida el objeto de estudio, el diseño de muestreo y las principales herramientas estadísticas empleadas.

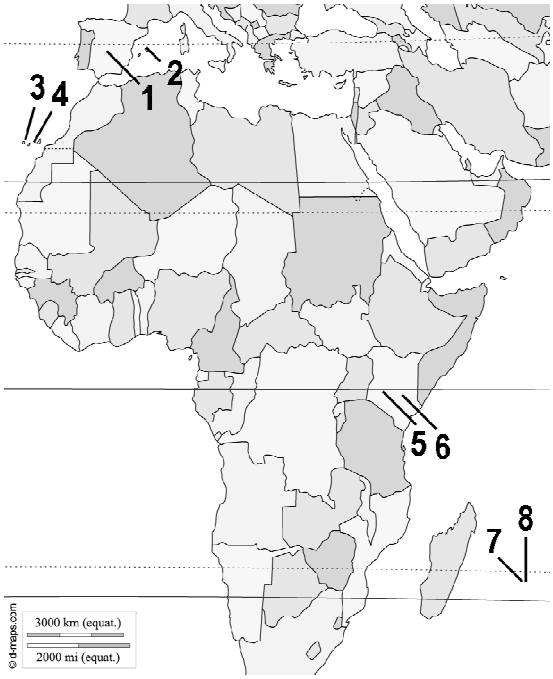

Figura 1. Poblaciones seleccionadas para llevar a cabo los experimentos. El capítulo II y el capítulo IV se limitan a las poblaciones 1 y 2 mientras que el capítulo III incluye todas las poblaciones. *Olea europaea* subsp. *europaea* (poblaciones 1 y 2), *Olea europaea* subsp. *guanchica* (poblaciones 3 y 4), *Olea europaea* subsp *cuspidata* (5, 6, 7 , 8).

Área y modelo de estudio.

Todos los capítulos que conforman esta tesis doctoral se han centrado en el estudio de la variabilidad de los caracteres funcionales de individuos adultos de *Olea europaea* L. La sección *Olea* del género homónimo está formada por el complejo *Olea europaea* con varios taxones distribuidoa desde Sudáfrica hasta China, a través de las montañas del Sahara, el archipiélago macaronésico y la cuenca del Mediterráneo. El taxón *Olea* se encuentra distribuido en un amplio número de hábitats desde ambientes áridos a bosques de nieblas en la región

macaronésica (*Olea europaea* L. subsp. *guanchica* P. Vargas *et al*.). En zonas subtropicales y tropicales se concentran la mayoría de las especies donde pueden formar un componente importante de la vegetación (Green 2002). Las poblaciones estudiadas abarcan un amplio gradiente latitudinal a escala global en las que se incluyen tres de las seis subespecies reconocidas (Vargas *et al*. 2001; Green 2002) (Figura 1; Tabla 2) y en la que se destacan poblaciones situadas en el límite de distribución debido a factores ambientales como el límite de tolerancia térmica en la parte central de la Península Ibérica (Vargas & Kadereit 2001; Rubio de Casas *et al.* 2006) o las impuestas por las bajas precipitaciones en el archipiélago canario (Pansiot & Rebour 1961). Análisis filogeográficos más recientes remiten a los cambios climáticos ocurridos en el Terciario y a el aislamiento geográfico como principales candidatos de la diferenciación en estas subespecies de *Olea europaea* (Besnard *et al.* 2009). A nivel de variabilidad funcional en la copa, se ha demostrado que la expresión de distintos caracteres a lo largo de la misma permite una división espacio-temporal en la captura de luz mediante ajustes complementarios en el rendimiento fotosintético, la inversión de biomasa y la arquitectura de la copa (García-Verdugo *et al.* 2009; Granado-Yela *et al.* 2011). Es más, la complementariedad funcional y estructural en respuesta a gradientes lumínicos entre posiciones de la copa se ha sugerido en este y otros siempre verdes mediterráneos (Werner *et al*. 2001; Rubio de Casas *et al.* 2007; Rubio de Casas *et al*. 2011).

Tabla 2. Código y características de las poblaciones seleccionadas

Pop. ID	Población	Latitud y Longitud		Subespecie	m.a.s.l. (m)	Declinación Magnética (°)
1	Aldea del Fresno (Madrid, España)	40.33 N	4.24 W	*Olea europaea subsp europaea*	500	1.71 W
2	San Luis (Menorca, España)	39.82 N	4.27 E	*Olea europaea subsp europaea*	50	0.59 E
3	Finca Amado (La Palma, España)	28.66 N	17.78 W	*Olea europaea subsp guanchica*	385	6.19 W
4	Anaga (Tenerife, España)	28.56 N	16.15 W	*Olea europaea subsp guanchica*	370	5.58 W
5	Gilgil (Gilgil, Kenia)	0.47 S	36.29 E	*Olea europaea subsp cuspidata*	2050	0.74 E
6	Nairobi (Nairobi, Kenia)	1.29 S	36.65 E	*Olea europaea subsp cuspidata*	1760	0.46 E
7	Tamarins (Isla de La Reunión, Francia)	21.06 S	55.25 E	*Olea europaea subsp cuspidata*	350	19.29 W
8	Palmistes Rouge (Isla de La Reunión, Francia)	21.16 S	55.47 E	*Olea europaea subsp cuspidata*	950	19.44 W

Diseño de muestreo.

En cada localidad se seleccionaron de cuatro a cinco individuos relativamente aislados, con signos de haber producido frutos para asegurar el muestreo de individuos adultos. Los experimentos de la presente tesis se desarrollan en individuos adultos para evitar un efecto ontogénico en la expresión de los caracteres funcionales propio de las plantas heteroblásticas (Allsopp 1967; Moreno-Alias *et al*. 2009). Cada copa se aproximó a un elipsoide de revolución y su hemisferio superior fue subsecuentemente dividido para obtener 12 puntos de muestreo por individuo (Fig. 2). Cada punto se obtuvo de la división del hemisferio superior de la copa en cuatro sectores orientados hacia los puntos cardinales (N, E, S, O) en los que se seleccionaron tres puntos de muestreo en un transecto oblicuo desde la parte externa de la copa hacia el centro geométrico de la misma (capas de la copa: fuera, medio y dentro) (1, 2, 3; Fig. 2). En cada capa y orientación se seleccionaron un número de hojas proporcional a su densidad. Se muestrearon 20, 10 y 5 hojas en las capas de fuera, medio y dentro respectivamente. En cada árbol se estimaron variables foliares para 140 hojas (35 hojas por sector x 4 sectores). Se evitó seleccionar hojas del extremo apical de las ramas para evitar medir hojas que no estuvieran totalmente expandidas. Para acceder a los distintos puntos de muestreo en la copa se emplearon escaleras telescópicas procurando la mínima perturbación de las mismas. Las dimensiones de la planta se estimaron mediante la altura, el diámetro mayor de su copa y el diámetro perpendicular al mismo. En cada sector se midió la distancia a la capa media y a la capa interior desde la capa más externa con una cinta métrica para establecer comparaciones entre puntos de muestreo y estudiar la profundidad de la copa. Las características climáticas de cada población se obtuvieron mediante extrapolación de la base de datos *worldclim* a una resolución de 5'. La precipitación y la temperatura constituyen dos determinantes clásicos de los caracteres funcionales a escala global desde un punto de vista ecológico y evolutivo (Walter 1994; Donoghue 2008). Todos los muestreos se llevaron a cabo durante tres años consecutivos desde 2011 hasta 2013.

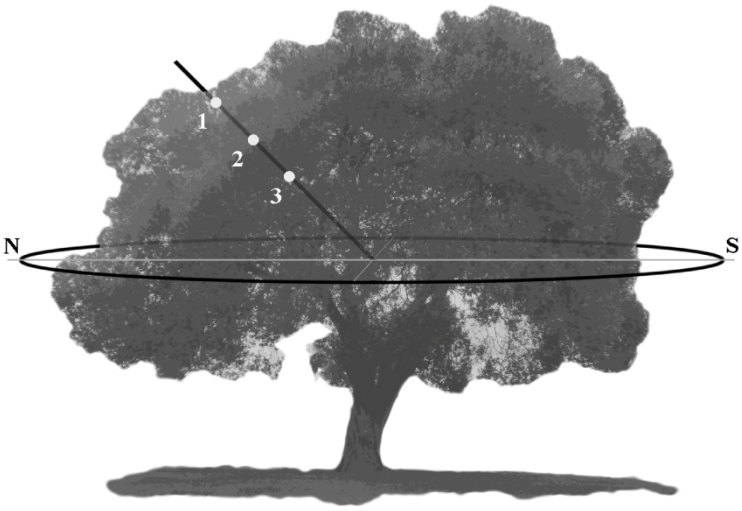

Figura 2. Representación del diseño de muestreo empleado en cada individuo. El transecto con los puntos de muestreo 1: Fuera, 2: Medio y 3: Dentro se estableció en cada uno de los cuatro sectores (Norte, Este, Sur y Oeste) para obtener un total de 12 puntos de muestreo por individuo.

Caracteres funcionales.

Previamente a la recolección de las hojas para estimar caracteres morfológicos y estructurales se midieron *in situ* los ángulos foliares de cada hoja para determinar su orientación y elevación. Los ángulos foliares obtenidos fueron empleados para estimar la superficie absoluta (*SAL*; cm^2) y relativa (*STAR*; %) de cada hoja potencialmente expuesta a la radiación solar directa, en un instante concreto y a lo largo de un día. La metodología empleada para abordar esta tarea se describe en el Capítulo I (Escribano-Rocafort *et al.* 2014). Para medición de los ángulos foliares se utilizaron dispositivos *Smartphone Nokia* (*Nokia N86*, *Nokia*, Spoo, Finlandia). La figura 3 muestra una representación de una de las posibles posiciones del dispositivo para medir los ángulos foliares deseados. De cada hoja se realizaron medidas del área, ancho, largo y peso seco para determinar el área específica de cada hoja (*SLA* de sus siglas en inglés) y la relación entre el ancho y largo de la hoja (*Lindex*). Ambas variables permiten aproximar la estructura y forma de la superficie fotosintética. Las hojas recogidas se escanearon (*HP-ScanJet* 3800; *Hewlett Packard*; Palo alto; CA; EEUU), se dispusieron en una estufa a 65ºC durante al menos 48 h y se pesaron en una balanza de precisión (*Mettler Toledo*,

Greifensee, Suiza). La estimación de la forma de la hoja se realizó mediante *ImageJ* (*NIH*, Maryland, EEUU).

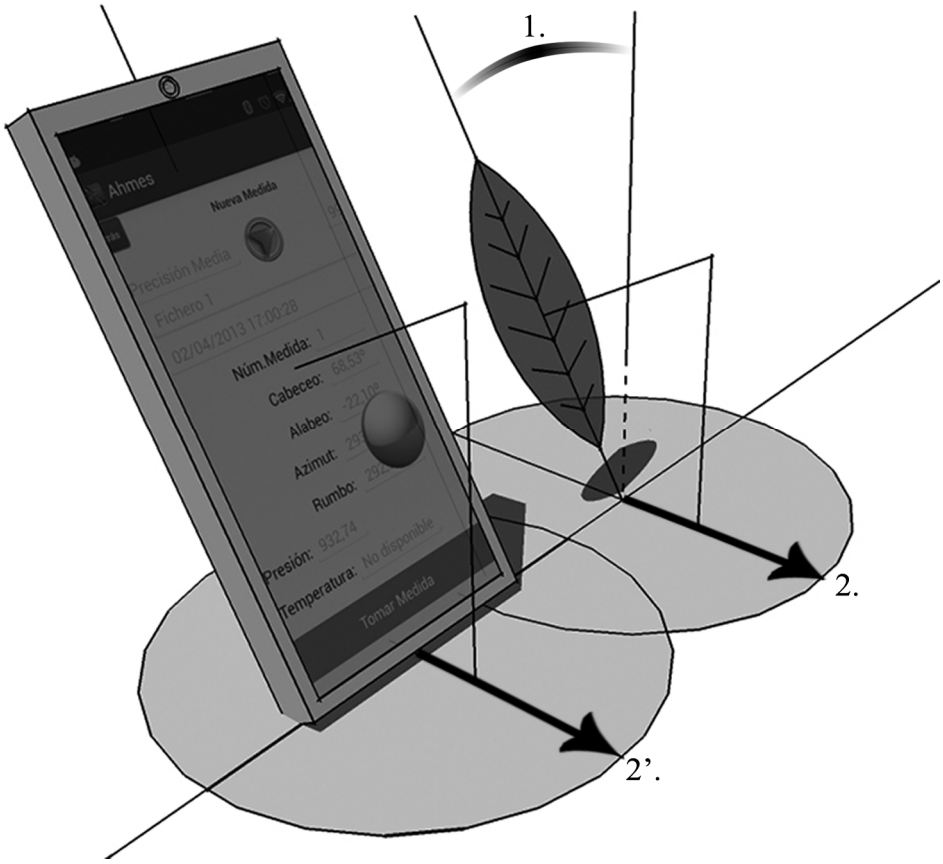

Figura 3. Disposición en paralelo del dispositivo respecto a la superficie foliar de interés para registrar la inclinación de la hoja respecto a la vertical (1) y la orientación respecto al norte de la superficie foliar (2 y 2') que definen la posición de la hoja (Capítulo I).

Análisis estadísticos.

Para abordar las preguntas de los diferentes capítulos se emplearon múltiples técnicas estadísticas. La naturaleza continua de las variables y la complejidad de la jerarquía de los muestreos suscitó el empleo de modelos lineales simples y mixtos (*LMs*, *LMMs*; capítulo II, III y IV; Pinheiro & Bates 2000; Cayuela *et al*. 2011) para identificar la relación entre las variables dependientes y las variables explicativas incluyendo factores aleatorios. Los modelos fueron empleados como base para realizar estimaciones de la partición de la varianza de los caracteres foliares a distintas escalas espaciales y temporales (ver Messier *et al*. 2010, Capítulo III y IV). Se emplearon análisis multivariantes (Capítulo III y IV) para evaluar simultáneamente los rasgos funcionales a nivel individual y poblacional

como *PCA* (Análisis de los componentes principales) y *PCA-between* para identificar la relación de las variables con los ejes principales de variación así como la inercia o variabilidad fruto de las distintas escalas. En la presente tesis los análisis estadísticos y la representación de los resultados se han llevado a cabo mediante diversas librerías del lenguaje de programación *R* (*R Core Team*, 2012) especificadas en cada capítulo.

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5. LISTADO DE MANUSCRITOS.

1. Simplifying data acquisition in plant canopies- Measurements of leaf angles with a cell phone.

   Adrián G. Escribano-Rocafort[1], Agustina B. Ventre-Lespiaucq[1], Carlos Granado-Yela[1], Antonio López-Pintor[2], Juan A. Delgado[2], Vicente Muñoz[3], Gabriel A. Dorado[4] y Luis Balaguer[1]

   Publicado en *Methods in Ecology and Evolution (2014) 5, 132–140.*

2. The modular structure of the tree crown enables splitting of tasks and optimization of light interception in *Olea europaea* L.

   Adrián G. Escribano-Rocafort[2], Agustina B. Ventre-Lespiaucq[2], Carlos Granado-Yela[1], Juan A. Delgado[2], Rafael Rubio de Casas[5] y Luis Balaguer[1]

   Enviado a *Annals of Botany*

3. Intraspecific variation in plant functional traits: magnitude and structucture of the variability for foliar traits at global scales in *Olea europaea* trees.

   Adrián G. Escribano-Rocafort[2], Agustina B. Ventre-Lespiaucq[2], Carlos Granado-Yela[1], Juan A. Delgado[2], Rafael Rubio de Casas[5], Adrián Escudero[6] y Luis Balaguer[1]

   Enviado a *New Phytologist*

4. Variability of light-related foliar traits across spatial and temporal scales in the Mediterranean evergreen *Olea europaea* L.

   Adrián G. Escribano-Rocafort[2], Agustina B. Ventre-Lespiaucq[2], Carlos Granado-Yela[1], Juan A. Delgado[2], Rafael Rubio de Casas[5], Adrián Escudero[6] y Luis Balaguer[1]

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CAPÍTULO I

Simplifying data acquisition in plant canopies- Measurements of leaf angles with a cell phone.

Publicado en la revista Methods in Ecology and Evolution 2014, 5, 132–140

ABSTRACT

1. Canopies are complex multilayered structures comprising individual plant crowns exposing a multifaceted surface area to sunlight. Foliage arrangement and properties are the main mediators of canopy functions. The leaves act as light traps whose exposure to sunlight varies with time of the day, date and latitude in a trade-off between photosynthetic light harvesting and excessive or photoinhibitory light avoidance. To date, ecological research based upon leaf sampling has been limited by the available technology, with which data acquisition becomes labour intensive and time-consuming, given the overwhelming number of leaves involved.

2. In the present study, our goal involved developing a tool capable of measuring a sufficient number of leaves to enable analysis of leaf populations, tree crowns and canopies.We specifically tested whether a cell phone working as a 3D pointer could yield reliable, repeatable and valid leaf angle measurements with a simple gesture. We evaluated the accuracy of this method under controlled conditions, using a 3D digitizer, and we compared performance in the field with the methods commonly used. We presented an equation to estimate the potential proportion of the leaf exposed to direct sunlight (*SAL*) at any given time and compared the results with those obtained by means of a graphicalmethod.

3. We found a strong and highly significant correlation between the graphical methods and the equation presented. The calibration process showed a strong correlation between the results derived from the two methods with a mean relative difference below 10%. The mean relative difference in calculation of instantaneous exposure was below 5%. Our device performed equally well in diverse locations, in which we characterized over 700 leaves in a single day.

4. The new method, involving the use of a cell phone, is much more effective than the traditional methods or digitizers when the goal is to scale up from leaf position to performance of leaf populations, tree crowns or canopies. Our methodology constitutes an affordable and valuable tool within which to frame a wide range of ecological hypotheses and to support canopy modelling approaches.

Key-words: survey measurements, plant strategies, leaf blade silhouette area, sunlight exposure, spatio-temporal characterization

1. INTRODUCTION

Canopies are complex multilayered structures resulting from the coalescence of individual plant crowns within any community, from forests to grasslands and from terrestrial to freshwater and marine environments (Moffett 2000). Canopies expose a multifaceted surface area of phytoelements, such as leaves and stems, which intercept sunlight, precipitation, wind, particulates and aerosols (Monteith 1973; Gutschick 1999; Huang *et al*. 2007; Asner & Martin 2011). Canopy processes (e.g. primary production, evapotranspiration, gas exchange, etc.) and concomitant phenomena such as heat absorption, light reflection, temperature regulation or erosion reduction are among the ecosystem functions supporting some of the most important ecosystem services (see for instance Lowman & Schowalter 2012). Canopy performance integrates multiple contributions and synergies across scales, from community overstorey to plant crown and to individual leaves (Barthélémy & Caraglio 2007). At the crown level, plant performance depends not only on the environmental conditions experienced by the plant but, to a large extent, on the modulation of the plant's environment by tree crown development and architecture (Rubio de Casas *et al*. 2007, 2011). The light environment within crowns is highly heterogeneous at the spatial and temporal scale. Spatially, tree crown structure mediates the exponential decrease in light intensity (Wang & Jarvis 1990; Uemura *et al*. 2006). Temporally, light intensity is determined by the interplay between crown anisotropy, the daily and seasonal motion of the sun and the atmospheric conditions (Granado-Yela *et al*. 2011). Foliage characteristics, arrangement and properties are the main mediators of the biological processes occurring within crowns (Hallé, Oldeman & Tomlinson 1978; Room, Maillette & Hanan 1994; Sterck & Bongers 2001).

Indeed, leaves are functional units that link global climate and ecosystem dynamics, participating in food webs, biogeochemical cycles and constituting an important microhabitat in the biosphere (Wright *et al*. 2004; Pincebourde &

Woods 2012). Leaf size and arrangement ultimately reflect functional strategies evolutionarily shaped to optimize light harvesting (Hansen 1917; Walter 1973; Lowman & Schowalter 2012). Overall light interception by leaves depends on abiotic factors (e.g. wind conditions, atmospheric transmissivity) and on biotic factors, such as leaf anatomy and position within the canopy (Campbell & Norman 1989; Terashima & Hikosaka 1995; Vogelmann, Bornman & Yates 1996; Smith *et al*. 1997; Gu *et al*. 2003). The leaves of some annuals or ephemerals form sparse canopies that track changes in solar elevation and azimuth throughout the day (Ehleringer & Forseth 1980). In denser canopies, however, solar tracking by the upper leaves reduces the light available to the lower ones, in turn reducing net canopy photosynthesis (Denison, Fedders & Harter 2010). Most species, particularly perennials, are static-leaved plants, that is, they maintain leaf orientation through the leaf life span (minimum variation in leaf angles caused by active or passive movements). These leaves act as fixed light traps whose exposure to sunlight varies with time of the day, date and latitude. In these cases, leaf position represents a tradeoff between photosynthetic light harvesting and excessive or photoinhibitory light avoidance, which acquires its full ecological and evolutionary meaning once contextualized within the geometry and dynamics of a plant's crown (Givnish 1988; Smith *et al*. 2004).Unfortunately, to date, ecological research on canopies and crowns based on leaf sampling has been limited by the available technology.

In many studies, data acquisition is labour intensive and time-consuming due to the overwhelming number of leaves present in tree crowns and their limited accessibility (Wang & Jarvis 1990; Parveaud *et al*. 2008). Field measurements of leaf angles have been customarily performed with clinometers, compasses, protractors, angle finders, rulers, plumb lines and callipers (hereafter, traditionalmethods; Comstock & Mahall 1985; Ehleringer & Werk 1986; Fleck *et al*. 2003; Granado-Yela *et al*. 2011) or with three-dimensional motion trackers or digitizing systems (hereafter, digitizers; Pearcy & Yang 1996; Sinoquet & Rivet 1997; Falster & Westoby 2003; Hanan & Wang 2004). Traditionalmethods are portable but require at least three sequential measurements (see below) to characterize a single leaf's spatial position, which increases data acquisition time

and the accumulated error. Additionally, traditional methods are frequently analogue, a fact that reducesmeasurement resolution. These disadvantages have been emphasized in previous studies (Jennings, Brown & Sheil 1999; Jonckheere *et al*. 2004; Seidel *et al*. 2011). Digitizers are highly accurate, precise and effective for characterizing leaves in reference to others (Falster & Westoby 2003). Digitizers, however, are difficult to implement under field conditions for a number of reasons. They require a static point of reference, are expensive and, in practical terms, are not portable because of their size and weight. Moreover, they are usually wired, which limits their use in the field.Wiring tends to impose movement constraints -particularly when working within the canopy- and limits the equipment's reach, which consequently restricts the data acquisition range. It also implies the relocation of the reference point within a single crown, increasing the time required formeasurements and for subsequent conversión of coordinates. Finally, digitizers most often require an external power supply, which increases expenses and can make its use in remote locations unfeasible. For all these reasons, when traditional methods and digitizers are used to describe forest canopies or tree crowns, they fail to characterize a representative number of leaves within a reasonable time, and consequently, any attempt to scale up from the leaf to higher functional and architectural levels will be considerably hindered, or even thwarted, by this severe drawback.

The aim of the present study involved developing a userfriendly, simple, fast, precise, digital, affordable, portable and highly autonomous tool capable of measuring a sufficient number of leaves to enable analysis of leaf populations, tree crowns and canopies. This tool should be sufficiently small, light and manageable to be used single-handed within the canopy. It should also measure all angles describing a leaf's position simultaneously without requiring an external reference point. We specifically tested whether a cell phone equipped with an ad hoc software application is more effective than both, traditional methods and digitizers. We evaluated the accuracy of this method under controlled conditions using a 3D digitizer and compared performance in the field with traditional methods. We presented an equation to estimate the potential proportion of the leaf exposed to direct sunlight at any given time and compared the results with those

obtained by means of a graphical method. Furthermore, we describe our research experience, highlighting the advantages and drawbacks of our method when used in intensive field campaigns at several sites.

2. MATERIALS AND METHODS

DEVICE IMPLEMENTATION

In order to measure the leaf lamina angles, we developed a specific application software to be implemented on a cell phone operating under Symbian OS *(Nokia N86, Nokia* Group, Espoo, Finland). This device incorporates a 3-axis accelerometer and a magnetometer that records its spatial position in relation to magnetic north (m) and to gravitational force (g) as XYZ coordinates (Fig. 1). These electromechanical sensors, however, have been common features in most of the commercialized cell phones for the past decade. Fitted with our software, the device acts as a 3D pointer with top/bottom, left/right and front/back side that enables the measurement of leaf angles with a single gesture (Fig. 1).

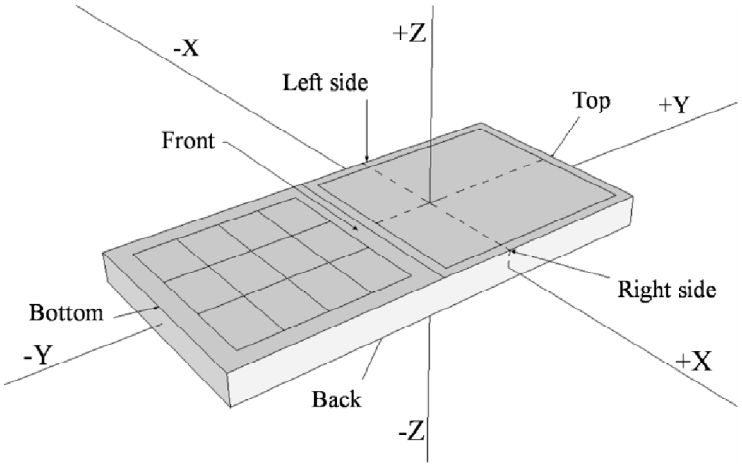

Figure 1. Device's coordinate system and equations which relate the three Euler's angles α: Atan (g_x / g_z), γ: Atan (g_z / g_y) ι: Asin ($|P_Y \times Q| / |P_Y| \cdot |Q|$) and β: Asin ($|P_z \times Q| / |P_z| \cdot |Q|$) where; P_Y and P_z are the axis projection of +Y and +Z axis, respectively, on a normal plane to g {$P_z = (g \times (+Z)) \times g$} and Q is the angle from magnetic north projected on a normal plane to g {$Q = (g \times m) \times g$}.

LEAF LAMINA CHARACTERIZATION

To describe leaf spatial position, we assume that leaves lie on a plane with adaxial/abaxial sides and a longitudinal axis running along the leaf midrib. Thus, a

leaf, as a three-dimensional object, can be parameterized with spherical coordinates (Fig. 2). This system allows us to calculate leaf lamina course angle and leaf inclination angle, which we used to estimate the area of the leaf lamina exposed to the sun in per cent of the total leaf area. Lamina course-angle (β) is the angle between north and the horizontal projection of a normal vector to the leaf lamina. Lamina inclination angle (ρ) is the angle of the maximum slope of the leaf from vertical. These two angles can be determined through a matrix of three vectors which comprises leaf pitch, roll and midrib azimuth-angle (α, γ, ι). Pitch angle (α) is the angle between the vertical and the midrib of the leaf lamina. Roll angle (γ) is the angle of rotation from horizontal along the longitudinal axis of the leaf (Fig. 2) and combined with α, defines the maximum slope of the leaf above horizontal or lamina inclination angle (ρ). Midrib azimuth-angle (ι) is the angle between magnetic north and the projection of the midrib from petiole insertion to the tip of the leaf. Together with α and γ, midrib azimuth-angle defines lamina course-angle (β) (Fig. 2).

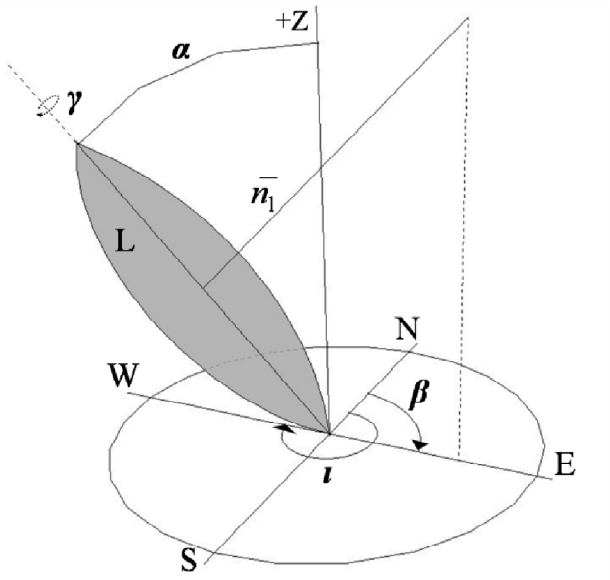

Figure 2. Leaf angles. α) spans from 0 to ±180 degrees where 0° and ±180° refer to a vertical leaf and ±90° to a horizontal leaf. (γ): spans from 0 to ±180 degrees. Negative values account for a right turn from petiole to leaf tip. In the figure, if γ = -90°, the reader should picture a leaf lamina facing North. (ι): Projection of leaf midrib vector from petiole insertion to leaf tip into polar

coordinates; 0° north, clockwise. (β): Projection of $\bar{n_l}$ vector into polar coordinates; 0° north, clockwise. $\bar{n_l}$ normal vector to the lamina surface. (L) Leaf lamina.

Using the above-described angles (α, γ, ι), the device mathematically determines a trihedron in space according to the following form:

$$\begin{pmatrix} \sin(\alpha)\cos(\iota), & \cos(\alpha)\sin(\gamma)\cos(\iota) - \cos(\gamma)\sin(\iota), & -\cos(\alpha)\cos(\gamma)\cos(\iota) - \sin(\gamma)\sin(\iota) \\ \sin(\alpha)\sin(\iota), & \cos(\alpha)\sin(\gamma)\sin(\iota) + \cos(\gamma)\cos(\iota), & -\cos(\alpha)\cos(\gamma)\sin(\iota) + \sin(\gamma)\cos(\iota) \\ \cos(\alpha), & -\sin(\alpha)\sin(\gamma), & \sin(\alpha)\cos(\gamma) \end{pmatrix}$$

Each column in the matrix is a vector; the first column accounts for the leaf midrib, the first and the second columns define the leaf plane and the third column is the normal vector to the leaf lamina surface. Thus, (ρ) and (β) can be calculated (Eqn. 1 and Eqn. 2) from pitch angle (α), roll angle (γ) and midrib azimuth-angle (ι):

$$\rho = asin(sin(\alpha)cos(\gamma)) \quad \text{[Eqn. 1]}$$

$$\beta = \iota + atan(tan(\gamma)/cos(\alpha)) \quad \text{[Eqn. 2]}$$

MEASURING LEAF ANGLES WITH A CELL PHONE

The cell phone must be set in parallel to the leaf, the leaf lamina orientation matching the frontal side (i.e. the screen in the device faces the same orientation as leaf lamina) and the tip of the leaf placed at the upper part of the cell phone (Fig. 3). Thus, the leaf angles match those read by the cell phone and can be recorded by pressing the selection key once. The instantaneous output of the accelerometer and magnetometer varies rapidly during operation of the software due to the high sensitivity of the sensors. The software application averages the last 100 values for the accelerometer and the last 25 values for the magnetometer in order to show a smooth display variation. The reliability of the measurement is visible by means of a green light displayed on the cell phone screen (when the device is held steady, values become stable in about 4 seconds). A characteristic beep sounds once a valid measurement is saved as text for further analysis. Data

can be downloaded via Bluetooth, a flash memory card (microSD), USB or send via email.

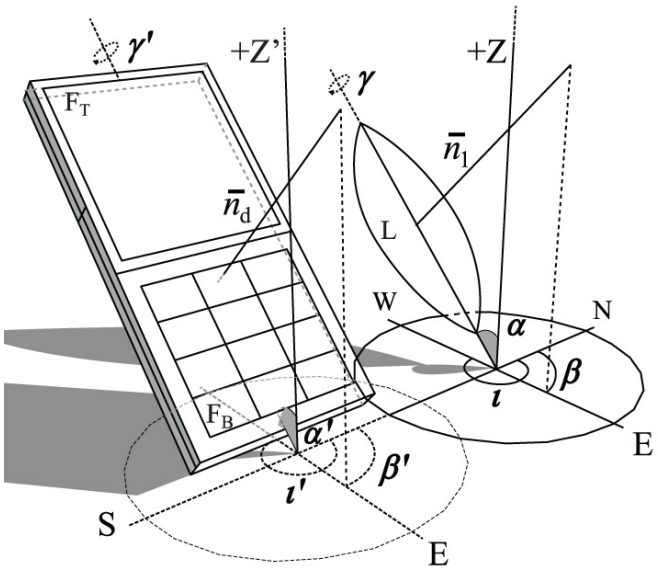

Figure 3. Device set in parallel to a leaf. Device and leaf lamina facing East (β=90°). Angles of interest are the same for the leaf and the device. $\alpha = \alpha'$, $\gamma = \gamma'$, $\iota = \iota'$ and $\beta = \beta'$. $\bar{n}_l$: normal vector to the leaf lamina surface, $\bar{n}_d$: normal vector to the device (parallel to each other), F_t: Top part of device's front side, F_b: Bottom part of device's front side, L: Leaf lamina. Insertion angles between leaf and petiole are parallel in this situation in which angle α is the same in leaf and petiole. Setting the device in parallel to a leaf with different insertion angle between petiole and leaf midrib will record α regarding leaf pitch and not petiole pitch.

LEAF EXPOSURE TO DIRECT SUNLIGHT

We used leaf angles to estimate the instantaneous silhouette area of the leaf blade (*SAL*), that is, the area of one side of the leaf blade that would receive direct sunlight, ignoring possible leaf overlaps (Granado-Yela *et al*. 2011). We calculated *SAL* through the cosine of the angle of the incidence (*cos(θ)*; Comstock & Mahall 1985; Ehleringer & Comstock 1987) of direct sunlight to a tilted surface, following a modified equation from Pearcy and co-workers (1989), which accounts for Earth's orbit and axial tilt (Eqn. 3). The equation involves (β) and (ρ) angles, relating to the spatial position of the leaf, and latitude (Φ), declination (δ) and hour angle (ω) relating to leaf location on Earth and sun relative position. Thus, for a given geographical coordinate (latitude and longitude), the day of the year and a period of time within the day, we can determine *cos(θ)* of direct sunlight to the leaf

lamina, where $SAL = 100\ cos(\theta)$ to obtain the proportion of leaf exposed to direct sunlight. Details of calculations and an example for distinct locations are provided in supplementary material S1.

$$Cos(\theta) = (sin(\delta)*sin(\Phi) + cos(\delta)*cos(\Phi)*cos(\omega))*cos(\rho)-sin(\delta)*cos(\Phi)*cos(\beta) + cos(\delta)*sin(\Phi)*cos(\omega)*cos(\beta) + cos(\delta)*sin(\omega)*sin(\beta))*sin(\rho) \quad \text{[Eqn. 3]}$$

VALIDATION OF SAL EQUATION

In order to evaluate the results obtained from the proposed estimation of leaf exposure (Eqn. 3), we recalculated the instantaneous silhouette area of the leaf blade (SAL_r) from the angles of the leaves included in the study by Granado-Yela *et al*. (2011). These authors measured the lamina angles of 308 leaves of *Olea europaea* L. by means of traditional methods. They calculated the instantaneous silhouette area of the leaf blade (SAL_g) graphically through AutoCAD for 250 leaves. We calculated Pearson's correlation coefficient for both estimates of leaf exposure.

CELL PHONE CALIBRATION

In order to assess the error in the measurement of the leaf angles caused by the inaccuracy of the cell phone sensors, we built a custom-made deck. The deck bears a hinge enabling different positions. The main panel has an inscribed circumference and a stand to hold a digitizer. Specifically, we used a 3D motion tracker (Fastrak, Pholemus, Vermont, USA). The deck was built without any metal parts to avoid electromagnetic interferences (Fig. 4). It was set on a range of elevations from the horizontal to the vertical plane every 5º. At each elevation, we made 36 measurements following the graduated notches in the drawn circumference (one measurement every 10º). Desired angles in the adjustable deck were fixed using the digitizer. Using trigonometric functions, we calculated the expected values for the study angles (α_r, γ_r, ι_r, ρ_r), which we compared with the measured ones (see details in Supplementary S2). Discrepancies between observed and expected values were assessed in terms of mean relative error. Likewise, we estimated the error of *SAL* obtained between observed angles (SAL_O) and expected angle values (SAL_E). Pearson correlations were performed between expected and obtained values.

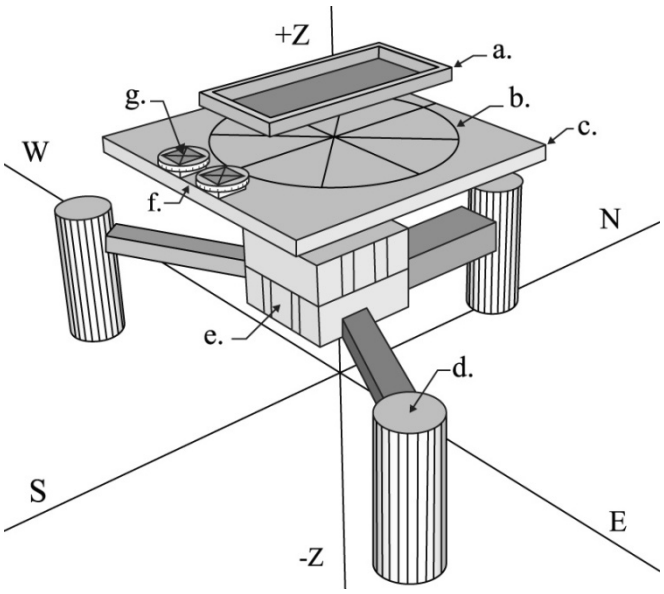

Figure 4. Adjustable deck outline used for calibration. a: cell phone holder (allows turn on Z axis), b: circumference inscribed in panel surface (notched every 10º), c: deck surface, d: supporting legs, e: hinge that allows elevation, f: space for digitizer, g: digitizer holders.

FIELD VALIDATION OF THE DEVICE

In order to evaluate whether our device could provide a tool as reliable as the traditional methods used in previous studies, but much easier to operate, we measured the leaf angles of 100 leaves using: a) a protractor and a compass, and b) our cell phone. Leaves were haphazardly chosen within the crown of ten wild olive trees (*Olea europaea* L.), approximately 1.5m high and located at the Alfonso XIII Royal Botanical Garden in Madrid (40°26′57″N, 3°43′41″W). Each leaf was measured with our device and then with a protractor (α and γ) and a compass (ι and β), as used elsewhere (Rubio de Casas *et al*. 2007; García-Verdugo *et al*. 2010; Granado-Yela *et al.* 2011; Rubio de Casas *et al*. 2011). We analyzed the differences between the measurements taken with the cell phone (α_C, γ_C, ι_C, ρ_C and β_C) and with the traditional methods (α_T, γ_T, ι_T, ρ_T and β_T angles) by means of Pearson correlations. In addition, we assessed the discrepancies in the instantaneous silhouette area of the leaf blade through a Pearson's correlation between those calculated from the angles measured with the cell phone (SAL_C) and those calculated from data obtained with traditional methods (SAL_T). Mean relative error between methods was quantified for each angle as performed for the

calibration process. We adopted the presented method during four field campaigns conducted in Aldea del Fresno (Madrid, Spain), San Luis (Mahón, Spain), Langalanga (Gilgil, Kenya) and Limuru (Nairobi, Kenya) which differed in accessibility and tree size. Effectiveness was described in terms of averaged ratio of measurements per hour, among other important considerations examined in the discussion.

3. RESULTS

VALIDATION OF SAL EQUATION AND CELL PHONE CALIBRATION

The values of instantaneous silhouette area of the leaf blade (*SAL*), calculated graphically by Granado-Yela *et al*. (2011) and by means of equation 3, were strongly correlated (R=0.98, $P < 0.05$; Fig. 5). Mean relative error was below 5% for all angles. Pitch angle (α) showed the biggest differences between measured and expected values with a standard deviation close to 5% (Table 1). Estimations of the response variable between expected values and the angles measured with the cell phone (SAL_E- SAL_O) differed by less than 2% with a standard deviation below 3% (Table 1).

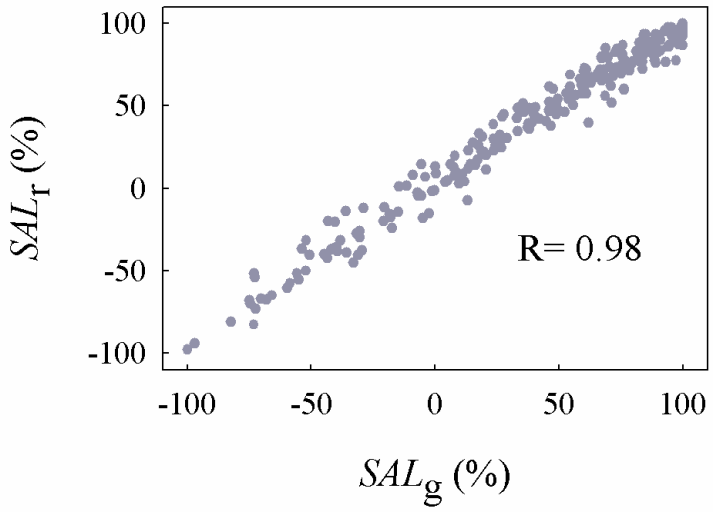

Figure 5. Correlation of recalculated *SAL* with equation 3 (SAL_r) and that obtained by graphical methods (SAL_g) in Granado-Yela *et al.* (2011). n = 250. Negative values indicate underside exposure.

Table 1. Mean relative error and *SD* (%) for each angle and leaf exposure. The error was calculated as the difference between the expected values (digitizer, traditional methods) and the cell phone measurements (observed) for each angle.

	Cell phone Vs. DT		*Cell phone Vs. TM*	
	Error (%)	SD (%)	Error (%)	SD (%)
Pitch (α)	4.1	5	4.2	3
Roll (γ)	1.6	1	5.4	5
MidribA. (ι)	1.5	0.3	3.4	2.8
L. Course (β)	2.2	2.7	3.8	2.5
Max. Slope (ρ)	2	1	3.7	3.1
SAL_E-SAL_O	1.5	2.5	--	--
SAL_T-SAL_C	--	--	5.5	6.8

DT: Digitizer, TM: Traditional Methods. SAL_E: estimations of *SAL* from expected angle values during calibration. SAL_O: estimations of *SAL* from angles measured by the device during calibration. SAL_T: estimations of *SAL* from angles measured by traditional methods during field validation. SAL_C: estimations of *SAL* from angles measured with our device during field validation. Cell phone Vs. DT n= 684 except *L.Course*, n = 648. Cell phone Vs. TM n= 100.

The expected angles (i.e. angles simulated by spatial geometry) and the angles measured with the cell phone were highly correlated in all cases ($R > 0.93$, $P < 0.05$; Fig. 6). Nevertheless, we detected a bias for the measurements of the pitch angle and roll angle due to cross axis sensitivity. Inclination angle (ρ), however, was not apparently affected by this bias (Fig. 6e). The values of β angle showed greater deviations when the adjustable panel was set near the horizontal plane (no specific orientation), but it remained constant at higher elevations (Fig. 6f).

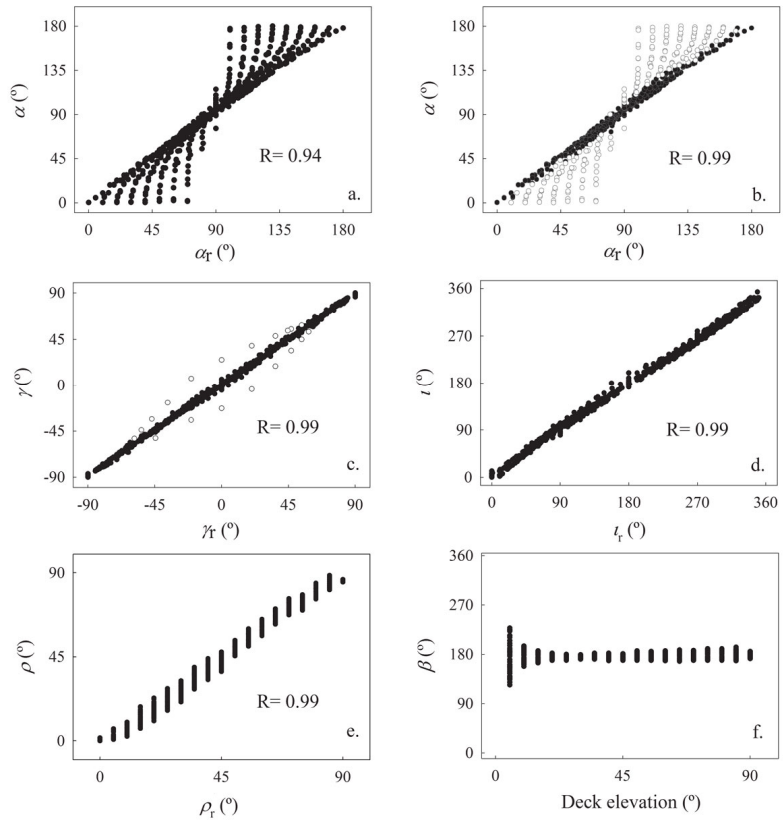

Figure 6. Calibration of the sensors. Correlation coefficients for the accelerometer [pitch angle (α, a-b) and roll angle (γ, c)], magnetometer [midrib azimuth-angle (ι), d], lamina inclination angle (ρ; e), and lamina course-angle (β; f). Unbiased (black) and biased (deviation from ideal correlation; white) values are shown for the pitch (a and b, respectively) and roll angle (c). Deviations from ideal correlation in 'a' and 'c' are minimized when accounting for the maximum slope angle (e). Lamina course-angle (f) was measured every 5º from 5º to vertical (90º). In Fig. 6a-e, n = 684; in Fig. f, n = 648

FIELD VALIDATION OF THE DEVICE

Estimations of the leaf angles measured with the cell phone and by means of traditional methods showed less than 6% of mean relative error for each angle (Table 1). Roll angle experienced the biggest discrepancies and standard deviation between methods. The mean relative error between the *SAL* calculated from angles measured with traditional methods and with our cell phone was 5.5% with a standard deviation below 7% (Table 1). A strong correlation was found for each angle between methods (R > 0.93; Fig. 7). Correlations between measurements were significant for all angles (P < 0.05, n =100; Fig. 7). The estimated *SAL*

between traditional methods and our cell phone showed a strong correlation (R= 0.95, $P < 0.05$, n =100) (Fig. 7f).

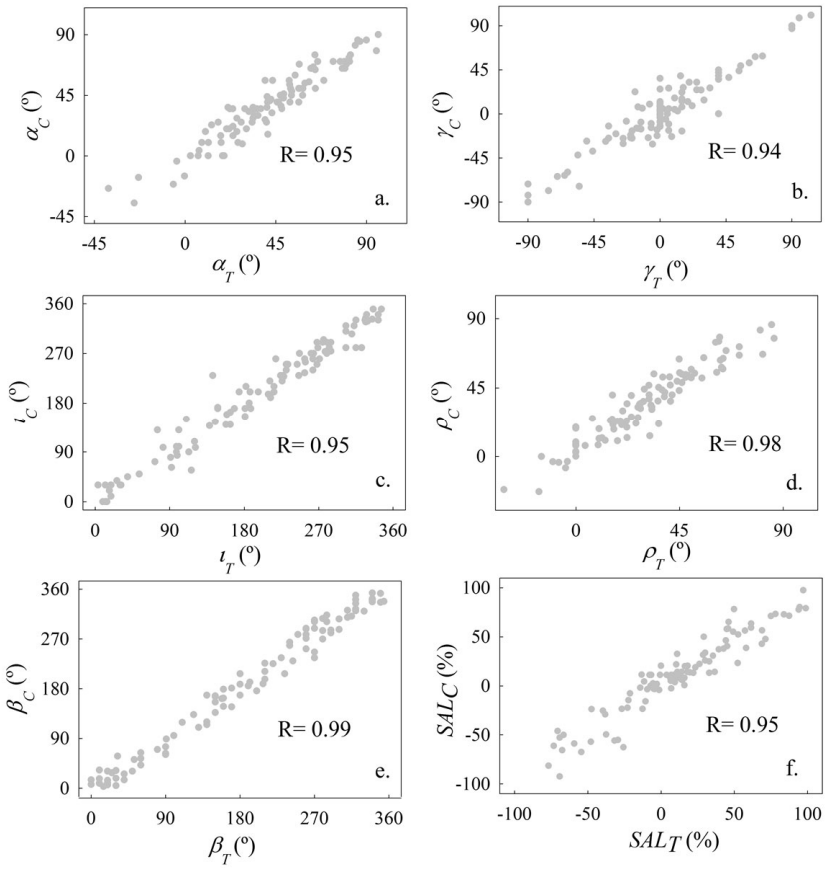

Figure 7. Relationship between traditional methods (α_T, γ_T, ι_T, ρ_T and β_T) and the cell phone (α_C, γ_C, ι_C, ρ_C and β_C) with the coefficient of correlation (R) given for each scatter plot. a: pitch angle, b: roll angle, c: midrib azimuth-angle, d: maximum slope, e: lamina course-angle, f: estimations of *SAL* between methods (SAL_T-SAL_C, negative values account for underside exposure), n = 100.

On average, we were able to obtain 146 ± 24 valid measurements per hour and cell phone during the field campaigns, where over 4000 leaves were measured. In practice, a single device could perform up to 720 measurements in a single day without running out of battery.

4. DISCUSSION

Our tests in the lab and under field conditions demonstrate that the present method provides accurate and reliable measurements of leaf angles and *SAL* estimations. The method described constitutes an advance in direct data acquisition based on a widespread, affordable, easy-to-use, portable and wireless methodology that enables leaves to be spatially monitored by any researcher, educator or student. Our method proved to be satisfactory and highly convenient for field designs involving planar leaves/leaflets (leaves and leaflets that can be broken down into planar elements), thus providing many advantages over methods reported in the literature. Nonetheless, the presented method may still require canopy lifters, scaffolds, or ladders to reach tree crowns and canopy elements.

Calibration and validation

The coefficients of correlation between the measurements taken with the cell phone and the expected values simulated by spatial geometry during the calibration process were strong and statistically significant for all angles and estimated *SAL*. Most values for all angles tested and *SAL* were tightly clustered around the expected ones, denoting great accuracy and precision (Fig. 6; Table 1). We found a strong linear relationship for all angles, although during the calibration process we detected a bias resulting from low cross-axis sensitivity in pitch and roll angles (Fig. 6a-6c). The cross-axis sensitivity is the measure of how much output is seen on one axis when acceleration is imposed on a different axis. It is a product of the 3-axis accelerometer architecture and is a key factor to be implemented and tested by manufacturers (Amarasinghe *et al*. 2006; Kal *et al*. 2006; Sankar *et al*. 2009). The sensor is most sensitive to changes in tilt when the axis involved is perpendicular to the acceleration and is least sensitive when it is parallel. Despite this fact, the effect was negligible in relation to our goals due to the combination of pitch and roll angles in the maximum slope-angle (ρ: Eqn. 1), which minimizes the aforementioned cross-axis sensitivity. Estimations of maximum slope-angle were strongly correlated to all tested methods, with small differences with regard to known values (Fig. 6e & 7d; Table 1). Measurements of lamina course-angle and midrib azimuth-angle were indeterminate in certain

positions (e.g. when pointing at zenith), but the trigonometric relationship between them enables these angles to be calculated with low error as occurs with maximum slope-angle. All these findings supported the reliability of the measurements taken by the cell phone, with low mean relative error in the estimation of the desired leaf angles.

The coefficients of correlation between the field measurements taken with the cell phone and traditional methods, and *SAL* estimated from them, were strong and statistically significant (Fig. 7). The differences between methods of angle measurement remained low in the validation process and for *SAL* estimations (Table 1). Likewise, the coefficients of correlation of *SAL* calculated between graphical methods and the equation presented were strong (Eqn. 3; Fig. 5). These findings support our method as a reliable tool for assessing the spatial position of leaves and for calculating potential *SAL* over time under field conditions.

Functional considerations: Opportunities and limitations

The method presented for estimating *SAL* does not account for leaf overlapping within canopy layers. Despite this limitation, we found it highly relevant to measure leaf angles and to estimate *SAL* in the whole canopy regardless of whether the leaves could be directly or indirectly exposed to wind, particulates, irradiation, or other effects. At the individual level, recent reports point towards spatial and temporal specialization through photosynthetic harvesting of complementary light resources (direct and diffuse radiation) and/or segregated time windows in woody plants, which can be explored with our method (Rubio de Casas *et al*. 2007; Granado-Yela *et al*. 2011). Indeed, optimization of leaf photosynthetic efficiency through modulation of leaf inclination angle, lamina orientation and lamina exposure will certainly help to scale photosynthesis from leaves to individual crowns and canopies, as suggested by Posada and co-workers (2009). The spatial position of leaves and their potential exposure to direct sunlight are relevant to many functional processes operating at the individual level (Givnish 1987; Smith *et al*. 1997; Falster & Westoby 2003; Pearcy *et al*. 2005; Granado-Yela *et al*. 2011). The mean relative error in *SAL* calculations were always below 10%, reaching maximum absolute values at full leaf exposure to the

sun. As *SAL* decreases, sunlight absorption is not expected to decrease in proportion to the reduction in the projected leaf surface, but rather at higher rates due to the expected increase in light reflection and the smearing of the incident photon flux over a larger leaf area.

Field experience

The field performance of our device during the research campaigns was remarkable. Its portability was crucial to our research, enabling tree crowns to be sampled and logistic requirements to be fulfilled in remote locations. We wish to stress the ease of travelling, particularly on regular commercial flights, with such an affordable and commonplace device. In the field the presented device was effortless to carry and to operate several meters above the ground. We found highly advantageous to take measurements with a single hand under these circumstances.

Use recommendations

In order to improve field estimations, we recommend avoidance of wind and magnetic interferences (e.g., metallic structures adjacent to the individuals selected, grounded conductive structures, power lines, etc) during measurements. Magnetic declination should be considered to correct for true north. For use in humid environments, we recommend that the device be placed in a re-sealable, transparent, plastic-bag which can protect the electronic components without interfering with measurements**.** There is a need to minimise interactions between crown and canopy elements and structural supplementary tools (e.g., ladders, lift platforms, etc) or between the above mentioned elements and the researchers themselves. We also highly recommend survey measurements during the day for leaves presenting sun-tracking behaviour. It should be highlighted that the present methodology can involve several devices working together. Efficient field campaigns can therefore be performed by a small work force in relation to time of sampling and coverage of measurements. Finally, the application included in the presented methodology was kept simple regarding software development in order to facilitate portability for the more common mobile operating systems; such Android, iOS, etc. (The Symbian version is available upon request and a free

Android version can be downloaded from Google Play website, *Ahmes*). Ultimately, users should estimate the state of preservation of the sensors and their resolution in order to successfully achieve specific aims. We strongly recommend determining whether the device's resolution is suitable for the desired design. This can be achieved by following a calibration process similar to that described herein.

5. CONCLUSIONS

We successfully avoided common constraints in canopy characterization by combining sensitivity, portability and speed of measurements in an affordable and relatively commonplace device. The tests performed support our method as a remarkable tool excelling in field campaigns. Our results demonstrate that the equation implemented in our methodology constitutes a firm estimation of potential instantaneous leaf exposure. The method presented involves direct field measurements providing valuable data in a wide range of ecological scopes (e.g., geometrical approaches, plant modelling, etc) and functional hypotheses.

We believe that our method is highly relevant in a wide range of scientific approaches. We briefly outline the potential applications in which our methodology could provide insight due to its versatility, even when some of its features are not necessarily involved, such as pollutant deposition/evaporation on planar surfaces, leaf microclimate (proxy for leaf-dwelling organisms), radial location through triangulation (namely, objects of interest within crowns: nests, epiphytes, plagues,..), slope characterization, termite mound irradiation patterns, spider-web spatial arrangement, and many others. To conclude, we would like to emphasize that the methodology presented can play an important role in ecophysiological and educational projects due its affordability to many institutions, researchers and students worldwide.

ACKNOWLEDGMENTS

We wish to thank MD Jiménez, A Vázquez, J Serrano, K Carrillo for their valuable assistance during the experimental design. Thanks to F Martínez and to Wasabi App Factory for their expertise and help with the programming. We are also grateful to the staff of the UCM *Taller Mecánico de CC. Físicas* (Physical Sciences Mechanical Workshop) for assisting in the calibration process. We also gratefully acknowledge two anonymous referees for their constructive comments. Special thanks to Mr Cormac de Brun for revision of the English. The presented methodology is a Spanish Patent Pending P201200481. This research was funded by the Spanish Ministry of Science and Education (project CGL2009-10392), through a FPI grant to A.G.E. (BES-2010-032767). We are also indebted to the Madrid Regional Govt. (project REMEDINAL-2, S2009/AMB-1783).

List of Abbreviations:

Magnetic north (m)

Gravitational force (g)

Latitude (ϕ)

Declination (δ)

Hour angle (ω)

Pitch angle (α)

Roll angle (γ)

Midrib azimuth-angle (ι)

Lamina course-angle (β)

Inclination angle (ρ)

Angle of incidence (θ)

Pitch angle calculated in calibration (α_r)

Roll angle calculated in calibration (γ_r)

Midrib azimuth-angle calculated in calibration (ι_r)

Lamina course-angle calculated in calibration (β_r)

Inclination angle calculated in calibration (ρ_r)

Silhouette area of the leaf blade 'Observed during calibration' (SAL_O)

Silhouette area of the leaf blade 'Expected during calibration' (SAL_E)

Pitch angle measured by means of traditional methods during validation (α_T)

Roll angle measured by means of traditional methods during validation (γ_T)

Midrib azimuth-angle measured means of traditional methods during validation (ι_T)

Lamina course-angle measured by traditional methods during validation (β_T)

Inclination angle measured by means of traditional methods during validation (ρ_T)

Pitch angle measured with the cell phone during validation (α_C)

Roll angle measured with the cell phone during validation (γ_C)

Midrib azimuth-angle measured with the cell phone during validation (ι_C)

Lamina course-angle measured with the cell phone during validation (β_C)

Inclination angle measured with the cell phone during validation (ρ_C)

Silhouette area of the leaf blade 'Traditional methods field validation' (SAL_T)

Silhouette area of the leaf blade 'Cell phone field validation' (SAL_C)

Silhouette area of the leaf blade 'graphical methods' (SAL_g)

Silhouette area of the leaf blade 'recalculated' (SAL_r)

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7. SUPPLEMENTARY MATERIAL-APPENDIX

S.1 Details of SAL

In order to estimate potential leaf lamina exposure we considered two coordinate vectors (Eqn. S1 & Eqn. S2). The first one accounts for Sun's beam vector and the second accounts for the normal vector to the leaf lamina. Both are unitary vectors whose length is the unity. In Euclidean space, the scalar product of two unit vectors is the product of their magnitudes and the cosine of the angle between them (Riley *et al.*, 2010).

$(\sin(\delta)*\sin(\phi)+\cos(\delta)*\cos(\phi)*\cos(\omega), -\sin(\delta)*\cos(\phi)+\cos(\delta)*\sin(\phi)*\cos(\omega), \cos(\delta)*\sin(\omega))$ [Eqn. S1]

$(\cos(\rho), \sin(\rho)*\cos(\beta), \sin(\rho)*\sin(\beta))$ [Eqn. S2]

The ratio of the surface exposed to the Sun can be calculated through the angle of incidence from the inner product of the aforementioned vectors. Results range from -1 to 1, where negative results indicate underside exposure and positive values represent leaf lamina exposure. Maximum exposure occurs when sunbeams are perpendicular to the surface of interest (angle of incidence is 1, 100% of *SAL*). The cosine of the angle of incidence is given by the formula in Eqn. 3, by which *SAL* values are calculated in the present study.

δ: Declination. Calculated following Spencer (1971). Equation S3

$((0.006918-0.399912*\cos(\eta)+0.070257*\sin(\eta)-0.006758*\cos(2\eta)+0.000907*\sin(2\eta)-$
$0.002697*\cos(3\eta)+0.00148*\sin(3\eta))*180/\pi$ [Eqn. S3]

$\eta= (N-1)*2\pi/365$ [Eqn. S3.1]

N: Number of days since midnight. Coordinated Universal Time as January 1st begins.

ϕ: Latitude. It spans from 0° (Equator) to ± 90 (Poles). North positive values and South negative values.

ω: Hour angle. One of the coordinates used in the equatorial coordinate system to assess the position of a point on the celestial sphere (Eqn. S4).

ω = (Solar Time -12)/15 [Eqn. S4]

ρ: Angle of elevation above horizontal. The effective angle lies within a 180° range. It spans from 0° (Horizontal, leaf lamina facing upwards) to 180° (Horizontal, leaf lamina facing downwards). It requires quadrant conversion from measured angles.

β: Lamina course refers to leaf lamina orientation in the earth's magnetic field. It requires quadrant conversion from measured values. It covers 360°, offset clockwise from south 0°.

All angles are introduced in radians. We present calculations of *SAL* (Eqn. 3) for fixed leaf angles (ρ, β) on representative days of the year at two different latitudes to highlight *SAL* dependence on latitude, day of the year and described angles (Fig. S1).

S.2 Calibration details

The panel surface acts as a leaf in which two new angles are defined. Panel elevation (μ) is defined as the elevation of the panel surface above horizontal μ[0°,90°] . Rotation (ν) is defined by a graduated circumference drawn on the panel surface every 10°, ν[0°,360°] (Fig. 4). The adjustable panel was immobilized with the panel surface horizontal (μ= 0º) and with ν= 0º facing North. Desired μ and ν were achieved using the described 3D motion tracker. Panel angles (namely, those corresponding to leaf angles) were measured every 0° to 360° at 10° intervals with the help of the inscribed circumference. Once 36 measurements were made, we proceeded to tilt the surface 5° in μ according to the digitizer and measured again every 10° in the circumference. We repeated these measurements until μ covered horizontal to vertical position [0°, 90°]. The digitizer was turned off after each tilt in μ to avoid interferences. The expected values of α_r, γ_r, ι_r and β_r are generated by the aforementioned μ and ν angles. If the device is set ν= 0°, the magnetic orientation of the +Y axis in the device (Fig. 1), defined as midrib azimuth-angle (ι), is equal to ι_0. The trihedron for ι_0= 0 results as follows;

$$\begin{pmatrix} cos(\mu)cos(\nu) & -cos(\mu)sin(\nu) & -sin(\mu) \\ sin(\nu) & cos(\nu) & 0 \\ sin(\mu)cos(\nu) & -sin(\mu)sin(\nu) & cos(\mu) \end{pmatrix}$$

With both trihedrons α and γ can be deducted (Eqn. S5 and Eqn. S6) from the described μ and ν angles.

$$\cos(\alpha) = \sin(\mu)\cos(\nu) \quad \text{[Eqn. S5]}$$

$$\sin(\gamma) = \sin(\mu)\sin(\nu)/\sin(\alpha) \quad \text{[Eqn. S6]}$$

This enabled us to calculate the expected values for each angle at different μ and ν values (Eqn. S7 to S11);

$$\boldsymbol{\alpha_r} = \text{acos}(\sin(\mu)\cos(\nu)) \quad \text{[Eqn. S7]}$$

$$\boldsymbol{\gamma_r} = \text{asin}(\sin(\mu)\sin(\nu)/\sin(\alpha_r)) \quad \text{[Eqn. S8]}$$

$$\boldsymbol{\rho_r} = 90° - \mu \quad \text{[Eqn. S9]}$$

$$\boldsymbol{\iota_r} = \beta_r - \text{atan}(\tan(\gamma_r)/\cos(\alpha_r)) \quad \text{[Eqn. S10]}$$

$$\boldsymbol{\beta_r} = \iota_0 + 180° \quad \text{[Eqn. S11]}$$

The angle β_r showed no variance during measurements due to the fixed orientation of the panel. To quantify measurement discrepancies between measured angles (e_i) and expected angles (e_i‘), we calculated the error in degrees (e_d, Eqn. S12).

$$e_d = \frac{1}{N}\sum_{i=1}^{N} d_i, \text{ in } [0, M/2] \quad \text{[Eqn. S12]}$$

where;

$$d_i = \min\{ Res(e_i - e_i', M), Res(e_i' - e_i, M)\} \quad \text{[Eqn. S12.1]}$$

and

$$\text{Res}(x, M) = x - [x/M]M \in [0, M) \quad \text{[Eqn. S12.2]}$$

Equation S5.2 is the residual function, the quotient’s remainder. All angles are comprised in the interval [0, M] with M values of 90°, 180°, 270° and 360°. To

solve β for α= 90° γ= 0° (lamina has no preferred orientation) we proposed an alternative β calculus regarding α, γ and ι (Eqn. S13). Likewise for α= 0°, ι uncertainty was assessed in terms of α, γ and β depending on γ= 0° (Eqn. S14.1) or γ= 90° (Eqn. S14.2).

$$\beta = \iota + \text{atan}(\tan(\gamma) / \cos(\alpha)) \quad [13]$$

$$\iota = \beta - \text{atan}(\tan(\gamma) / \cos(\alpha)) \quad [14.1]$$

$$\iota = \beta - \text{acot}(\cot(\gamma) * \cos(\alpha)) \quad [14.2]$$

S.3 Further development

We believe that a free and open source mobile operating system such as Android will make our methodology available to a greater number of Smartphone users and will contribute to further implementations. Indeed, we are currently developing the application under the aforementioned operating system, which will make the methodology freely available to any potential user (forthcoming). Furthermore, future users may experience improved cross-axis sensitivity (for devices featuring a gyroscope) and will be capable of checking the state of the sensor state on request. Despite these benefits, users should check the state of preservation of the desired device and follow a calibration process before applying the methodology in order to achieve successful measurements.

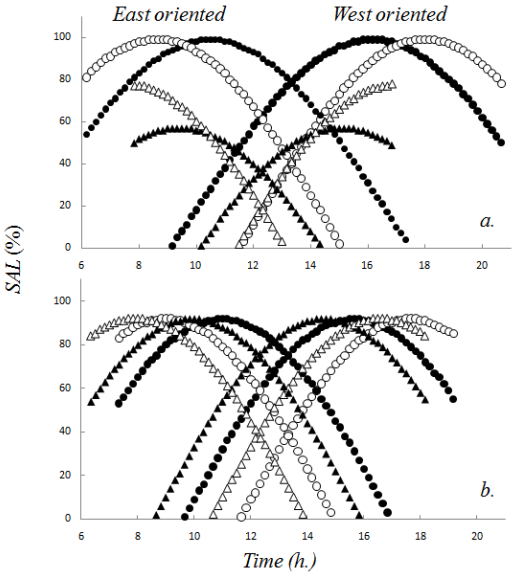

Figure S1. Daily integrated values of *SAL*. Leaves facing East (early in the day reach maximum SAL values) and West (greatest SAL values are achieved late during the day) are depicted to show

segregated time windows in relation to leaf orientation. Two inclination angles are shown; 35º (black) and 65º (white). *SAL* was calculated for two days during the year corresponding to June 21st (circles) and December 22nd (triangles) to capture seasonality. Two latitudinal locations were selected, a. 40º and b. 0º, to capture seasonal and daily interactions.

CAPÍTULO II

The modular structure of the tree crown enables splitting of tasks and optimization of light interception in *Olea europaea* L.

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ABSTRACT

- The spatial arrangement and the expression of foliar syndromes within tree crowns can reflect the coupling between crown form and function in a given environment. Specialization of the photosynthetic surfaces among crown subunits could lead to the optimization of the light interception strategy at the crown scale in high irradiance environments.

- We quantified eight morpho-functional foliar traits across twelve crown positions of isolated evergreen sclerophyllous trees in the field. Specifically, we investigated whether light interception patterns and the phenotypic expression of foliar traits differ among crown positions.

- We found a strong complementarity among crown positions in the daily patterns of potential light interception related to a fine-tuned expression of foliar phenotypes. Light interception at the outer layer was below 60% of the displayed surface with maximum interception during morning periods. Daily interception increased towards the inner layer and showed a consecutive maximization from east to west positions within the crown matching the sun path trajectory. Light interception was highly variable at the outer layer during the day while the middle and inner layer showed a lower reduction.

- Our results reveal a strong spatiotemporal specialization across the tree crown. We suggest that trees may intercept light efficiently by a complementary display of leaf angles among crown positions and a differential carbon investment to minimize the deleterious effects of high irradiance.

Keywords: crown architecture, leaf angles, light interception, splitting of tasks, structural photoprotection.

1. INTRODUCTION.

Trees are able to adjust the expression of foliar traits in response to the environment and within-crown gradients. Environmental heterogeneity across tree crowns could promote the expression of phenotypic differences in foliar traits to meet limitations of the experienced environment and the prevalence of a set of coordinated traits (individual´s phenotypic syndrome) that affect the fitness of the whole plant (see Jurik *et al.*, 1979; Howell *et al.*, 2002; Falster & Westoby, 2003; Pearcy *et al.*, 2005; Marks & Lechowicz, 2006; Valladares & Niinemets, 2008; Wilson & Nussey, 2010). The variability in leaf traits within canopies is functionally relevant. The expression of plasticity in architectural traits such as leaf angle or size can have profound consequences for daily light interception (Howell *et al.*, 2002; Falster & Westoby, 2003). Indeed, plasticity in the expression of leaf inclination angles alters light properties and transmission within the tree crown enhancing whole individual carbon gain (Uemura *et al*. 2006). Modifying light transmission and light properties can confer trees advantages such as higher degree of control on photosynthesis, transpiration, energy balance (Pearcy & Yang, 1996) and further alter biotic interactions such as competition for light (Hikosaka & Hirose 1997). Considering the modular nature of plants, the phenotypic response of the whole tree could be enhanced by the integrated expression of leaf phenotypes among functional modules of the tree crown (de Kroon *et al*., 2005; Granado-Yela *et al*., 2011). The most and least exposed leaves within the tree crown (outer and inner layer hereafter) constitute a classic example of functional subunits that are spatially segregated and specialized in the harvesting of a heterogeneous resource, e.g. the within-crown light gradient (Sack *et al.*, 2006) but the realized phenotypic diversity will necessarily be constrained by other abiotic and biotic factors. As a consequence, the expression of leaf attributes related to light interception may be adjusted to the patterns of irradiance and concomitant stress of a local environment.

In long-lived trees a differential phenotypic expression of leaf traits across crown positions can facilitate the control of resource uptake. Intrinsically, tree growth generates local differences in the light environment experienced by each portion

of the crown. In isolated trees the efficiency of light interception is strongly dependent on the environmental conditions and on leaf number, size, shape, leaf angles, leaf angle distribution and leaf dispersion, which in turn are affected by crown shape and structure (Niklas, 1989; Ackerly & Bazzaz, 1995; Farnsworth & Niklas, 1995; Planchais & Sinoquet, 1998; Farque *et al*., 2001; Falster & Westoby, 2003). The spatial position of the photosynthetic surfaces and their location within the crown greatly affect light interception at the whole crown scale. Among other factors, the potential light capture achieved by a flat leaf (or any flat surface with defined tilt and orientation angle) during a certain day and at given latitude is determined by the angle of incidence of the sunbeams to the leaf blade. The mismatch between the sunbeam and the normal vector to the plane of the leaf, impose a reduction in the potential light capture due to diurnal changes in the azimuth and altitude of the sun (Granado-Yela *et al*., 2011). However, the estimation of the potential reduction in the surface intercepting light is trivial once the spatial position of the leaf is defined for a given time and latitude. In high irradiance environments with concomitant stress such as high temperatures and drought, an increase in factors that provide self-shading constitute crucial attributes of plant layout (Howell *et al*., 2002; Pearcy *et al.* 2005). Specifically for those species that are not able to modify drastically the leaf's spatial position once the lamina is fully expanded, leaf shape, structure and leaf angles are determinant in light interception (Falster & Westoby, 2003).

Minimizing photoinhibition and the negative impact on photosynthesis with steep angles appears to compensate for potentially low daily carbon gains in high irradiance environments (Falster & Westoby, 2003). At the crown scale, optimization of the light interception efficiency can lead to spatial specialization and temporal complementarity related to the environment experienced by each portion of the crown. The leaf length-to-width ratio has a positive effect on light interception while vertical leaf angles can reduce high heat loads during midday, minimize photoinhibition and enable light penetration to inner layers (Ehleringer & Werk, 1986; Valladares & Pugnaire, 1999; Werner *et al.*, 2001; Werner *et al.*, 2002; Pearcy *et al.* 2005; Sarlikioti *et al.,* 2011). The expression of distinct leaf phenotypes is elicited to enable a spatiotemporal specialization in the light

interception efficiency across the tree crown by means of complementary adjustments in the photosynthetic performance, biomass investment and crown architecture (Granado-Yela *et al.*, 2011). Indeed, structural and functional complementarity in response to light gradients among crown positions has been suggested in Mediterranean evergreens such as *Quercus coccifera* L. (Werner *et al.*, 2001; Rubio de Casas *et al.*, 2007) and *Olea europaea* L. (Granado-Yela *et al.*, 2011; Rubio de Casas *et al.*, 2011).

Despite the expected within-crown heterogeneity, field designs under natural conditions aimed at characterizing leaf traits and daily patterns of light interception efficiency are often restricted to saplings or to extreme positions of the light gradient (Valladares *et al.* 2005; Gratani *et al.*, 2006; Sack *et al.*, 2006; Rubio de Casas *et al.*, 2007; Rubio de Casas *et al.*, 2011). However, the expression and effects of phenotypic plasticity are expected to occur in reproductive adults under natural conditions (Cavender-Bares *et al.*, 2004; Bouvet *et al.*, 2005). Therefore, our first aim was to provide a detailed characterization of the variation in foliar traits across crown positions in adult trees in the field. We believe that it is advisable to identify the degree of specialization of leaves in a spatio-temporal context to explore light optimization strategies at the crown scale and to assess the functional relationship among crown positions, Moreover, estimates of light interception at the crown scale are required in individual-based models of vegetation dynamics (e.g. Moorcroft *et al.*, 2001; Falster *et al.*, 2011). We hypothesized that foliar specialization would lead to divergent phenotypes and light interception patterns across crown positions enabling optimization of light interception at the crown scale. To test this hypothesis, we conducted systematic field measurements of a set of functional foliar traits that are related to light interception. Namely, we characterized leaf angles, area, dry weight and shape (ratio of leaf length to leaf width) at different depths within the crown of 10 *Olea europaea* L. trees growing in two populations with contrasting climates but similar radiation levels. We investigated whether morpho-functional leaf traits are complementary among crown positions and discuss to what extent phenotypic differences among leaves within the canopy can lead to splitting of tasks among crown positions in terms of light interception during the day. To accomplish this

task we estimated the absolute and relative leaf surface intercepting light at any given time at five evenly distributed periods during the day. Therefore, variability in the displayed leaf surfaces can be used to describe the potential light interception across the tree crown. Moreover, combined with the relative investment in area over dry mass can provide insight to identify complementary strategies intended to optimize light capture from the leaf to the whole crown level (Granado-Yela *et al*., 2011). Therefore, the measured variables may contextualize the shape, structure, relative position and distribution of the photosynthetic surfaces within the crown, which in turn, may be involved in the regulation of light interception, photosynthesis and photoprotection at the whole crown level (Werner *et al*., 2001; Werner *et al*., 2002; Granado-Yela *et al*., 2011).

2. MATERIALS AND METHODS.

Species and study site.

We studied reproductive adults of *Olea europaea* L. ssp. *europaea* (wild olive tree), an evergreen, sclerophyllous and long-lived tree distributed throughout the Mediterranean Basin. This species develops flat leaves that do not track sun movements and shows signs of foliar specialization between the extremes of the light gradient within the tree crown (Granado-Yela *et al.*, 2011; Rubio de Casas *et al*., 2011). The present study was conducted in two natural populations located on a Mediterranean island (*SL*; Menorca, Spain) and in the central Iberian Peninsula (*AF*, Madrid, Spain). The two populations occur at equivalent latitudes, thus experiencing similar day length and sun elevation angles (Table 1). In each population, we sampled five individuals that had previously borne fruits. Sampling was conducted on isolated trees during one mid-summer day (July-August, 2011) to ensure the complete development of leaves in both populations as recommended for woody species under most of the Mediterranean climate in the northern hemisphere (Garnier et al. 2001). However, the *AF* population was located on a sloped terrain (~30º, south aspect) and the surrounding terrain decreased direct sun irradiance during the final hours of daylight. Photosynthetically active radiation (PAR) was recorded in each population throughout the day (summer 2011) to quantify irradiance levels. We used a

sunshine sensor (BF5- Sunshine sensor, Delta-T Devices, Cambridge, UK) coupled with a data logger (HOBO U30, Onset Computer, MA, USA). To ensure total exposure to light and to obtain representative values, the sensor was placed 2.55 m above ground on sampling days.

Sampling design.

Each individual canopy was assimilated to an ellipsoid defined by the maximum diameter of the canopy and its longest perpendicular diameter. Sampling was conducted along the North-South and East-West diameters of the upper half of the ellipsoid, excluding the lower parts of the crown to avoid both self-shading and the effect of cattle grazing (i.e., only the upper semi-ellipsoid was considered). Each sampling axis (*sector* hereafter) was labelled according to its cardinal direction: N, E, S and W and subdivided in three *layers*: the outermost layer of leaves (*Outer*), the middle layer of leaves (*Middle*) and the deepest layer where leaves were present (*Inner*) (Fig. 1). Sampling points for the middle and inner layers were set in an oblique direction from the outer part of the crown towards its geometrical centre. We used a ladder to reach sampling points avoiding crown disturbance. The distance between sampling points along each sector (from the outer to the inner layer) was measured to ensure that all sampling points were comparable and that individuals had similar crown depths (Table 1). To account for the decrease in leaf density from the outermost layer to the inner layer we sampled 20, 10 and 5 leaves at the outer, middle and inner layers of each sector, respectively (n = 35 leaves per sector). Only fully expanded leaves were measured. The total number of sampled leaves was 1,400 (10 individuals × 4 sectors × 35 leaves).

Leaf traits.

The relative position of leaves was obtained by measuring in situ three leaf angles using a smartphone (*Nokia N86, Nokia*, Spoo, Finland) following Escribano-Rocafort and collaborators (2014). By placing the cell phone parallel to the desired leaf, leaf elevation angle, midrib-roll angle and leaf azimuth can be easily recorded and stored. The elevation angle of the leaf's midrib was combined with the roll angle around the leaf's midrib and transformed to estimate a leaf's surface

inclination angle to the horizontal plane (Leaf tilt; L_{tilt}; *0º horizontal, 90º vertical*) (Eqn. 1; Escribano-Rocafort *et al.*, 2014). The third measured angle is the orientation of the leaf lamina to true north, i.e. the projection of a normal vector to the leaf's adaxial surface (Leaf azimuth, L_{azi} ; 0º North, 90º East, 180º South, 270º West). Ltilt and Lazi were used to estimate the absolute and relative area intercepting light (see light interception estimations below). Measurements were conducted in the absence of wind. Histograms of leaf angles at each crown sector and layer can be found in Supplementary materials (Figure S1, S2).

After characterizing leaf's spatial position we measured six light-related morpho-functional on the same leaves to define the shape and structure of the leaves. We scanned all collected leaves using a scanner (HP-ScanJet 3800; Hewlett Packard; Palo alto; CA; USA) to estimate the one-side area of the leaf blade (cm^2: *Larea*), the leaf width (cm: *Lwidth*) and the leaf length (cm: *Llength*) using ImageJ (NIH, Maryland, USA). Leaves were then oven-dried at 65 °C for 48 h and weighed to calculate leaf dry weight (g: *LDW*) with a precision balance (Mettler Toledo, Greifensee, Switzerland). From these measurements we calculated the two remaining traits: the specific leaf area (*SLA* = *leaf area* by *LDW*) and leaf shape (*Lindex* = Leaf length to leaf width ratio).Plant size (*biovolume*) was estimated as:

$$biovolume = (2/3)\pi(D/2)(d/2)h$$

to account for allometric differences between individuals. Where (*D*) and (*d*) are the diameters used to define the canopy ellipsoid and (*h*) is plant height. We used *biovolume* as a size proxy because the total aerial size of wild olives can be approximated with a semi-ellipsoid (Rubio de Casas *et al.*, 2011). Due to missing data the lowest *n* considered was 1219 for leaf width, leaf length and leaf index.

Light interception.

To examine the potential ability of each leaf to capture light, we calculated the silhouette area of the leaf blade as a measure of leaf area intercepting light (*SAL* = projection of the leaf blade x leaf area; cm^2) and the silhouette-to-leaf area ratio (*STAR* = Projected area /leaf area) which relates the relative area of the leaf with the angle of incidence of the incoming radiation (Carter & Smith, 1985; Oker-

Blom & Smolander, 1988; but see Granado-Yela et. al 2011). Both variables combine the sun path trajectory and the spatial position of an isolated leaf without considering leaf overlapping. Nonetheless, they define the maximum potential area and proportion of the leaf displayed to capture light at a given time (*SAL* = leaf area, *STAR* = 1) and, hence, a hypothetical limit of net photosynthetic rates due to the displayed leaf area and the angle of the incidence of the incoming radiation. Comparisons between *SAL* and *STAR* values estimated over the day period can further provide when the potential maximum is achieved during the day. To account for light interception variance during the day period we calculated *SAL* and *STAR* among crown positions at five evenly distributed periods (SAL_t and $STAR_t$) and as the integral of each leaf over time (from sunrise to sunset) on the day of the measurements (SAL_d and $STAR_d$). To assess the daily silhouette area of the leaf blade (SAL_d) and the daily silhouette to area ratio ($STAR_d$) we calculated the integral of the leaf projection from sunrise to sunset of each leaf among crown positions. Calculations considered leaf angles (L_{azi} corrected for magnetic declination at each population), location (geographic coordinates) and the sun position for a given time (see Eqn. 3; Escribano-Rocafort *et al*., 2014). We integrated this equation over time for one of the measurement days (July 31st), every 2 min. The equation used is sensitive to determine whether the exposure occurs in the adaxial or abaxial surface of the leaf (indicated by positive and negative signs, respectively). To assess the variation within the day period, *SAL* and *STAR* were calculated at five evenly distributed periods during the day (SAL_t, $STAR_t$; *T1* to *T5* in which *T3* equals solar noon) to account for representative sun elevation and azimuth angles at distinct day periods (Fig. 1).

Statistical analyses.

We investigated differences in SAL_d, $STAR_d$, SAL_t, $STAR_t$ and leaf traits among crown positions and day periods using linear mixed effects models (*LMM*). All final models were fit with restricted maximum likelihood (*RELM*) using the *nlme* package in R software (R Development Core Team, 2008). We determined model selection based on Akaike's information criteria (*AIC*) obtaining the most explicative model penalized for the number of estimated parameters, which includes all significant effects. We included in all models the variables *biovolume*

and population as random intercept to account for differences in leaf exposure due to allometry and local conditions (Rubio de Casas *et al*., 2011). The most complex model included *Crown Sector* (with four levels; *North*, *East*, *South* and *West*), *Crown Layer* (three levels; *Outer*, *Middle* and *Inner*) and *Day period* as fixed factors. The most complex random term was plant *biovolume* nested within population. With the aim to assess differences between and within day periods, we performed linear mixed models for SAL_t and $STAR_t$ adding the fixed term *Day period* (ordered factor with five levels; *T1* to *T5*). Multiple comparison tests were performed with the *multcomp* R-package (Bretz *et al.*, 2004). Leaf angles were transformed to radians and the remaining leaf traits were log-transformed to improve normality in the analyses. Normality and homocedasticity assumptions were checked graphically in the final models (a detailed summary of the models can be found in supplementary materials Table S1,8).

3. RESULTS.

Crown structure and leaf traits.

Tree canopy structure was comparable across individuals and populations in spite of small differences in the light environment between populations (Table 1). Trees in the *SL* population were slightly taller than trees in the *AF* population (Table 1) but that small difference was accounted for by including *biovolume* as a random term in all subsequent analyses. Leaf traits differed significantly among crown positions except in the ratio leaf length to leaf width (*Lindex*), the silhouette area of the leaf blade over the course of the day SAL_d and the daily silhouette to area ratio $STAR_d$ (Table 2; see Table S1,8 for details of the statistical models). All crown sectors and layers showed a mean leaf azimuth L_{azi} angle oriented towards east-south aspects except for the middle and inner layer of the *North* and *West* axes, which were facing north-east and south-west respectively (Table 3). The outer layer showed more vertical leaf tilt angles (L_{tilt} ~ 60º) in addition to the middle layer at the *South* and *West* sectors. The inner layer showed the lowest L_{tilt} angles (~ 45º) except at the *South* sector were L_{tilt} values were similar to that displayed in the middle layer (Table 3).

Despite an effect of the interaction between crown sector and crown layer, leaf area was similar among layers and sectors with maximum differences within the *West* sector (2.59 ± 0.05cm^2 in the outer layer compared to 2.92 ± 0.08 cm^2 in the middle layer) (Table 3). Specific leaf area revealed significant differences between layers (*SLA*, Table 3). The outer layer at the *North*, *East* and *West* sectors showed the lowest *SLA* values (~ 47 cm^2 g^{-1}) followed by leaves in the outer layer at the *South* sector (52.08 ± 0.86 cm^2 g^{-1}). Leaves in the inner layer showed the highest values of *SLA*. *Lindex*, a descriptor of the leaf's shape, did not show an interaction between crown sectors and layer (*Lindex*, Table 2). Leaves at the *East* and *South* sectors were the most elongated as opposed to leaves occurring at the *North* sector. *Lindex* differed between crown layers showing the highest values in the outer layer and the lowest in the inner layer (Table 3).

Light interception across the tree crown over time.

The integrated values of SAL_d and $STAR_d$ (product of the interaction of leaf angles and sun-path over the chosen day) only showed significant differences among crown layers (Table 2). Leaves of the inner layer displayed a greater SAL_d and $STAR_d$ than leaves in the middle and outer layers (Table 3), thus experiencing a smaller potential reduction in the intercepting surface due to sun path trajectory and leaf angles. On average leaves in the inner layer displayed a surface area of 0.14 cm^2 and 4% more $STAR_d$ than the outer layer during the chosen day. The silhouette area of the leaf blade (SAL_t) and the silhouette to total area ratio ($STAR_t$) showed a significant effect of the triple interaction among crown sector, layer and day period (Table 4). Leaves at each sector and layer were distinctly intercepting light during the selected periods. The outer layer of the crown showed a sustained adaxial exposure from *T1* to *T4*. However, SAL_t values remained below 2 cm^2 in all crown sectors and reached their maximum between *T2* to *T3* (mid-morning and midday; triangles and solid lines upper row, Fig. 2). Therefore, leaves at the outer layer experienced a sustained reduction of the exposed surfaces during the day congruent with avoidance of midday irradiance and optimization of light interception at the crown scale. On the contrary, middle and inner layers displayed distinct SAL_t patterns across crown sectors over the course of the day achieving full interception and hence, experiencing a lower reduction than the outer layer

(circles-dashed, square-dotted lines upper row, Fig. 2). In the *North* sector, the inner and middle layer showed a similar daily SAL_t pattern to the outer layer. The remaining sectors showed greater SAL_t values (above 2 cm^2) and variance during the day. Leaves of the middle and inner layer at the *East*, *South* and *West* sectors reached maximum *SAL*t at consecutive periods during the day from T2 to T4 enabling spatiotemporal complementarity of the productive processes (bottom row, Fig. 2). The variation in SAL_t over the chosen periods showed an increasing trend from the outer layer to the middle and inner layer of the crown across crown sectors, except for the *North* sector, which resulted in the opposite trend (upper to bottom row, Fig. 2).. Likewise, as leaf area showed little variation, we obtained similar $STAR_t$ patterns among crown positions (bottom row Fig. 2). Maximum $STAR_t$ values reached up to 0.6 in the outer layer whereas middle and inner layers showed a maximum $STAR_t$ of 0.8 (except in the *North* sector; Fig. 2).

Crown layers and sectors as a function of leaf angles (L_{azi} and L_{tilt}) had similar $STAR_d$ over time (integrated values from sunrise to sunset). Average leaf angles per crown sector and layer showed that $STAR_d$ differ by around 5-6% between the inner and outer layer along the day. However, sun path trajectory from East to West determines that all leaves oriented towards East or West (Lazi ~ 90º or 270º) will experience the greatest variance in the exposed surface during the day. Specifically, if they display high L_{tilt} angles as shown by the outer layer (except the *South* sector), the inner and middle layer of the *East* sector (all facing NE to SE orientations) and the inner and middle layer of the *West* sector (oriented towards West). These positions in the crown are maximizing potential light interception (the angle of incidence of the sun beam is perpendicular to the plane of the leaf) either in the morning or the afternoon periods due to the displayed $L_{azi.}$ Despite small variation in $STAR_d$ values at the different crown positions during the day, leaves at the middle and inner layers of the *North* sector and in all layers of the *South* sector experienced; a greater reduction in the maximum surface to intercept light ($STAR_d < 1$) and less variability over time. The spatial configuration of the photosynthetic surfaces across crown positions enables spatio-temporal complementarity of light interception and may enable optimization of light capture at the whole crown level.

4. DISCUSSION.

We found high variance in leaf inclination and azimuth angles across crown positions, which resulted in complementary patterns of light interception during the day among crown sectors and layers. Despite the fact that leaf angles differed among crown positions, the potential leaf area intercepting light was similar among sectors and showed small differences between crown layers during the day. However, displayed leaf angles determined distinct temporal patterns of light interception at contrasting day periods. *Outer* leaves showed the greatest reduction in the exposed surface (specifically during midday). On the contrary, *middle* and *inner* layers showed a lower potential reduction on the exposed surfaces and a sustained interception during the whole day due to the displayed leaf angles. Additionally, our results showed that the distribution of leaf angles, specific leaf area and leaf length-to-width ratio among crown positions may enable greater penetration of the irradiance to deeper crown layers. Based on these findings, we suggest that the phenotypes of modules experiencing different light environments at the leaf level are specialized so as to optimize light interception in the given environment. Therefore, the distinct expression of leaf attributes among crown positions may enable specialization and temporal complementarity in light interception at the whole-crown scale in *Olea europaea* trees.

The expression of functional leaf phenotypes within the tree crown can enhance light interception at the whole crown level. Among other factors, light interception and light use efficiency are highly dependent on the relative spatial position, shape and structure of the phososynthetic surfaces (Niklas, 1989; Ackerly & Bazzaz, 1995; Farnsworth & Niklas, 1995; Planchais & Sinoquet, 1998; Farque *et al*., 2001; Falster & Westoby, 2003). Sunlight capture efficiency of a single leaf and, thus, photosynthetic potential can be defined on an area-mass basis, which relates the investment in potential leaf area to capture light to biomass allocation to structural and biochemical components. Leaves with low *SLA* may experience a lower net photosynthetic capacity than leaves with higher *SLA* due to limitations in gas diffusion at the cell level and light absorption among chloroplasts (Terashima & Hirosaka 1995; Reich, Ellsworth & Walters, 1998). Leaf's spatial

position (inclination and orientation angles) in addition to the degree of aggregation of leaves influences the potential of a single leaf to intercept direct sunlight and the penetration of light to lower layers of the crown (Falster & Westoby, 2003). Leaf shape and internode length also have an impact on the distribution of light within the crown. Elongated leaf shapes (high length to width ratio) can contribute to light capture efficiency decreasing self shading among leaves and enhancing light penetration to deeper layers (Takenaka 1994; Pearcy *et al.* 2005; Sarlikioti *et al.*, 2011). In high irradiance environments leaf attributes such as low *SLA*, high leaf length-to-width ratio and high inclination angles at the outer layer of the crown can reduce heat loads and photoinhibition risks while having a positive effect on light interception at the whole individual level (Ehleringer & Werk, 1986; Valladares & Pugnaire, 1999; Werner *et al.*, 2001; Werner *et al.*, 2002). Optimization of the light interception efficiency can be achieved by means of spatial specialization and temporal complementarity related to the environment experienced by each portion of the crown. Complementarity in light interception patterns during the day may, ultimately, enable complementarity of productive processes such as carbon fixation, accumulation and carbon export (Granado-Yela *et al.*, 2011).

As shown by our results, different leaf inclination and orientation angles across the tree crown positions can lead to complementary patterns in the light interception strategy during the day. Without considering self-shading due to leaf overlapping, leaf inclination and orientation angles can yield complex patterns of light interception for a given day (Valladares, 1999). A previous study in one individual of the current species has shown a significant correlation in the light interception patterns, carbon assimilation and export between crown positions experiencing the extremes of the light gradient (Granado-Yela *et al.* 2011). Despite the fact that our measurements do not consider self-shading from adjacent layers and other phytoelements, the *inner* and *middle* layer showed near optimal leaf inclination angles to maximize light interception in the studied location (L_{tilt} ~40º, Mehleri *et al.* 2010). In turn, leaf azimuth varied matching, on average, the crown sector in which the leaf was located. This configuration enables a consecutive achievement of maximum interception during the day by each crown

sector and potentially constant assimilation even at high irradiance periods such as midday. On the contrary, the *outer* layer of leaves were experiencing less variation in the light interception patterns due to high L_{tilt} values and L_{azi} facing East and SE orientations. Such relative spatial position can enabled sustained carbon assimilation throughout the day despite the diurnal variation in solar light intensity and in the angle of incidence (Granado-Yela *et al.*, 2011). Our results showed that leaves in the *outer* layer are potentially experiencing a decrease 5-6% higher in the exposed leaf area than the *inner* layer due to cosine's law during the day and that do not achieve full exposure at any given time. Optimization theories suggest that the investment in leaf area over dry weight across crown positions should be proportional to the intercepted light (Field 1983; Farquhar 1989). Accordingly, the *outer* layer of leaves showed lower *SLA* values than the *inner* and *middle* layers. The observed configuration of the *outer* layer of the crown (more vertical leaves, low *SLA*, high length-to-width ratio, low SAL_d and $STAR_d$) suggests a trade-off between maximizing interception and avoiding excessive radiation loads, which could lead to photoinhibition during high irradiance periods.

Minimizing the negative impact of photoinhibition by displaying vertical leaf angles (structural photoprotection) can compensate for low daily carbon gains and may enable interception of non-saturating *PFD* in high irradiance environments (Ehleringer & Werk, 1986; Valladares & Pugnaire, 1999; Werner *et al.*, 2001; Falster & Westoby, 2003). This strategy should be similar across crown sectors, however according to our measurements the *outer* layer at the *South* sector (expected to endure higher irradiance and heat loads in the northern hemisphere) showed greater interception area at *T3* than at any other day period. An explanation for this paradoxical finding (i.e., maximizing light interception at central hours of the day and yet being less exposed than other sectors) may rely upon structural photoprotection. This sector showed lamina azimuth (L_{azi}) values oriented towards SE aspects. Facing South with high leaf inclination angles may contribute to the avoidance of dynamic photoinhibition by ensuring that full area interception is not reached at any given time. However, avoidance of direct sunlight by the *outer* leaves of the *South* sector during *T3* would result in long-

lasting high intensity irradiance pulses reaching the *inner* and *middle* layers. Such pulses may be at least as harmful for the *middle* and *inner* layer as they are for the *outer* layer. Consequently, *inner* and *middle* layers in the *South* sector showed similar leaf tilt angles than the outer layer. This is congruent with previous findings of a minimal phtoinhibition during midday and a subsequent recovery of assimilation in the afternoon (Granado-Yela *et al*., 2011). Increases in factors that provide self-shading constitute crucial attributes of plant survival in high irradiance environments despite their ability to adjust physiologically to stress (Valladares & Pearcy, 1997; Howell *et al*., 2002; Pearcy *et al.* 2005). In summary, we suggest that the spatial configuration of leaves in the *South* sector may contribute to minimize photosynthetic midday depression, chronic photoinhibitory losses and may allow a lower investment in costly biochemical-dissipating mechanisms (Tenhunen *et al*., 1981; Valladares & Pearcy, 1998; Pearcy *et al*., 2005). In addition, such configuration may enable a greater investment in specific leaf area intended to optimize light capture.

The steeper angles of outer leaves in the other sectors are also congruent with phenotypic integration across the canopy. If the outer layers of the less exposed areas of the canopy allow a higher fraction of the radiation to penetrate into deeper layers the use of light and whole crown photosynthesis might be more efficient (Watson & Witts, 1959; Verhagen *et al*., 1963; Terashima & Hikosaka, 1995). The effects of non-random leaf orientation have been shown to enhance water use efficiency at high solar angles and to procure an equivalent or higher carbon gain compared to other possible orientations in herbaceous and tree species (Jurik et al. 1990; James & Bell et al. 2000) and even linked to reproduction (Werk & Ehleringer 1986). The environmental heterogeneity across the tree crown of isolated trees depends on the exogenous conditions but also on the modification of the within-crown environment caused by the plant. As a consequence, crown performance depends not only on the environment in which the tree grows but, to a large extent, on the modification of the environment caused by the tree crown growth and its architecture (Rubio de Casas *et al*., 2007; Rubio de Casas *et al*., 2011).

5. CONCLUSIONS.

Our results show functional variation at different scales from the leaf to the crown level. Moreover, this functional differentiation is in agreement with spatiotemporal integration across the tree crown. Indeed, plants adapt morphologically and physiologically to subtle environmental differences at smaller scales than the individual level (de Kroon *et al*., 2005; Esteso-Martínez *et al*., 2006; Niinemets, 2009), which could result in interactions between functionally differentiated subunits and potentially in optimized performance at the organism level (de Kroon *et al*., 2005; Kawamura, 2010; Granado-Yela *et al*., 2011). However, we should bear in mind that tree crowns are complex multifunctional structures that may be subjected to several pressures such as development, biomechanic, hydraulic and allocation constraints (Pearcy *et al.*, 2005). Our results show that light interception can be optimized at the crown scale by means of the divergence of temporal patterns within the tree crown and specialization of the photosynthetic surfaces at different spatial scales. Structural photoprotection in high irradiance environments can be advantageous by minimizing the negative impacts of photoinhibition and through an increase of photosynthetic performance under low incidence angles. A deeper understanding of the function of the tree crown requires additional research to determine to what extent tree crowns modify the experienced environment to optimize photosynthetic performance at the crown scale. Moreover, the potential consequences in terms of fitness of phenotypic variability across the canopy remain to be assessed.

ACKNOWLEDGMENTS

We wish to thank MD Jiménez, A López-Pintor, A Vázquez and K Carrillo for their expertise and help during the design and field sampling and H Huber and H During for their great contributions in the final stage. This research was funded by the Spanish Ministry of Science and Education (project CGL2009-10392), and through a FPI grant to A.G.E. (BES-2010-032767). We are also indebted to the Madrid Regional Govt. (project REMEDINAL-2, S2009/AMB-1783).

6. TABLES AND FIGURES.

Table 1. Tree traits and radiation characteristics in the studied populations (mean ± 1SE).

Location	Coordinates	Altitude (m. a. s. l.)	Direct radiation ($\mu mol\ day^{-1}$)	Diffuse radiation ($\mu mol\ day^{-1}$)	Plant Heigh* (*m*)	Short Diameter* (*m*)	Long Diameter* (*m*)	Inner Layer* (*m*)	Middle Layer* (*m*)
Aldea del Fresno (AF)	40° 19' N,4° 14' W	690	2034 ± 4	369 ± 1	2.90 ± 0.13	3.31 ± 0.36	3.65 ± 0.49	1.36 ± 0.12	1.08 ± 0.09
San Luis (SL)	39° 49' N,4° 16' E	91	1919 ± 2	446 ± 1	3.30 ± 0.18	4.23 ± 0.36	4.66 ± 0.25	1.30 ± 0.06	0.83 ± 0.05

* $n = 5$

Table 2. Summary statistics for the best linear mixed model fitted to leaf traits, the integrated silhouette of the leaf blade (SAL_d) and silhouette to total area ratio ($STAR_d$) across the tree crown with the fixed effects of *Crown Sector*, *Crown Layer* and the interaction between them.

	numDF	*Leaf azimuth*	*Leaf tilt*	*Leaf area*	*LDW*	*Leaf width*	*Leaf length*	*SLA*	*Lindex*	SAL_d	$STAR_d$
Sector	3	23.15***	2.49	0.89	3.40*	2.71*	2.05	13.51***	8.19***	-	-
Layer	2	6.49**	29.81***	1.16	38.14***	32.07***	15.80***	233.86***	80.04***	23.38***	26.17***
Sector : layer	6	10.93***	4.46***	3.56**	2.97**	2.84**	2.98**	4.28***	1.44	-	-

numDF, Numerator degrees of freedom. *Biovolume* was included in all models as a random factor. The standard deviation of the random components of each model and further details are provided in the supplementary materials (Table S1,10). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; underscored values are marginally non -significant, $0.05 < P < 0.1$.

Table 3. Mean values ± 1SE for leaf traits per *Crown Sector* and *Crown Layer*. Silhouette area of the leaf blade (SAL_d) and silhouette to total area ratio ($STAR_d$).

Crown Sector	*Crown Layer*	*Leaf azimuth (º)*	*Leaf tilt (º)*	*Leaf area (cm²)*	*LDW(g)*	*Leaf width (cm)*	*Leaf.length (cm)*	*SLA ($cm^2 g^{-1}$)*	*L.Index*	*SALd cm²*	*STARd*
North	*Outer*	116.18 ± 25b	61.94 ± 2.84d	2.77 ± 0.06ab	0.061 ± 0.002e	0.88 ± 0.02bc	4.01 ± 0.06bc	46.36 ± 0.64e	4.76 ± 0.11Aa	0.45 ± 0.02C	0.16 ± 0.01C
	Mid	21.1 ± 35.31ab	49.25 ± 4.78ab	2.78 ± 0.09ab	0.051 ± 0.002bcd	0.91 ± 0.02b	3.90 ± 0.08ab	55.65 ± 1.18bc	4.49 ± 0.14Ab	0.47 ± 0.04B	0.17 ± 0.01B
	Inner	23.29 ± 36.78ab	41.72 ± 4.01a	2.55 ± 0.14ab	0.044 ± 0.003a	0.89 ± 0.02ab	3.60 ± 0.14a	61.23 ± 1.71a	4.09 ± 0.16Ac	0.54 ± 0.05A	0.21 ± 0.02A
East	*Outer*	109.18 ± 27.81ab	60.44 ± 3.65d	2.72 ± 0.05ab	0.058 ± 0.001ce	0.84 ± 0.01a	4.10 ± 0.05b	47.84 ± 0.51e	5.03 ± 0.09Ba	0.45 ± 0.02C	0.16 ± 0.01C
	Mid	106.01 ± 21b	54.76 ± 4.58bcd	2.76 ± 0.10ab	0.051 ± 0.002ab	0.90 ± 0.02bc	3.85 ± 0.07ab	54.44 ± 0.98cd	4.43 ± 0.10Bb	0.55 ± 0.04B	0.2 ± 0.01B
	Inner	106.73 ± 21.18b	48.79 ± 4.46ac	2.92 ± 0.17ab	0.052 ± 0.004abc	0.92 ± 0.03b	3.92 ± 0.13ab	58.27 ± 1.49a	4.32 ± 0.12Bc	0.61 ± 0.06A	0.22 ± 0.02A
South	*Outer*	169.14 ± 27.06a	62.69 ± 4.47d	2.71 ± 0.04ab	0.055 ± 0.001bcd	0.85 ± 0.01ac	4.06 ± 0.05b	52.08 ± 0.86d	4.92 ± 0.09Ba	0.41 ± 0.02C	0.15 ± 0.01C
	Mid	197.21 ± 21.07ad	55.36 ± 4.67bcd	2.68 ± 0.08ab	0.048 ± 0.001ad	0.88 ± 0.02ab	3.84 ± 0.06ab	56.96 ± 0.98bc	4.53 ± 0.12Bb	0.51 ± 0.03B	0.19 ± 0.01B
	Inner	174.69 ± 14.06ab	57.71 ± 3.94bcd	2.85 ± 0.12ab	0.049 ± 0.002ab	0.92 ± 0.03b	3.91 ± 0.10ab	59.32 ± 1.27ab	4.32 ± 0.13Bc	0.57 ± 0.04A	0.21 ± 0.02A
West	*Outer*	96.86 ± 28.81ab	57.99 ± 3.88cd	2.59 ± 0.05b	0.055 ± 0.001be	0.83 ± 0.01a	3.93 ± 0.05bc	47.54 ± 0.72e	4.84 ± 0.08ABa	0.44 ± 0.02C	0.17 ± 0.01C
	Mid	262.51 ± 24.04cd	60.06 ± 3.1cd	2.92 ± 0.08a	0.055 ± 0.002bde	0.92 ± 0.02b	4.02 ± 0.07bc	55.12 ± 1.04c	4.52 ± 0.12ABb	0.49 ± 0.04B	0.17 ± 0.01B
	Inner	274.75 ± 21.78c	45.91 ± 4.33ab	2.77 ± 0.12ab	0.048 ± 0.002ab	0.94 ± 0.03b	3.73 ± 0.12ac	58.82 ± 1.46ac	4.08 ± 0.15ABc	0.62 ± 0.05A	0.22 ± 0.01A

Levels with different letters indicate significant differences ($\alpha < 0.05$). Upper and lower case letters indicate an absence of interaction between factors. Leaf angles $n = 1394$; leaf area $n = 1252$; *SLA* and *LDW* $n = 1247$; leaf width, leaf length and leaf index $n = 1219$; SAL_d $n = 1252$; $STAR_d$ $n = 1394$. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 4. Summary statistics for the best linear mixed model fitted to the instantaneous silhouette of the leaf blade (SAL_t) and to the silhouette to total area ratio ($STAR_t$) displayed within the crown with the fixed effects of *Crown Sector* (NESO), *Crown Layer* (*Outer*, *Middle* and *Inner*), *Day period* (T1 to T5) and the interactions between crown sector and crown layer, day period and crown sector, day period and crown layer and the triple interaction.

		SAL_t		$STAR_t$	
	numDF	*F* -value	*P* -value	*F* -value	*P* -value
(Intercept)	1	105.2	**<.0001**	322.43	**<.0001**
Sector	3	1.85	0.14	1.08	0.35
Layer	2	13.86	**<.0001**	13.11	**<.0001**
Day period	4	284.64	**<.0001**	323	**<.0001**
Sector : day period	12	42.58	**<.0001**	39.5	**<.0001**
Sector : layer	6	0.55	0.77	1.44	0.2
Layer : day period	8	12.44	**<.0001**	10.68	**<.0001**
Sector : layer : day period	24	17.94	**<.0001**	14.73	**<.0001**

The standard deviation of the random component was as follows: *biovolume* = 0.20 and *biovolume* = 0.04 for the fitted model to the silhouette area of the leaf blade (SAL_d) and the silhouette to total area ratio ($STAR_d$), respectively. Statistically significant values are indicated in bold text. numDF, numerator degrees of freedom; Denominator degrees of freedom SAL_t,= 6192, $STAR_t$ =6901

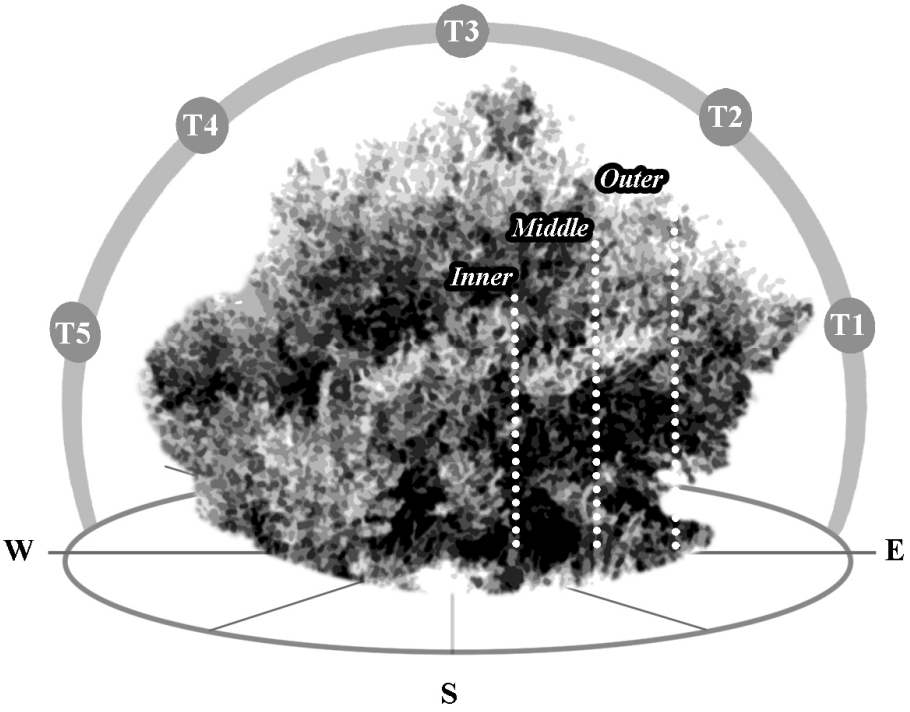

Figure 1. Sampling points within the tree crown and schematic representation of the considered day periods. Sampling of *outer*, *middle* and *inner* layers were conducted at *North*, *East*, *South* and *West* sectors. Each sector spanned 90° leaving the main cardinal point in the bisector angle of the crown sector. We sampled 140 leaves per tree crown. The yellow line depicts sun path with the chosen day periods (T1 to T5) for the instantaneous silhouette area of the leaf blade (SAL_t) and the silhouette to total area ratio ($STAR_t$) simulations.

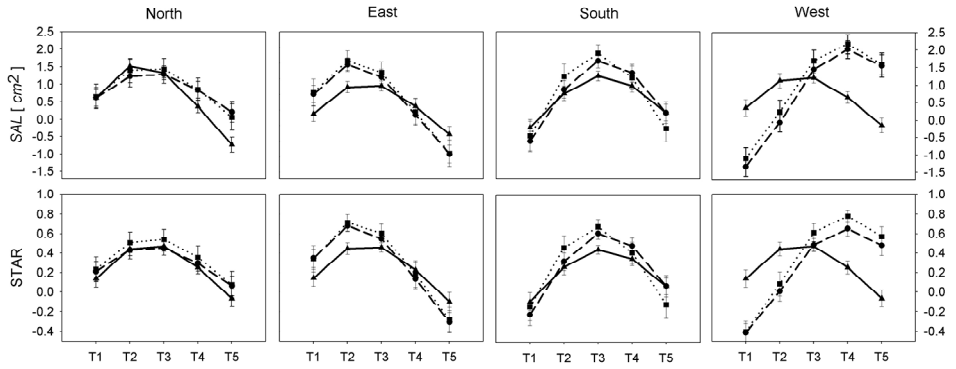

Figure 2. Interaction plot of the best fit model for SAL_t (upper row) and $STAR_t$ (lower row) among *Crown Sectors* (columns) and *Crown Layers* (symbols and lines; triangle-solid *outer*, circle-dashed *middle* and square-dotted *inner*) over the simulated times (*T1* to *T5*). Adjusted means ± 2SE. Negative *SAL* and *STAR* values indicate abaxial interception. Models [*SAL~ C.Sector x C.Layer x Day Period + (1|biovolume)*] *n*= 6260; [*STAR~ C.Sector x C.Layer x Day Period + (1|biovolume)*] *n*= 6970.

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8. SUPPLEMENTARY MATERIAL

Tables S1,10 Details of linear mixed models

Figure S2 Histograms of lamina azimuth per crown sector and layer binned each 10°.

Table S1 Summary statistics for the best linear mixed model fitted to leaf area with the fixed effects of crown sector and crown layer, the interactions between them and biovolume as random effect. Denominator degrees of freedom = 1232.

Random effects	**Variance**	**SD**	
Biovolume	0.14	0.25	
Fixed effects	**Estimate**	**SE**	**t-value**
(Intercept)	1.002	0.059	17.019
C.SectorNorth	-0.103	0.052	-1.963
C.SectorSouth	-0.002	0.052	-0.044
C.SectorWest	-0.031	0.052	-0.583
C.LayerMiddle	-0.044	0.045	-0.968
C.LayerOuter	-0.037	0.041	-0.901
C.SectorNorth : C.LayerMiddle	0.16	0.064	2.489
C.SectorSouth : C.LayerMiddle	-0.015	0.064	-0.236
C.SectorWest : C.LayerMiddle	0.108	0.064	1.679
C.SectorNorth : C.LayerOuter	0.138	0.059	2.355
C.SectorSouth : C.LayerOuter	0.009	0.059	0.146
C.SectorWest : C.LayerOuter	-0.017	0.059	-0.288

Table S2 Summary statistics for the best linear mixed model fitted to LDW with the fixed effects of crown sector and crown layer, the interactions between them and biovolume as random effect. Denominator degrees of freedom = 1227

Random effects	**Variance**	**SD**	
Biovolume	0.111	0.298	
Fixed effects	**Estimate**	**SE**	**t-value**
(Intercept)	-3.054	0.058	-52.757
C.SectorNorth	-0.148	0.063	-2.365
C.SectorSouth	-0.038	0.064	-0.598
C.SectorWest	-0.028	0.063	-0.438
C.LayerMiddle	0.027	0.054	0.497
C.LayerOuter	0.154	0.05	3.098
C.SectorNorth : C.LayerMiddle	0.174	0.077	2.265
C.SectorSouth : C.LayerMiddle	-0.012	0.078	-0.154
C.SectorWest : C.LayerMiddle	0.09	0.077	1.176
C.SectorNorth : C.LayerOuter	0.203	0.07	2.894
C.SectorSouth : C.LayerOuter	-0.026	0.071	-0.364
C.SectorWest : C.LayerOuter	-0.002	0.07	-0.034

Table S3 Summary statistics for the best linear mixed model fitted to leaf width with the fixed effects of crown sector and crown layer, the interactions between them and biovolume as random effect.

Random effects	**Variance**	**SD**	
Biovolume	0.142	0.136	
Fixed effects	**Estimate**	**SE**	**t-value**
(Intercept)	-0.1	0.052	-1.931
C.SectorNorth	-0.026	0.03	-0.894
C.SectorSouth	-0.001	0.029	-0.048
C.SectorWest	0.019	0.029	0.649
C.LayerMiddle	-0.034	0.025	-1.379
C.LayerOuter	-0.091	0.023	-4.039
C.SectorNorth : C.LayerMiddle	0.045	0.036	1.247
C.SectorSouth : C.LayerMiddle	-0.016	0.035	-0.449
C.SectorWest : C.LayerMiddle	0.013	0.035	0.383
C.SectorNorth : C.LayerOuter	0.075	0.033	2.265
C.SectorSouth : C.LayerOuter	0.014	0.032	0.446
C.SectorWest : C.LayerOuter	-0.026	0.032	-0.801

Denominator degrees of freedom = 1199

Table S4 Summary statistics for the best linear mixed model fitted to leaf length with the fixed effects of crown sector and crown layer, the interactions between them and biovolume as random effect.

Random effects	**Variance**	**SD**	
Biovolume	0.078	0.156	
Fixed effects	**Estimate**	**SE**	**t-value**
(Intercept)	1.344	0.035	38.442
C.SectorNorth	-0.098	0.034	-2.877
C.SectorSouth	-0.001	0.033	-0.044
C.SectorWest	-0.049	0.033	-1.5
C.LayerMiddle	-0.01	0.028	-0.346
C.LayerOuter	0.053	0.026	2.057
C.SectorNorth : C.LayerMiddle	0.104	0.041	2.51
C.SectorSouth : C.LayerMiddle	0.001	0.04	0.024
C.SectorWest : C.LayerMiddle	0.094	0.04	2.334
C.SectorNorth : C.LayerOuter	0.069	0.038	1.814
C.SectorSouth : C.LayerOuter	-0.005	0.037	-0.129
C.SectorWest : C.LayerOuter	0.009	0.037	0.245

Denominator degrees of freedom = 1199

Table S5 Summary statistics for the best linear mixed model fitted to leaf index with the fixed effects of crown sector, crown layer and biovolume as random effect. Denominator degrees of freedom = 1205.

Random effects	**Variance**	**SD**	
Biovolume	0.185	0.154	
Fixed effects	**Estimate**	**SE**	**t-value**
(Intercept)	1.431	0.063	22.649
SecNorth	-0.058	0.013	-4.526
SecSouth	-0.006	0.012	-0.493
SecWest	-0.025	0.012	-2.062
LayMiddle	0.063	0.014	4.416
LayOuter	0.148	0.013	11.326

Table S6 Summary statistics for the best linear mixed model fitted to SLA with the fixed effects of crown sector and crown layer, the interactions between them and biovolume as random effect. Denominator degrees of freedom = 1227.

Random effects	**Variance**	**SD**	
Biovolume	0.123	0.134	
Fixed effects	**Estimate**	**SE**	**t-value**
(Intercept)	4.05	0.046	88.865
C.SectorNorth	0.059	0.028	2.106
C.SectorSouth	0.025	0.029	0.888
C.SectorWest	0.011	0.028	0.376
C.LayerMiddle	-0.068	0.024	-2.775
C.LayerOuter	-0.192	0.022	-8.614
C.SectorNorth : C.LayerMiddle	-0.025	0.035	-0.719
C.SectorSouth : C.LayerMiddle	0.015	0.035	0.418
C.SectorWest : C.LayerMiddle	0.001	0.034	0.024
C.SectorNorth : C.LayerOuter	-0.081	0.032	-2.579
C.SectorSouth : C.LayerOuter	0.049	0.032	1.543
C.SectorWest : C.LayerOuter	-0.024	0.032	-0.771

Table S7 Summary statistics for the best linear mixed model fitted to lamina tilt with the fixed effects of crown sector and crown layer, the interactions between them and biovolume as random effect. Denominator degrees of freedom = 1373.

Random effects	**Variance**	**SD**	
Biovolume	0.104	0.365	
Fixed effects	**Estimate**	**SE**	**t-value**
(Intercept)	0.854	0.061	13.988
C.SectorNorth	-0.123	0.073	-1.683
C.SectorSouth	0.152	0.073	2.09
C.SectorWest	-0.052	0.073	-0.711
C.LayerMiddle	0.099	0.063	1.572
C.LayerOuter	0.197	0.058	3.419
C.SectorNorth : C.LayerMiddle	0.027	0.089	0.307
C.SectorSouth : C.LayerMiddle	-0.145	0.09	-1.615
C.SectorWest : C.LayerMiddle	0.144	0.089	1.616
C.SectorNorth : C.LayerOuter	0.15	0.082	1.841
C.SectorSouth : C.LayerOuter	-0.117	0.082	-1.44
C.SectorWest : C.LayerOuter	0.01	0.082	0.122

Table S8 Summary statistics for the best linear mixed model fitted to lamina azimuth with the fixed effects of crown sector and crown layer, the interactions

between them and biovolume as random effect. Denominator degrees of freedom = 1373.

Random effects	**Variance**	**SD**	
Biovolume	0	1.606	
Fixed effects	**Estimate**	**SE**	**t-value**
(Intercept)	2.186	0.227	9.624
C.SectorNorth	0.782	0.321	2.435
C.SectorSouth	0.865	0.321	2.692
C.SectorWest	2.147	0.321	6.685
C.LayerMiddle	-0.018	0.278	-0.066
C.LayerOuter	0.544	0.254	2.142
C.SectorNorth : C.LayerMiddle	-0.25	0.393	-0.635
C.SectorSouth : C.LayerMiddle	0.344	0.394	0.871
C.SectorWest : C.LayerMiddle	-0.332	0.393	-0.844
C.SectorNorth : C.LayerOuter	-1.027	0.359	-2.857
C.SectorSouth : C.LayerOuter	-0.462	0.359	-1.287
C.SectorWest : C.LayerOuter	-2.13	0.359	-5.929

Table S9 Summary statistics for the best linear mixed model fitted to SALd with the of crown layer as fixed effect and biovolume as random effect. Denominator degrees of freedom = 1241.

Random effects	**Variance**	**SD**	
Biovolume	0.138	0.296	
Fixed effects	**Estimate**	**SE**	**t-value**
(Intercept)	0.588	0.051	11.497
LayMiddle	-0.066	0.027	-2.435
LayOuter	-0.152	0.025	-6.15

Table S10 Summary statistics for the best linear mixed model fitted to STARd with the of crown layer as fixed effect and biovolume as random effect. Denominator degrees of freedom = 1241

Random effects	**Variance**	**SD**	
Biovolume	0.029	0.097	
Fixed effects	**Estimate**	**SE**	**t-value**
(Intercept)	0.213	0.011	18.535
LayMiddle	-0.028	0.008	-3.368
LayOuter	-0.052	0.008	-6.838

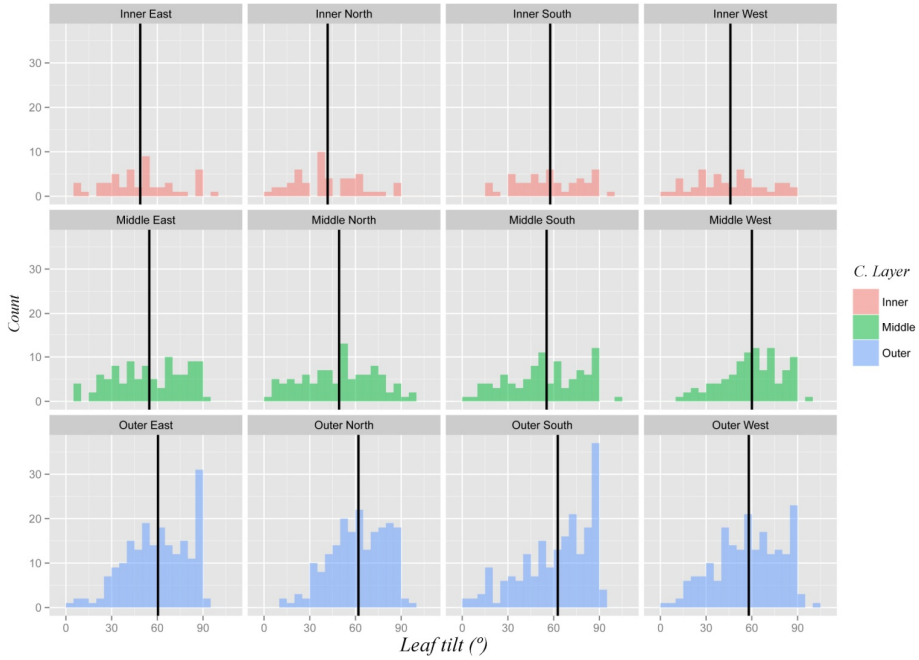

Figure S1. Histograms with means (black lines) of leaf tilt per crown sector and layer binned each 5°. Leaf tilt was transformed to radians and treated as an axial variable (0º- 180º) for mean calculations

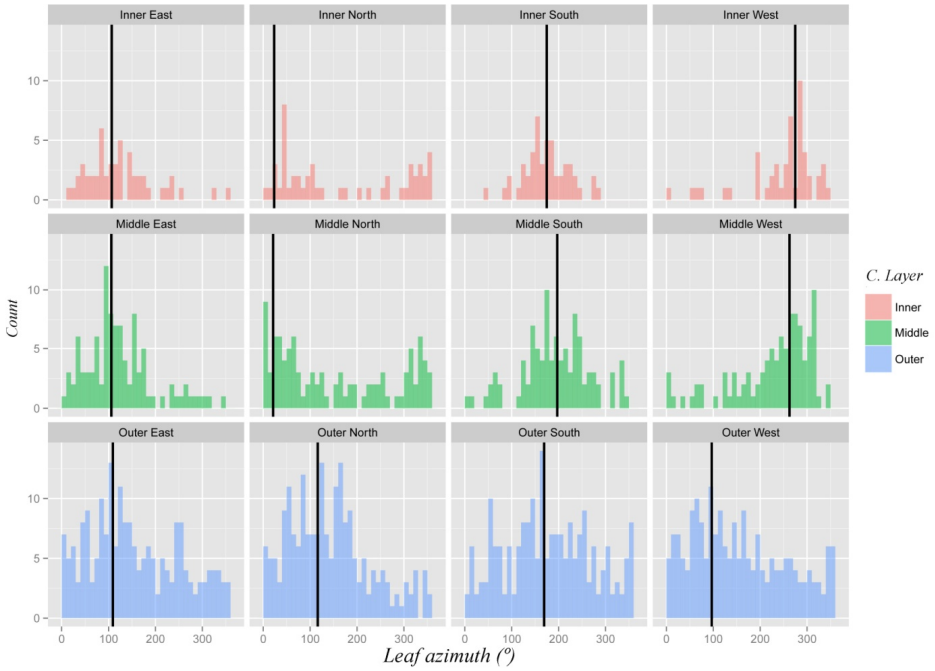

Figure S2. Histograms with means (black lines) of lamina azimuth per crown sector and layer binned each 10°. Leaf azimuth was transformed to radians and treated as a circular variable (0º- 360º) for mean calculations.

CAPÍTULO III

Intraspecific variation in plant functional traits: magnitude and structucture of the variability for foliar traits at global scales in *Olea europaea* L. trees.

Enviado a New Phytologist

ABSTRACT

- Natural selection can only work if there is functional variation (FV) among individuals. Even more, intra-specific and intra-individual variance in functional traits plays a crucial role at multiple ecological scales; from individual adaptation to community assemblage and dynamics. However, our understanding of the magnitude and distribution of FV across scales in tree canopies is limited and the influence of FV in specific responses to environmental gradients remains poorly assessed.

- Here we addressed variance of leaf and crown traits across multiple nested ecological scales in *Olea europaea* L. trees. We sampled eight populations over a wide latitudinal gradient (~60º) and analyzed the variability in light-related foliar traits within and among individuals and populations. We investigated the relationship of FV of multiple traits with environmental variables.

- The variance partitioning for leaf traits showed two main sources of variation: within canopy (> 90% of total variance in some cases) and population (25- 60% variance). Multi-trait analyses revealed that variability in the chosen traits was highly correlated to plant and leaf size and to leaf shape among populations. FV for multiple leaf traits among populations was strongly correlated to the annual variance of irradiance, temperature and precipitation over the latitudinal gradient.

- The magnitude and structure of FV found for light-related foliar traits across spatial scales highlight the relevance of including FV in ecological studies, specifically the variability within individuals. We conclude that the annual distribution of irradiance, temperature and precipitation (seasonality) are major drivers of FV in leaf and crown attributes across global gradients in *Olea europaea* trees.

Keywords: intraspecific variability, variance partitioning, foliar traits, variability across scales, tree functional traits,

1. INTRODUCTION

Plant functional traits are any measurable morphological, physiological or phenological characteristic from the cell to the whole plant scale that affects plant growth, reproduction or survival (Lavorel et al. 1997; Violle et al. 2007; Violle & Jiang 2009). Plant functional traits constitute an indirect link between plant fitness and the environment and are useful to answer relevant questions in ecology (McGill et al. 2006; Ackerly et al. 2007). Indeed, plant functional traits have been used at the species and community levels to investigate ecosystem functioning, diversity structure and dynamics, and to address the effect of the environment in species or community performance (Lavorel et al. 1997; Reich et al. 2003; Wright et al. 2004; Diaz et al. 2004, 2007; de Bello et al. 2010). Furthermore, plant functional traits can be used to build predictive tools such as computer trait-based models (Sitch et al. 2003; Norberg et al. 2001; Savage, Webb & Norberg 2007). Functional ecologists have historically assumed that functional plant traits differ more significantly among than within species (Garnier et al. 2001; Roche et al. 2004). However evidence has been accumulated during the last years highlighting the relevance of intraspecific trait variation (Violle et al. 2012) especially in species-poor communities (Niinemets *et al.* 2014; Niinemets 2015). As a consequence, trait based analyses are currently improving their ability to unveil community processes by incorporating such variability (Siefert 2012; Kraft *et al*. 2014). Indeed, there is mounting evidence that suggests a significant effect of within species variability on ecosystem functioning and community assembly and dynamics (Booth & Grime 2003; Boege & Dirzo 2004; Crutsinger *et al*. 2006; Lecerf & Chauvet 2008). However, recent advances in the field only consider some of the potential sources of variation in traits: within and between populations. This is surprising since it is well known that intraespecific trait variation is organized in multiple scales, often even within individuals (Herrera, 2009; Albert et al. 2010b).

Intraspecific trait variability is the differential expression in the attributes of individuals of a given species that arises from genetic diversity and phenotypic response at different spatial scales (Albert et al. 2011). Functional variability

within species (FV) can be partitioned in three nested components; intraspecific variation among populations (FVpop), intraspecific variation among individuals of a given population (FV_{BI}) and the intraspecific variability within individuals (for instance, variability between leaves of an individual, FV_{WI}). Assessing FV across scales can provide valuable information about how individuals of a given species respond to the environment and the potential diversity of functional responses among individuals. In fact, FV is essential to evolutionary processes. Thus natural selection can only take place if there are functional differences among individuals including differences not only in average values but also variation within individuals (Futuyma 2009). FV as a substratum for evolution should not be quantified only in terms of response curves between functional traits and environmental gradients but should account for the interaction among different scales of phenotypic variability (Ryser & Eek, 2000; Sugiyama, 2003; Albert et al., 2010a) For instance, differences in functional traits among populations of a single species (high FVpop) can be due to the existence of different genotypes and to a different response of the genotypes to the environment. Likewise, differences among individuals of a given population (high FV_{BI}) can be due to genetic differences among individuals, to a different response of the genotypes to the environment or to different biotic interactions experienced by each individual. Lastly, differences in the expression of functional traits within individuals can be due to developmental contingency but also to environmental gradients at the individual scale (Albert *et al.*, 2011).

Despite the importance of the FV partitioning across the three nested components, FV_{WI} remains poorly investigated in trees due to the fact that they form complex multilayered structures product of the modular development of plants, its relationship with the environment and the reciprocal modulation of the environment by the different phytoelements (Sultan, 1987; Petit & Hampe 2006). Indeed, among plant forms trees are expected to show high FV_{WI} (Richardson et al., 2001; Messier et al., 2010). Unfortunately, our understanding of FV_{WI} in trees remains poorly investigated in spite of its relevance in ecological processes. In order to improve trait-based analyses it is critical to know how the all these scales assume variance in plastic traits. Without such information predictive trait-based

models are neglecting the role of FV in mechanisms controlling the coexistence of species, habitat filtering, niche differentiation and ecosystem functioning (Albert *et al*., 2010; Jung *et al*. 2010).

Individuals of long-lived plants are expected to respond to environmental heterogeneity through the expression of phenotypic plasticity (Bradshaw, 1965; Sultan, 1987; Petit & Hampe 2006) and the prevalence of a set of coordinated traits (individual´s phenotypic syndrome) that affect the fitness of the whole plant (Marks & Lechowicz, 2006; Wilson & Nussey, 2010). For instance the quantity and quality of the irradiance experienced at different crown positions in a tree can lead to the expression of different leaf phenotypes within the canopy (Sack *et al.* 2006) but the realized phenotypic diversity will necessarily be constrained by some abiotic and biotic factors. As a result, intra-canopy gradients in light quality and quantity can lead to the emergence of clearly differentiated leaf phenotypic syndromes (i.e., sun and shade leaves; Jurik, Chabot & Chabot 1979; Valladares & Niinemets, 2008). The variability in leaf traits within canopies as a response to local cues is functionally relevant. The expression of plasticity in architectural traits such as leaf angle or size can have profound consequences for daily light interception (Howell et al., 2002; Falster & Westoby, 2003). Indeed, plasticity in the expression of leaf inclination angles alters light properties and transmission within the tree crown enhancing whole-individual carbon gain (Uemura *et al*. 2006). Modifying light transmission and light properties can confer trees advantages such as higher degree of control on the available resources (Bellingham & Sparrow, 2000) and further alter biotic interactions such as competition for light (Hikosaka & Hirose 1997). As a consequence, FV in light related traits may be highly sensitive to seasonal changes in irradiance across latitudinal gradients in widespread tree species. Predictive models of functional plant responses can only be fully developed once multi-scale trait variation is incorporated and the main sources of variation identified. For instance, plant traits related to light interception and photosynthesis should vary along latitude but also depend on the irradiance, temperature and precipitation experienced by individual plants within latitudes. From an ecological and evolutionary perspective temperature and precipitation have been suggested as major determinants of plant

leaf traits at global scales (Walter 1994; Donoghue 2008). Although individuals might potentially respond similarly to the distribution of the irradiance within the tree crown, environmental filters (such as periods of low water availability, or exposure to extreme annual temperatures) should constrain trait variability within crowns and influence the whole crown strategy.

With this in mind we examined the partition of the variance in -related functional traits of the evergreen tree *Olea europaea* L. (wild olive) across nested scales. *O. europaea* are long-lived trees that naturally extend over a large latitudinal-geographic range from the Mediterranean Basin to South Africa covering contrasting environments. Evidence of complementarity in the primary productive mechanisms such as light interception, carbon fixation and carbon accumulation/export in individual tree crowns of this species has been previously reported, highlighting the relevance of phenotypic responses within individuals on whole plant fitness (García-Verdugo et al. 2010; Granado-Yela et al. 2011; Rubio de Casas et al. 2011). Therefore, *Olea europaea* provides an ideal model to analyze the role of within-species variation in broad-scale latitudinal gradients and to assess the relationship with environmental factors such as temperature, irradiance and precipitation. We selected eight populations ranging from range edge and peripheral populations (thermal and rainfall limits) to tropical and subtropical populations in both hemispheres. Namely, we conducted analyses on light-related foliar (SLA, leaf angles, leaf shape, silhouette area of the leaf blade, projection of the leaf blade) and whole plant (plant height, crown diameters, crown depth) across six ecological scales in which the irradiance can vary(*Site /Population /Tree /Crown sector /Crown layer /Leaf*) This nested structure was used as random intercept in linear mixed models as a baseline for comparisons to assess the partitioning of FV.

We specifically tried to respond the following questions: 1. How do plant functional traits implied in the photosynthetic response vary across scales within this species? 1.1. How is the FV distributed within and among traits? Is there a trend in the variance partitioning among sites for any of the light related traits? 2. Which foliar and whole plant traits better describe FV in multiple trait analyses?

Which is the relationship among foliar and whole crown traits? To which extent the different scales participate in the variability of multiple traits? 3. Does FV of multiple traits rank individuals and populations consistently according to a latitudinal gradient? Is the expression in the set of measured traits related to climatic conditions experienced at the population level?

2. MATERIALS AND METHODS

We conducted our study in eight populations of *Olea europaea* L. occurring at four evenly distributed sites from 40ºN to 21ºS (Fig 1). Populations covered large fraction the whole latitudinal range of the species and included three subspecies (Vargas *et al.* 2001; Green, 2002) (Table 1). *Olea europaea* is a long-lived evergreen with flat leaves with signs of light-related expression of plasticity within the tree crown that do not track sun movements (Granado-Yela *et al.*, 2011; Rubio de Casas *et al.*, 2011). Despite that indirect gradients such as latitude should not be considered to describe ecological patterns (Körner, 2007) we find highly relevant to assess FV between latitudes (sites; L21, L0, L28 and L40) as the studied range entails distinct daily and seasonal climatic patterns due to Earth's geometry and declination. Latitudinal differences can thus, have direct implications on FV in our model species and light depending traits. Northernmost populations (L40, L28) included the thermal tolerance limit imposed by minimal winter temperatures (Vargas and Kadereit, 2001; Rubio de Casas et al., 2002) (*O.europaea* subsp *europaea*, AF) and the lower rainfall limit of the species (Pansiot and Rebour, 1961) (*O.europaea* subsp *guanchica*, FA, AN). On the contrary, *O.europaea* subsp *cuspidata,* (L0, L21) thrive in locations with less seasonal variation (Table 1). In each population we sampled five well-developed trees (except in GG and PR where four trees were sampled) with signs of fruit production in previous years. Trees were relatively isolated except in NB where the vegetation in the near vicinity was as tall as the measured individuals. Samplings at each site were conducted during three consecutive years from 2011 to 2013. We obtained a set of climatic variables at the population scale level calculated through *NOAA* web calculator and extrapolated from *worldclim* database at .5' spatial resolution from each population (Table 1).

Sampling design.

We divided the upper half of each crown into two sampling axes centred at cardinal directions (N-E-S-O) to obtain four crown sectors (crown sectors hereafter). Then we subdivided each sector into three layers: the outermost layer of leaves (*Outer*), the middle layer of leaves (*Middle*) and the deepest layer where leaves were present (*Inner*) (Fig. 1b). Sampling points for the middle and inner layers were set in an oblique direction from the outer part of the crown towards its geometrical centre. To account for the decrease in leaf density from the outermost layer to the inner layer we sampled 20, 10 and 5 leaves at the outer, middle and inner layers of each sector, respectively (n = 140 leaves per individual). The total number of sampled leaves was 5320 (38 trees × 4 sectors × 35 leaves). All measurements were performed on fully expanded leaves in the course of a single day, under no-wind conditions with the aid of telescopic ladders. Ladders were set to avoid crown disturbance.

Leaf traits

The relative position of leaves within the crown was obtained by measuring in situ three leaf angles using a smartphone (Nokia N86, Nokia, Spoo, Finland) following Escribano-Rocafort *et al*. (2014). By placing the cell phone parallel to the desired leaf we obtained the leaf's surface inclination angle to horizontal (Leaf tilt, L_{tilt}; 0° horizontal, 90° vertical) (Eqn. 1; Escribano-Rocafort *et al*. 2014) and the orientation of the leaf lamina to true north, i.e. the projection of a normal vector to the leaf's adaxial surface (Leaf azimuth, L_{azi}; 0° North, 180° South, clockwise) (Fig. 1c).

After leaf angle measurements we collected and scanned all leaves (HP-ScanJet 3800; Hewlett Packard; Palo Alto; CA; USA) to estimate the one-side area of the leaf blade, leaf width and leaf length using ImageJ (NIH, Maryland, USA). Leaves were then oven-dried at 65 °C for 48 h and weighted to calculate leaf dry weight with a precision balance (Mettler Toledo, Greifensee, Switzerland). From these measurements we calculated the specific leaf area (*SLA* = leaf area by dry weight) and a leaf shape (*Lindex* = leaf length-to-width ratio). On the leaf economic spectrum *SLA* is considered a key trait related to the photosynthetic

function and carbon investment. Lindex constitutes a leaf shape descriptor linked to within crown environment, leaf gas exchange, and structural constraints (Rubio de Casas *et al*. 2011).

To examine the potential ability of each leaf to intercept light, we calculated the silhouette area of the leaf blade (SAL_d: cm^2) and the ratio of the silhouette to total leaf area ratio ($STAR_d$: *%*), which relates leaf irradiance to incident radiation (Carter & Smith, 1985; Smith & Hughes, 2009). To assess SAL_d and $STAR_d$ we calculated the integral of the leaf projection from sunrise to sunset of each leaf at three annual situations determined by the elevation of the sun within populations. Namely, we estimated $STAR_d$ and SAL_d during; a) the day of the year with maximum midday sun's elevation angle (Solstice of June for L28 and L40, solstice of December for L21 and the March equinox for L0), b) the day of the year with minimum midday sun's elevation angle (Solstice of December for L28 and L40, solstice of June for L21 and solstice of June for L0) and c) the day of the year with intermediate midday sun's elevation angle to the previous described situations (March equinox for L21, L28 and L40 and a day between the March equinox and the summer solstice for L0). Chosen dates were assumed to represent the annual variability in sun path trajectories and therefore the variability in $STAR_d$ and SAL_d (*STAR*Hi, *STAR*Med, *STAR*Lo and *SAL*Hi, *SAL*Med and *SAL*Lo). Calculations considered the two leaf angles (L_{azi} was corrected for magnetic declination at each location), the location of the populations (geographic coordinates) and the sun position every two minutes for the mentioned '*a- c*' situations at each location. The equation is sensitive to determine whether the exposure occurs in the adaxial or abaxial surface of the leaf (indicated by positive and negative signs, respectively). To estimate *SAL* and *STAR* during the day both adaxial and abaxial intercepting surface were added up. Values around 0 indicate that the leaf is equally intercepting light by both sides. We compared *SAL* and *STAR* values between days of equivalent midday sun's elevation angle for L0 to assure consistency of calculations and interpretation of the variability. In order to enable FV comparisons among sites from northern and southern latitudes the *north* and *south* levels from the crown sector at L0 and L21 were exchanged.

At the individual level we measured maximum tree height, crown perpendicular diameters and the distance between leaf sampling points at each sector (crown depth; from the outer to the inner layer) to have a measure of crown depth averaged per individual. Plant height and crown diameters are positively correlated with the competitive ability and plant performance. For the studied species plant size was also correlated with leaf phenotypic traits such as leaf area or leaf index (Rubio de Casas *et al*. 2011). Crown depth was related to the costs of producing leaves at the outermost part of the crown. Crown depth was related to the costs of producing leaves at the outermost part of the crown. Early works established a notion of trees producing deeper crowns at increasing latitudes (lower sun elevation angles) as being beneficial in terms of light interception (Terborgh 1985; Kuuluvainen 1992) although this notion has been challenged recently in a theoretical work (Vermeulen 2014). The variance partitioning for the short diameter of the crown and for crown middle depth across scales can be found in the supplementary materials (Figure S1).

Single trait analysis

We assessed the intraspecific variability in leaf and crown traits across six ecological scales; 1 - among leaves within crown layers; 2- among crown layers within crown sectors; 3- among crown sectors within a tree; 4- among trees within a population, 5- among populations within a site, and 6- among sites. To assess the scaling of the variance of the selected traits we fitted linear mixed models to all traits including the six nested scales as a random intercept. We also conducted the analyses independently to each site excluding the higher hierarchical scale (*site*).Variability was estimated by maximizing the restricted log-likelihood (*REML*). Leaf traits were *log* transformed except for leaf angles which were transformed to radians to improve normality. Due to missing data the lowest *n* considered for leaf traits was 4621 leaves from 38 individuals.*Multiple traits analysis*To account for the expression of trait syndromes at the individual level, which ultimately influences plant fitness (Reich *et al.* 2003; Wilson & Nussey 2010) we conducted multivariate analyses on leaf and crown traits (Albert et al. 2010b; Boucher et al. 2013). First, we performed a *PCA* to describe the relationship among the studied leaf traits. Secondly, we run *PCA-between* at the

different scales to obtain the inertia of all considered scales and at each site separately and to observe whether the variance was maintained among sites. Thirdly, we added the main axes for foliar traits from *PCA-between* trees to crown traits to assess the variance between populations and sites at the individual level. Confidence intervals for *PCA-between* were obtained by drawing 1000 pseudorandom subsets (three leaves and individuals) and by calculating the lower and upper percentiles (2.5 and 97.5 respectively). The relationship with environmental variables was determined by linear regression of the principal components (*PCA-between* leaf traits at the population level can be found in Table S1). Subsets were obtained maintaining the hierarchical structure which enabled balanced analyses. All *PCA* were conducted on scaled variables. All analyses were performed using R (R development Core Team 2007) using multiple packages (*nlme, ape, ade4, ggplot2, plyr*).

3. RESULTS

Variance partitioning for foliar traits

The variability in functional traits was unevenly distributed across nested scales for leaf and crown traits (Fig. 2, 3 and 4). The analyses of the variance partitioning when considering all scales revealed three groups of leaf traits based on the FV_{WI} and FVsite. The first comprised leaf structure (*SLA*) and leaf length to width ratio (*Lindex*) and showed c. 25% of FVWI and c. 50%-60% of FVsite respectively. On the contrary, leaf angles (L_{tilt}, L_{azi}) and the silhouette to area ratio (*STAR*) showed up to 90% of the variability within individuals and a negligible (absence or below 10%) variability at the *Site* scale (greater overlap in the leaf attributes among s*ites* than differences between their mean values Fig. 2, 3). The combination of leaf angles with the actual leaf area invested to intercept light (*SAL*) formed the third group showing c. 50% of FV_{WI} and 25% of FVsite. The variance among trees and populations (FV_{BI}, FVpop) was below 10% in all traits except for Lindex (FV_{BI} ~ 12%) and *SAL* (FVpop ~15%). The distribution of the variance for *SAL* and *STAR* was maintained during the three considered midday sun elevation angle situations (high, med and low) except for a decrease in FVpop for *SAL*Lo (from 15% for SAL*Hi* and *SAL*Med to ~7% for *SAL*Lo). However,

within individuals the variance was higher (~25%) for *SAL*Lo and *STAR*Lo at the crown sector scale in detriment of the variance at the crown layer, than for *SAL* and *STAR* during high and medium midday sun elevation angles (below 10%, Fig. 3).

The partition of the variance in each site was rather similar among sites for leaf angles and *STAR*. These traits showed a major part of the variance within individuals (FV_{WI} above 80%). On the contrary, *SLA*, Lindex and *SAL* showed different patterns in the variance partitioning among sites. The variance between populations for *SLA* was higher at L21 and L40 (FV_{BI} c. 25%) than for L0 and L28 sites. The variance among individuals for *SLA* was around 10-15% whereas in L0 was absent. Opposite to the main trend observed for all scales the FV_{WI} for *SLA* in each site reached more than 50%. The FVpop for Lindex was negligible in all sites except for L40 in which the variability between populations was c. 12%. *Lindex* showed the highest variability among individuals with values around 50% in L21 and L40 and c. 25% in L0 and L28 sites. However, FV_{WI} for Lindex was higher at each site (50 to 75%) than when all scales were simultaneously considered. The variance partitioning for *SAL* differed among sites, specifically at high and medium midday elevation angles. The variability between populations for SALhi and SALmed were higher (FVpop ~25%) in L0 and L21 than in L28 and L40 sites where FVpop were negligible. The FV_{WI} for *SAL* showed an increasing latitudinal trend from L0 site (c. 50%) towards the site L40 (above 95%). FV_{WI} also increased from high to low midday sun elevation angles in L0 and L21 sites.

Variance partitioning for tree traits

The variance partitioning for crown traits differed among the measured traits and scales (Fig. 4). When considering all scales FVsite was c. 50% for *Hmax* and *crown depth* whereas remained below 15% for crown diameters. However, FVpop was similar for *Hmax* and crown diameters (above 25%) and higher than for crown depth (below 15%). FV_{BI} was above 15% for *Hmax*, above 30% for crown depth and above 50% for crown diameters. The partition of the variance differed

in each site for all crown traits. FVpop was virtually non-existent for *Hmax* in the Site L28, for crown diameters in L0 and for crown depth in L21 and L40 *sites*.

Variance partitioning for multiple foliar traits

The first axis of variation for leaf traits (39% of the variance) was mainly related to leaf area, leaf width and the invested area to intercept light at high and medium midday sun elevation angles (*SAL*Lo, *SAL*Hi above 80%, $p < 0.001$). The second axis (16% of variance) was correlated to *STAR*Hi and leaf tilt (86% and -81%, respectively, $p < 0.001$, Fig. 5a). Within the foliar trait space, individuals were segregated on the first axis accordingly to a latitudinal gradient that discriminated temperate sites from equatorial ones which displayed greater leaf areas, wider leaves, more area dedicated to intercept light during high midday sun elevation angle (Fig. 6a). The variability on foliar traits due to differences among *sites* (inertia of *PCA-between sites*) was similar to that among populations, accounting for one third of the total variance (ALL, Table 2). The higher differences among the levels of the studied scales occurred at the *Tree* scale (*PCA-between trees*, 37%) Within trees, the variance for foliar traits due to differences among *crown sectors* and *layers* remained below 10%. At each *site* the inertia of the *PCA-between* populations was lower than when including all scales. The *site* L0 showed the highest variance between populations (11%) whereas the variance in foliar traits between populations in L28 was 1%. *PCA-between trees* also showed lower variance at each *site* separately (13-20%) (Table 2). The variance due to differences within individuals showed and increased trend from L0 to L40 *sites* at the crown sector scale with higher values than when including all scales. Conversely, L0 showed the lowest variance at the *crown layer* scale (3%) while at the remaining *sites* went up to 6%. In order to conduct analyses on all measured leaf and crown traits we retained the two first axes from the *PCA-between trees*. The first axis from the *PCA-between trees* for foliar traits (63% of the variance) was defined by leaf area, the leaf area potentially exposed (all sun's elevation angle situations) and leaf width (Fig. 5b). The second axis (21% of the variance) was determined by leaf length and *Lindex*.

Variance partitioning for multiple foliar and tree traits

The *PCA* conducted on leaf and crown traits explained 51%, and 26% of the variance. The main axis was defined by plant height and the leading axis of the *PCA-between trees* on foliar traits (correlations above 0.8). The second axis was explained by *crown depth* (Fig. 5c). The variance due to differences between *sites* and *populations* were 55% and 67% respectively. At the *tree* scale, *PCA-between trees* for all traits retained two axes that explained 63% and 20% of the variance (Fig. 5d). The first axis of the *PCA-between* populations for all traits was defined by tree height, the leading axis of foliar traits (leaf size, potential area exposed) and the distance to the middle layer (63% of the variance). The second axis explained 20% of the variance (correlation above 0.85) and was defined by leaf length and *Lindex* (the second axis retained from *PCA-between trees* on foliar traits).

FV Relationship with environmental variables

The two axes from the *PCA-between* populations on crown and leaf traits were regressed against radiation, temperature and precipitation variables at the population level. The first axis was positively and significantly (α<0.05) correlated to irradiance, diffuse light, cloudiness, altitude above sea level and isotermality (Table 3) whereas negatively correlated to temperature seasonality, the mean temperature of the driest quarter and the optimal elevation angle of a flat surface (oriented towards the equator) that maximizes light interception during the year (Table 3). The second axis was negatively correlated to the precipitation of the driest quarter, annual precipitation, precipitation of the driest month, precipitation of the warmest quarter and precipitation seasonality (marginally non significant $0.09 > p > 0.05$) (Table 3).

4. DISCUSSION

Functional Variance (FV) magnitude and structure is, as expected, larger and more complex than previously thought. Our study revealed two patent patterns in the structure of the FV for the studied leaf traits characterized by the contrasting variability among sites. Leaf traits related to leaf's shape and structure (*Lindex*, *SLA*) showed up to 65% of the variance at the site scale. On the contrary, leaf traits related to leaf's spatial position and light interception (L_{tilt}, L_{azi}, *STAR*)

showed virtually no variance among sites and high variability within individuals (above 75%). The variance partitioning within individuals showed an increasing trend from equatorial (L0) to temperate sites (L40) in detriment to the variance between individuals and populations. FV structured individual trees and populations accordingly to a latitudinal gradient from equatorial populations towards higher latitudes. Such distribution was strongly correlated to annual variation in temperature, irradiance and precipitation.

Although available paradigms in the field of functional ecology are based on the relative simple assumption than within species trait variability is negligible and it can be characterized by measuring the trait in a very reduced set of individuals with almost no replicates within them (Garnier et al. 2001; Roche et al. 2004), recent evidences points out that such a variability is relevant and even critical for many ecological and evolutionary processes (Booth & Grime 2003; Boege & Dirzo 2004; Crutsinger *et al*. 2006; Lecerf & Chauvet 2008). FV within individuals in foliar traits may constitute a fundamental feature of plant function that may enable individuals and species to overcome abiotic filters such as excess of irradiance, extreme annual temperatures or low water availability. Our results clearly support this idea linking leaf form and function to environmental variation at many spatial scales and call for the formulation of a completely different framework in which variability in plant traits is accounted for at the individual scale. Even more, they suggest that such variability may be critical for individual responses to shifting environmental conditions but also in the assembly of communities as a whole.

We found that variability within individuals was at least one quarter of the total variance for all traits except for *Lindex*. Likewise, the crown layer scale constituted above 10% of the variance in the majority of traits. Thus, the variation in leaf attributes appears to be non-randomly distributed within the spatial position of the tree crown.. These results are in agreement with previous studies that found complementarity of the productive photosynthetic processes among groups of leaves exposed to contrasting conditions and spatio-temporal splitting of tasks (Granado-Yela *et al*. 2011). Nevertheless, a large part of FV_{WI} remained

unexplained. It is likely that other processes acting at the leaf level such as leaf's micro-environmental conditions (self-shading, degree of aggregation of leaves) may drive variation in leaf angles and potential light interception attributes. Indeed, light interception efficiency is directly related to leaf clumping and leaf area to total crown area in small trees and shrubs (Duursma *et al*. 2012). Strikingly, only FV_{WI} for *SAL* (combination of leaf angles and leaf area, c. 50%) was similar to those values found in other traits in the literature (Albert *et al*. 2010a) Nonetheless, *SAL* and *STAR* revealed an increasing trend in FV_{WI} associated to both the site scale (higher FV_{WI} at higher latitudes) and midday sun elevation angle (higher FV_{WI} during *low* annual midday sun elevation angle). This finding suggests a greater influence of filters operating at the leaf level such as the experienced within-canopy light gradient, manifesting a spectrum of FV within individuals of a species in one side of the leaf economic spectrum (Wright *et al*. 2004; Niinemets *et al*. 2014).

Leaf attributes greatly overlap among individuals and between populations (FV_{BI}, FVpop and FVsite) except for *SAL*, *SLA* and *Lindex* (3-15%). Despite the fact that it is not possible to distinguish between ecotypic and plastic sources of variation in field and non-manipulative studies (Niinemets, 2015), the variance partitioning among trees at each individual site was considerably high for Lindex (c. 25% of the variance or even higher) whereas *SLA* and *SAL* showed higher FVpop. The variance partitioning for these traits was not conserved when the analyses where conducted at each site independently. High FV_{BI} and low FVpop for *Lindex* may reveal a strong genetic component on leaf elongation attributes. It is the only trait that does not conserve trait variability at the tree scale since individuals of the same population are expected to endure similar environmental conditions. Likewise, *SLA* can be associated to the environment as shown by low FV_{BI} values (except at site L28) and medium values of FVpop (up to 25%). FVsite was high for *SAL*, *Lindex* (above 50%) and SAL (25%) indicating that variation in these traits can be potentially influenced by ecological processes such as climatic conditions operating at the *site* scale (Messier *et al*. 2010). Ultimately, multiple trait analyses revealed a similar variance across sites, populations and trees (30-40%) and a very low contribution of c. 5% of the variance due to differences

within individuals. This result indicates that populations and individuals integrate a wide range of environmental heterogeneity and/or genetic variation. Even more, the variance within individuals was higher when the variance partitioning was explored independently at each site. This result reveals that the spatial distribution of leaf attributes within the tree crown may be adjusted at each site in which prevails either variation among crown sectors (equatorial populations) or among crown layers (the remaining populations). We propose that the crown irradiance patterns may drive the differences in FV at the crown sector and layer scales due to differences in the apparent sun path trajectory at each site. Nevertheless, despite including a wide geographical range, the annual sun path trajectories greatly overlap among sites which may foster the magnitude of FV_{WI} that occur at each site.

The main axes of variation in foliar traits were related to leaf size, leaf tilt and light interception at high solar elevation angles during the year. The axes that maximized the variation between individuals were also highly correlated to leaf shape and structure and explained 80% of the variance in the leaf and crown attributes at the individual scale (Fig. 5a, b). *SLA* and plant height shared the main axis and direction of variance which is coherent with previous findings (Diaz *et al*. 2004; Gross *et al*. 2007) differentiating an end formed by the biggest plants (taller with greater crown diameters and less crown depth) with bigger leaves with high SLA that are able to intercept more light (greater *SAL*) at any situation during the year (NB, GG). As shown in previous studies, *SLA* and Lindex constitute a part of the leaf economic spectrum of variation among plants related to light capture efficiency (Takenaka 1994; Niinemets 1999; Ryser & Urbas 2000). Indeed, *SLA* and Lindex were highly correlated to the main axes of variation at the individual level (above 0.7, p <0.001). Plants experiencing stressful conditions, such as drought and high irradiance levels develop thick, smaller leaves with steeper leaf angles than plants growing in less adverse conditions (Gratani & Bombelli, 2000). In these relatively sunny and dry environments, increased maintenance costs at the expense of vegetative growth tends to reduce plant size and indirectly reproductive performance (e.g. Samson & Werk, 1986; Weiner *et al*., 2009; García-Verdugo, 2010). Thermal tolerance limits imposed by winter

minimum temperatures experienced in AF population (Vargas and Kadereit, 2001; Rubio de Casas et al., 2002) coupled to high radiation periods with low water availability should lead to strong directional selection pressures for increased resistance to drought and high radiation, whereas in less seasonal sites (L0) competition is expected to be stronger and lead to phenotypic divergence for niche partitioning between interacting individuals, thus resulting in increased trait variance (Cornwell, Schwilk & Ackerly 2006). Populations are expected to differ in relation to the importance of biotic and abiotic limiting factors (Gaston, 2009) nonetheless leaf attributes in the studied populations appear to be heavily related to the environment. Even more, at the population scale leaf and crown traits highly correlate with annual variation in irradiance, temperature and precipitation. Despite wide geographical distances the main axis of variation showed that populations at L21 and L28 sites were similarly ranked between the two extremes represented by populations at L0 and L40 sites. However, populations at L28 site despite thriving at similar absolute latitude than L21 reaches the lower rainfall limit of the species (Pansiot and Rebour, 1961). Interestingly our study showed that leaf shape highly correlates with the second component of variance between individuals and populations and with low rainfall and high precipitation seasonality. Elongated leaf shapes may therefore provide certain advantages related to water economy in these populations. The link between functional traits and climatic variables such temperature and precipitation at global scales along with high levels of FV_{BI} and FVpop in equatorial populations support historical theories postulating that the absence of seasonality would lead to greater stability of the populations across wide latitudinal gradients (MacArthur 1972; Schemske et al. 2009). Even more, we showed that rather than annual mean values, the annual distribution of irradiance, temperature and precipitation might define leaf shape and structure attributes rather than annual mean values.

5. CONCLUSIONS

In the present study we have quantified the intraspecific trait variation for single and multiple leaf and crown traits and showed the relationship between traits and environmental gradients across nested ecological scales. From the single traits

analyses we conclude that FV within individuals constitutes a relevant part of the variance partitioning, specifically when sites where considered independently. Including how FV is distributed quantitatively across scales may constitute a valuable input in trait based models to identify mechanism controlling habitat filtering and niche differentiation. Multiple trait analyses revealed that plant trait variation respond to foreseeable factors such as annual variation in irradiance, temperature and precipitation. Finally, we believe that results presented here highlight the need to revise our current approach to the study of trait variation across global scales and that provide a valuable input for trait-based models.

ACKNOWLEDGEMENTS

Special thanks to A Vázquez, M Nicholson, S Pichillou, C Weyns and Mamen for their help and advice on the field. Thanks to J Messier for sharing the R script to develop the bootstrap process and CI estimations. This research was funded by the Spanish Ministry of Science and Education (project CGL2009-10392), through a FPI grant to A.G.E. (BES-2010-032767). We are also indebted to the Madrid Regional Govt. (project REMEDINAL-2, S2009/AMB-1783)

6. TABLES AND FIGURES

Table 1. Population coordinates and environmental characteristics.

Population	Pop_ID	Lat.	Long.	Site	m.a.s.l. (m)	Annual Mean Temperature (°C)	Mean Diurnal Range (°C)	Isothermality	Temperature Seasonality	Annual Precipitation (mm)	Precipitation Seasonality	Cloudiness (%)	Clear-sky days	Insolation (kWh/m2 day)*	Diffuse light (kWh/m2 day)*
Palmistes Rouge (La Reunion Island)	PR	21.16 S	55.47 E	L21	950	18.40	6.30	5.20	217.30	1557.00	50.00	50.20	3.83	5.93	1.60
Tamarins (La Reunion Island)	TM	21.06 S	55.25 E	L21	350	22.00	6.10	5.20	211.70	1414.00	54.00	50.20	3.83	5.93	1.60
Nairobi (Kenya)	NB	1.29 S	36.65 E	L0	1760	17.40	11.50	7.30	124.60	973.00	76.00	60.20	1.25	5.92	1.89
Gilgil (Kenya)	GG	0.47 S	36.29 E	L0	2050	16.90	16.30	8.20	74.00	677.00	38.00	63.80	0.17	5.77	1.93
Anaga (Canary Islands)	AN	28.56 N	16.15 W	L28	370	18.00	7.00	4.60	266.20	424.00	80.00	50.50	2.83	5.62	1.50
Finca Amado (Canary Islands)	FA	28.66 N	17.78 W	L28	385	18.40	6.60	4.40	276.80	384.00	84.00	50.20	1.42	5.79	1.43
San Luis (Balearic Islands)	SL	39.82 N	4.27 E	L40	50	16.80	7.10	3.30	499.00	612.00	53.00	48.40	5.92	4.82	1.35
Aldea del Fresno (Central Spain)	AF	40.33 N	4.24 W	L40	500	13.80	10.70	3.50	659.80	389.00	34.00	52.20	4.92	4.39	1.45

Extrapolated from WorldClim at .5’ resolution and from NOAA webcalculator

*Incident on a horizontal surface

Table 2. Inertia of *PCA-between* for foliar traits on all data (ALL) and on each *site*.

	ALL	***L0***	***L21***	***L28***	***L40***
Site	27.8 [27.23- 28.76]	-	-	-	-
Population	31.86 [31.69- 33.41]	10.99 [9.19- 12.29]	7.86 [5.39- 8.72]	1.15 [1.01- 1.84]	4.33 [3.35- 5.05]
Tree	37.78 [37.76- 39.64]	20.1 [18.06- 21.96]	17.68 [15.94- 20.42]	12.93 [12.64- 16.17]	15.13 [14.06- 17.6]
Crown Sector	1.63 [1.64- 2.16]	5.68 [5- 6.69]	4.54* [4.98- 7.98]	3.39 [3.4- 5.79]	2.51* [2.74- 5.15]
Crown Layer	5.62* [3.66- 4.71]	3.66 [3.04- 5.23]	6.91 [5.67- 8.95]	6.45 [5.97- 9.47]	6.64 [6.49- 9.4]

Table 3. Correlation between the main axes of variation for crown and leaf traits at the population level against climatic variables.

Axis1 PCA-Between populations	r^2	*p*
Isotermality	0.77	**0.002**
Diffuse light	0.76	**0.003**
Altitude	0.51	**0.026**
Cloudiness	0.46	**0.038**
Isolation	0.42	**0.047**
Mean Temperature Coldest Quarter	0.37	0.064
Annual Optimal Angle	0.79	**0.002**
Temperature Seasonality	0.67	**0.008**
Mean Temperature Driest Quarter	0.56	**0.02**
Annual clear-sky days	0.37	0.064
Axis2 PCA-Between populations		
Precipitation Driest Quarter	0.41	0.051
Precipitation Seasonality	0.32	0.082
Precipitation Driest Month	0.31	0.089
Precipitation Warmest Quarter	0.3	0.091
Annual Precipitation	0.29	0.095

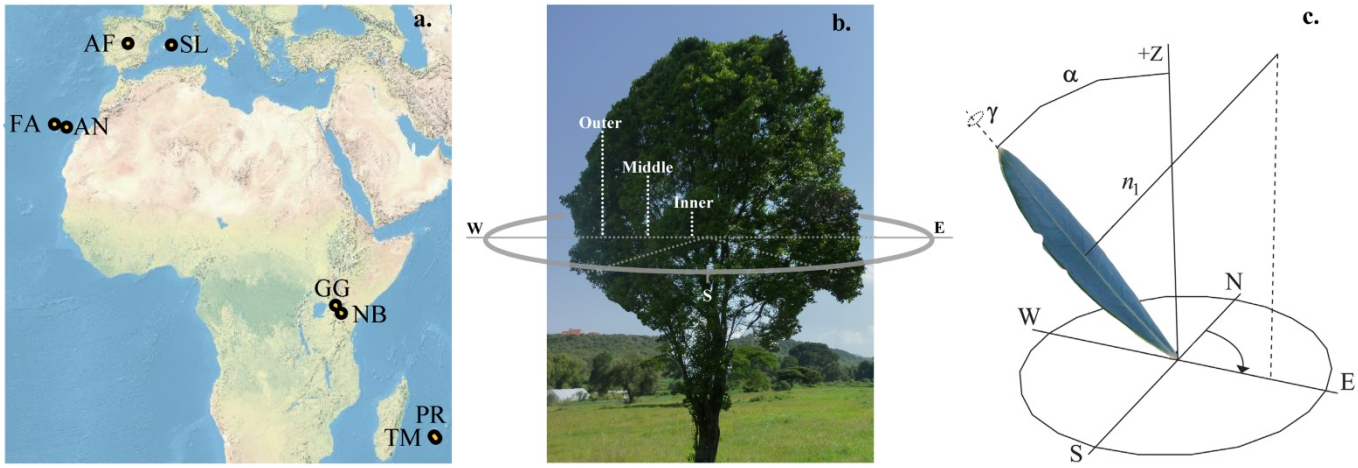

Figure 1. Location of the studied populations along a latitudinal gradient (a), sampling design within trees (b) and detail of leaf angles (c). Sampled populations span from 40°N to 21°S. Each tree was divided into four sectors according to main cardinal points (NESO) and into three layers at each sector (Outer, middle and inner layer) where leaves were sampled. Leaf's spatial position was determined by three leaf angles; the inclination angle of the leaf's midrib above horizontal (alpha), the roll angle of the leaf's midrib (gamma) and the orientation of a normal vector (n_1) to the leaf lamina (Leaf azimuth, from 0° North to 180° South, clockwise). The inclination of the leaf surface (Leaf tilt, from 0° horizontal to 90º vertical) was calculated through combining alpha and gamma angles following equation 3 from Escribano-Rocafort et al. (2014).

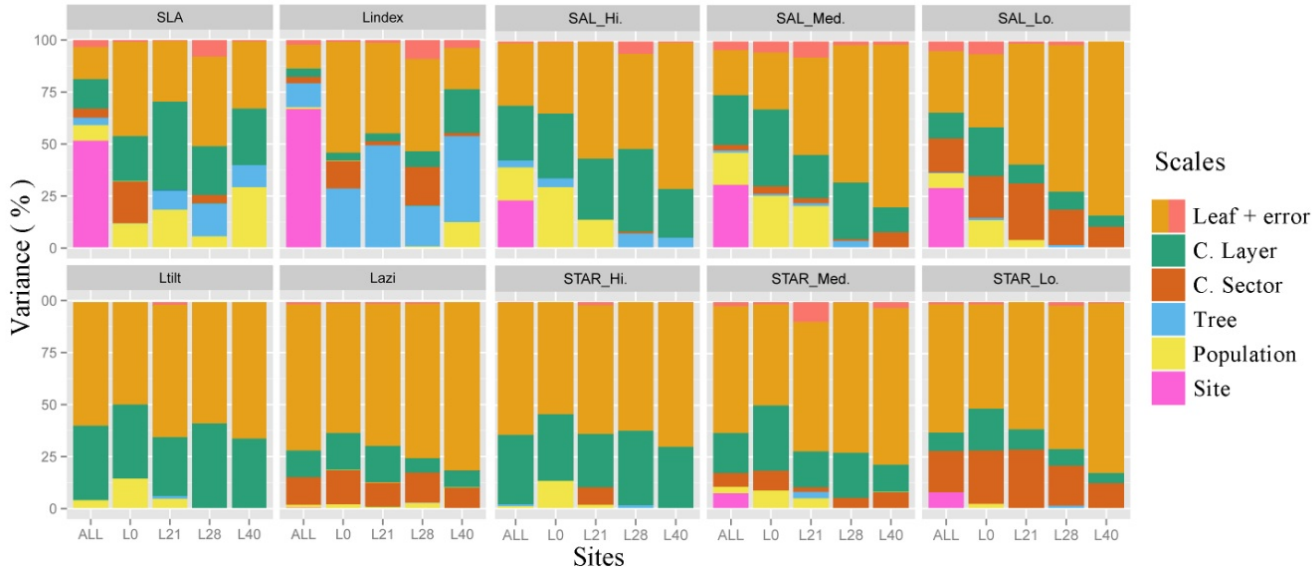

Figure 2. Variance partitioning across nested scales for specific leaf area, leaf length to width ratio (Lindex), leaf angles; inclination of the leaf surface above the horizontal (Ltilt) and orientation of the leaf lamina (Lazi) and the projected leaf area (*SAL*) and the silhouette ratio to leaf area (*STAR*) on all data (ALL) and in each *site* (L0 to L40). Leaf Trait~1, random= ~1| Site/ Population/ Tree/ C.sector/ C.layer/ Leaf. SAL and STAR were calculated for high to low midday sun elevation angles during the year (high midday sun elevation angle during the summer solstice and low elevation angle during the winter solstice in the northern hemisphere and vice versa in the southern). For L0 (equatorial population) high midday sun elevation angle occur during the equinoxes, medium elevation angles occur between solstices and equinoxes and 'low' midday sun elevation angles occur during solstices (March equinox, June solstice and a situation in between were chosen for L0 calculations). The variance partitioning for SAL and STAR was investigated on all data (ALL) and in each *Site*. Leaf Trait~1, random= ~1| Site/Population/Tree/C.sector/C.layer/Leaf

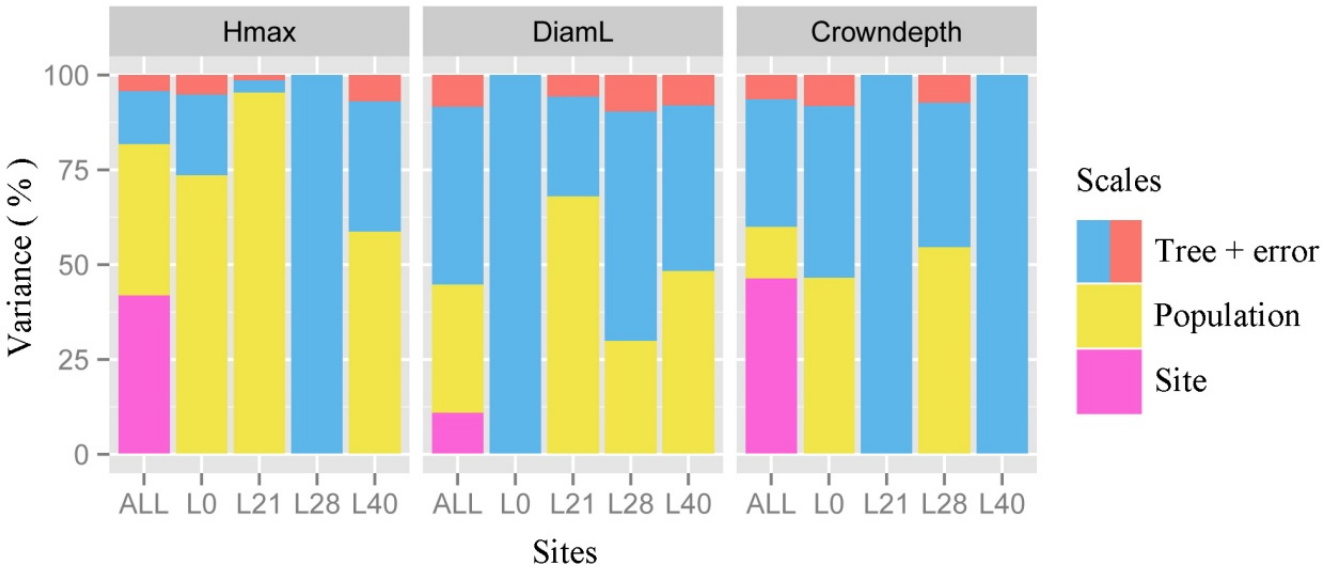

Figure 4. Variance partitioning across nested scales for tree max height (Hmax), the longer diameter of the tree crown (DiamL) and the crown depth from the outer layer towards the inner layer where leaves were present (Crowndepth) on all data (ALL) and in each Site. Crown Trait~1, random= ~1| Site/Population/Tree

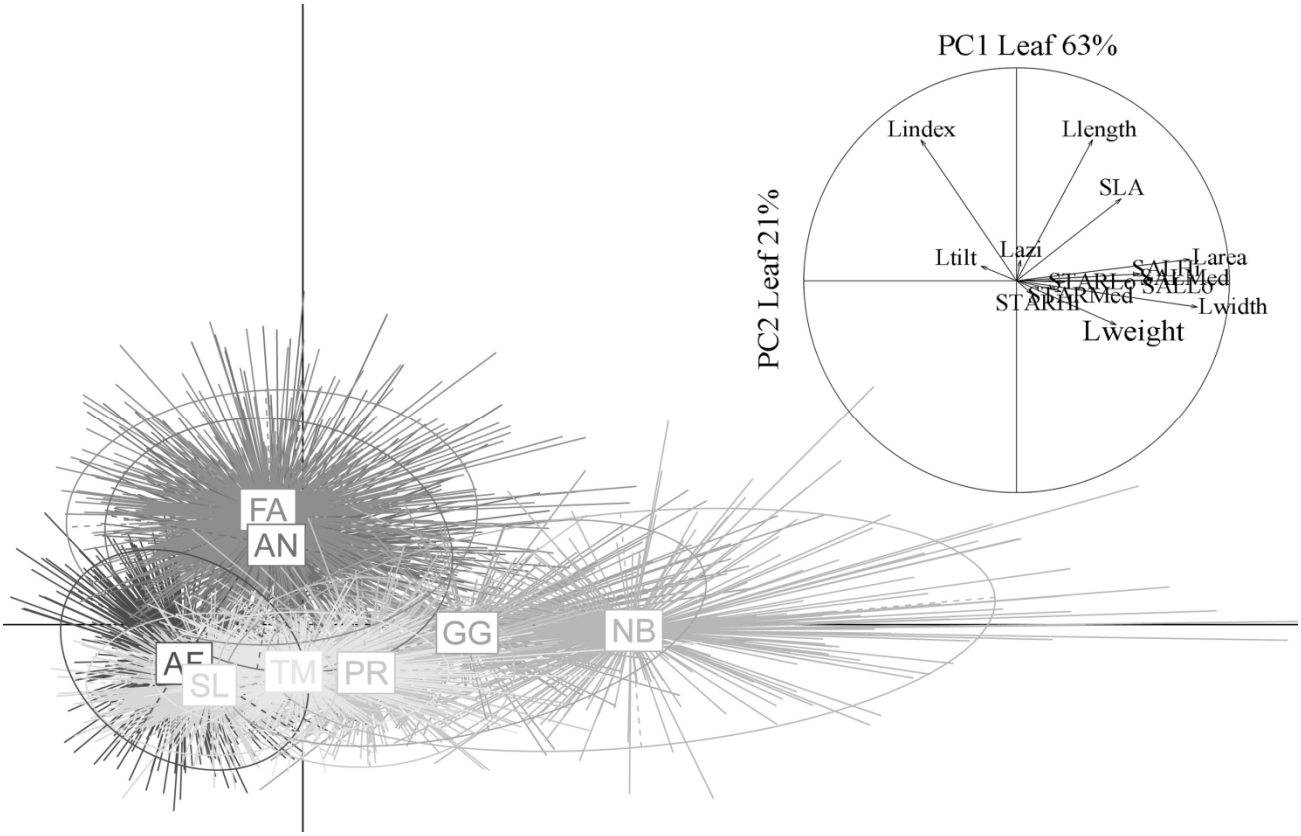

Figure 5. Multidimensional structure within the foliar trait space. Correlation circles and the two-first principal components (PC1, PC2; upper right) from *PCA-between* populations for foliar traits (inner left). Population codes as in Figure 1.

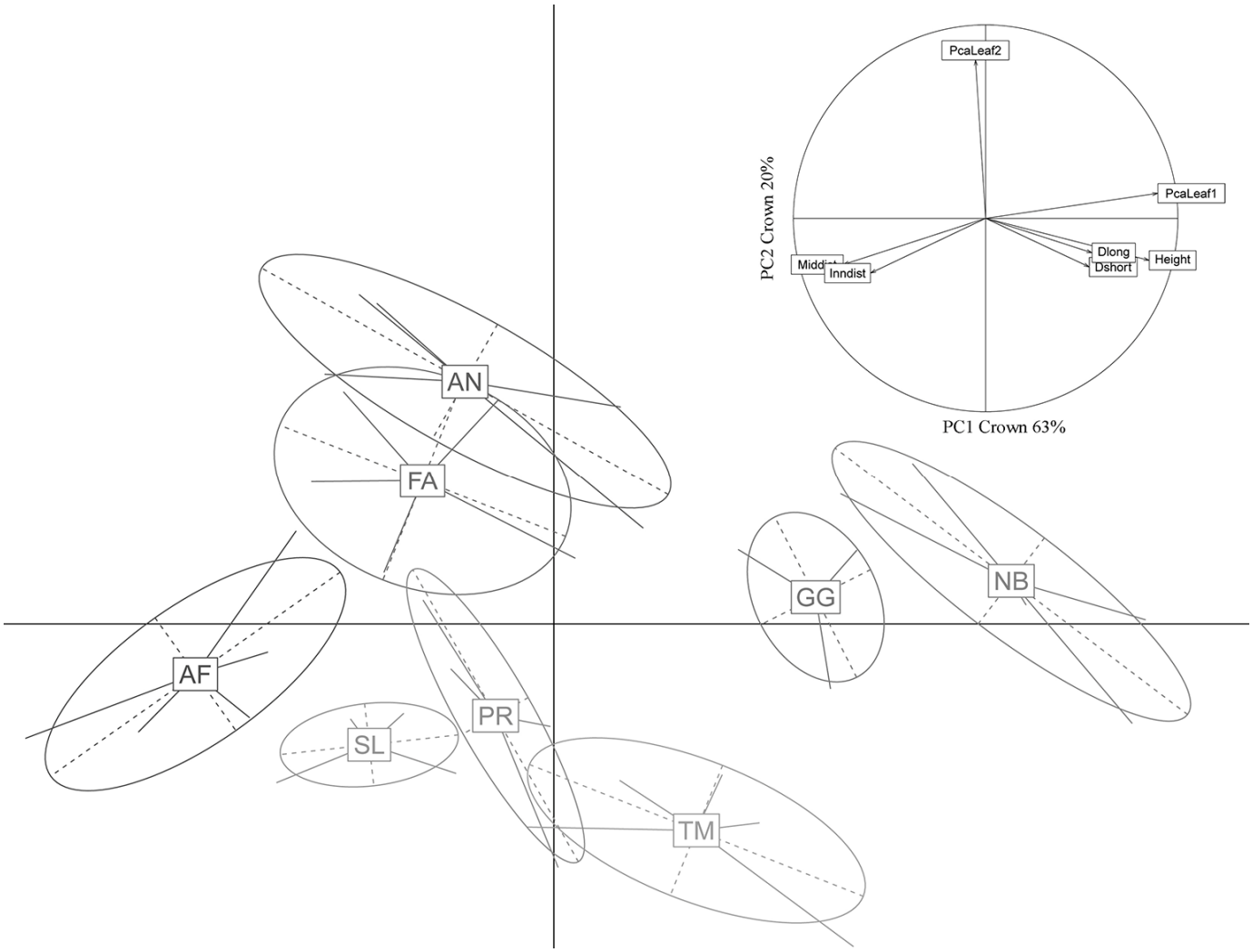

Figure 6. Multidimensional structure for foliar and tree traits. Correlation circle and the two-first principal components (PC1, PC2; upper right) from *PCA-between* populations for tree traits and the two first principal components for foliar trait variance between populations (inner left). Population codes as in Figure 1

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8. SUPPLEMENTARY MATERIALS

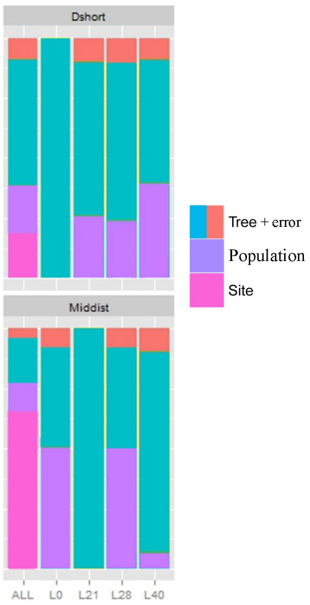

Figure S1. Variance partitioning across nested scales the shorter diameter of the tree crown (DShort) and the crown depth from the outer layer to the middle layer on all data (ALL) and in each Site.

Table S1. Regression of *PCA-between population* scores on leaf traits against climatic variables.

Axis1 PCA-Between Leaf traits	r^2	*P*
diffus	0.8	0.002
Isotermality	0.77	0.003
m.a.s.l.	0.72	0.005
Cloudiness	0.56	0.02
insolation	0.31	0.088
AnnOptAngle	0.75	0.003
Mean Temp Driest Quarter	0.74	0.004
MeanTempWarmestQ	0.57	0.018
Temp Seasonality	0.54	0.022
clear-sky days	0.42	0.048
Axis2 PCA-Between Leaf traits		
AnnPrecp	0.48	0.033
PrecSeasonality	0.46	0.039
PrecDriestQuarter	0.41	0.053

CAPÍTULO IV

Variability of light-related foliar traits across spatial and temporal scales in the Mediterranean evergreen *Olea europaea* L.

Enviado a Trees Structure and Function

ABSTRACT

- The variability in space and time of a given set of traits can describe the biological activity of plants and influence individual performance and the properties of communities and ecosystems. However, seasonal variance in foliar traits of tree species has been poorly addressed in temperate latitudes despite its potential implications in fundamental processes such as light interception strategies.

- We partitioned the variance for a set of light-related foliar traits across six nested scales in two populations of the evergreen tree *Olea europaea* L. Trees were sampled at two contrasting seasons (*summer* vs. *winter*). We conducted single and multivariate analyses to assess the variability within and among individuals and populations during these two periods and investigated whether physiological adjustments in leaf attributes alter the structure of the variance partitioning across scales.

- Differences in the mean trait values between seasons were significant for most but the variance between seasons was low or absent except for the potential leaf surface invested to intercept light. Principal component analyses revealed that differences between seasons account for ~5% of the variance and that the tree scale held up to 20% of the variability in the measured set of traits.

- Despite significant differences between traits measured at contrasting seasons, the variance partitioning was mainly conserved between seasons for most traits. Seasonal variability was c. 25% for the potential surface invested to intercept light. The high levels of intraspecific variance across spatial scales among and within individuals challenge the use of mean trait values as species descriptor in trait based models.

Keywords: functional traits, variance across scales, leaf attributes, Mediterranean evergreen, temporal variability,

1. INTRODUCTION

There is a growing interest on quantifying the intraspecific variation of plant functional traits (FV) across scales due its effects on ecosystem processes (Booth & Grime 2003; Boege & Dirzo 2004; Crutsinger *et al*. 2006; Lecerf & Chauvet 2008). A critical assumption in functional ecology is that differences in FV within species is insignificant compared to differences among species (Garnier *et al*. 2001; Roche et *al*. 2004). Nonetheless, evidence suggest that intraspecific FV should not be neglected, especially in communities dominated by one or few species and those subjected to low resource availability (Violle *et al*. 2012; Niinemets 2014). Indeed, including FV in computer trait-based analyses improve their ability to address community processes (Sitch *et al*. 2003; Norberg *et al*. 2001; Savage, Webb & Norberg 2007; Kraft *et al*. 2014; Siefert 2012). The structure of intraspecific FV, from lower to upper organizational scales, can be decomposed into three elements; the variability within individuals (FV_{WI}), the variability among individuals (FV_{BI}) and the variability among populations (FVpop). The differences in these three components arise from diverse spatio-temporal scales and hierarchical levels; from leaves to individuals and populations (Albert *et al*. 2011). However, despite the advances made to assess how the variance is partitioned across different nested spatial scales (Messier *et al.* 2010; Violle *et al.* 2012) there is a lack of studies providing how FV is structured in a temporal framework. This is surprising since temporal heterogeneity experienced at the leaf and individual scales are assumed to shape many biological processes. For instance, the daily and/or annual patterns of irradiation appear to be critical to plant function affecting phenomena such as light interception, the photosynthetic efficiency and ultimately, the whole-crown light caption strategy and plant performance. In fact, the temporal patterns in the light gradient within canopies may play a relevant role in the expression of distinct leaf attributes shaping the within-canopy economic spectrum (Niinemets *et al*. 2014).

Individuals of long-lived plants such as many woody species are expected to respond to environmental heterogeneity through the expression of different leaf phenotypes and the prevalence of a set of coordinated traits (individual´s

phenotypic syndrome) that affect the fitness of the whole plant (Bradshaw 1965; Sultan 1987; Petit & Hampe 2006; Marks & Lechowicz 2006; Wilson & Nussey 2010). In this sense it is well known that intra-canopy gradients in light quality and quantity can trigger the expression of different leaf phenotypic syndromes (i.e., sun and shade leaves; Jurik, Chabot & Chabot 1979; Kull and Niinemets 1993, Terashima & Hikosaka 1995; Niinemets 2001; Valladares & Niinemets 2008). Indeed, among plant functional types, trees show high levels of functional variability within individuals (Messier *et al*. 2010). The spatial variation in light related attributes across the tree crown such as leaf shape, structure and position can alter light properties, light transmission and influence daily light interception (Howell *et al*. 2002; Falster & Westoby 2003; Uemura *et al*. 2006). In fact, modulating light interception within the crown can confer trees advantages such as enhancing whole individual carbon gain (Uemura *et al*. 2006), displaying higher degree of control on the available resources (Bellingham & Sparrow 2000) and further altering biotic interactions such as competition for light (Hikosaka & Hirose 1997).

In high irradiance environments subject to additional sources of stress such as low water availability and extreme temperatures found in Mediterranean climates, the expression of leaf attributes dedicated to modulate light interception within the crown can be advantageous. For instance, the expression of thick and narrow leaf shapes along with vertical leaf angles at the outer crown can contribute to avoid the negative impact of direct light interception during high irradiance periods in Mediterranean regions (Niinemets 1999, Valladares & Pugnaire 1999, Rubio de Casas *et al*. 2011, Granado-Yela *et al*. 2011). The dominant ecosystems in the Mediterranean region are arid or semi-arid characterized by a dry period during summer and low temperatures during winter that may limit plant growth (Specht 1969, Di Castri 1973; Sperlich *et al*. 2014). In this sense and without considering climatic factors such as cloudiness or wind, light interception for woody species whose leaves are not able to track/avoid direct radiation are heavily influenced by the annual and daily irradiance patterns in a given location. At increasing latitudes the differences between seasons increase in terms of day length and maximum sun elevation angles. Therefore, species thriving in Mediterranean region are

subjected to diurnal and annual variation of the patterns of irradiance which generate periods of stressful conditions encompassed with very high or low temperatures and low water availability that have direct consequences on the photosynthetic function. Given that seasonal variation in leaf traits are crucial to understand plant and vegetation responses to distinct environmental conditions (Sperlich *et al*. 2015) here the question arises as whether light-related leaf traits and their variance across scales differ between contrasting seasons and day periods and what is the magnitude of the temporal scales of variation in those traits.

In the present paper, we examine the variance partitioning of light-related leaf traits across nested spatial scales (*Population/ Tree/ Crown Sector/ Crown Layer/ Leaf*) between two contrasting seasons in terms of irradiance, temperature and water availability. We conducted our research with data gathered from previous studies in two populations of the sclerophyllous tree *Olea europaea* L. in the Mediterranean region. Both populations occur at the same latitude assuring similar sun path trajectories and hence similar annual irradiance patterns. We expect that individuals of both populations would be facing similar constraints in the expression of leaf attributes within the crown due to spatial heterogeneity (product of the interaction of the phytoelements with the environment) and to temporal variation in the patterns of irradiance within the day and between seasons. We tested whether leaf structure, leaf shape, leaf angles and potential light interception differ between seasons and investigated the variance partitioning across scales for each trait and on a multitrait basis. In addition, we hypothesize that the annual (season) and daily (time) variation in the irradiance patterns will have a significant effect on the potential light interception of the leaves without considering leaf overlapping.

2. MATERIALS AND METHODS

We included in our study two populations of *Olea europaea* L. trees occurring at similar latitude with and hence, similar sun path trajectory (40ºN). Trees were relatively isolated and showed signs of fruit production in previous years. Leaf traits were sampled in the same individuals (nine in total) in two consecutive seasons during July 2011 and February 2012. Leaf shape (leaf length-to-width ratio), leaf structure and leaf angles (leaf tilt and leaf azimuth) were measured across two spatial scales within individuals; crown sector and crown layer. The crown sector scale is product of the division of the upper part of the crown into four sections centred at compass points (four levels NESO). Each crown sector was subdivided into three crown layers: the outermost layer of leaves (*Outer*), the middle layer of leaves (*Middle*) and the deepest layer where leaves were present (*Inner*) in an oblique direction towards the geometrical centre of the crown. Inner and outer canopy layers of wild olive trees show pehenotypic variation in their leaves that correlates with light availability at the population level (García-Verdugo *et al*. 2010; Rubio de Casas *et al*. 2011). To account for the decrease in leaf density from the outermost layer to the inner layer we sampled 20, 10 and 5 leaves at the outer, middle and inner layers of each sector, respectively (n = 140 leaves per individual). The total number of considered leaves was 2520 in two populations (2 Seasons × 9 Trees × 4 Crown sectors × 35 leaves).

Leaf traits

The relative position of leaves was obtained by measuring in situ two leaf angles using a smartphone (Nokia N86, Nokia, Spoo, Finland) following Escribano-Rocafort *et al.* (2014). By placing the cell-phone parallel to the desired leaf lamina we obtained the leaf's surface inclination angle to horizontal (Leaf tilt, L_{tilt}; 0° horizontal, 90° vertical) (Eqn. 1; Escribano-Rocafort *et al*., 2014) and the orientation of the leaf lamina to true north, i.e. the projection of a normal vector to the leaf's adaxial surface (Leaf azimuth, L_{azi}; 0° North, 180° South, clockwise) (Fig. 1c). Measurements corresponding to a population and a season were taken on fully expanded leaves during a single non-windy day. After leaf angle measurements we collected and scanned all leaves (*HP-ScanJet* 3800; *Hewlett*

Packard; Palo alto; CA; USA) to estimate the one-side area of the leaf blade, leaf width and leaf length using ImageJ (NIH, Maryland, USA). Leaves were then oven-dried at 65 °C for 48 h and weighted to calculate leaf dry weight with a precision balance (Mettler Toledo, Greifensee, Switzerland). From these measurements we calculated the specific leaf area (*SLA* = leaf area by dry weight) and a leaf shape (*Lindex* = leaf length to width ratio). On the leaf economic spectrum *SLA* is considered a key trait related to the photosynthetic function and carbon investment. *Lindex* constitutes a leaf shape descriptor linked to within crown environment, leaf gas exchange, and structural constraints (Rubio de Casas *et al*. 2011).

To examine the potential ability of each leaf to intercept light, we calculated the silhouette area of the leaf blade (*SAL*: cm^2) and the ratio of the silhouette to total leaf area ratio (*STAR*: %), which relates leaf irradiance to incident radiation without considering leaf overlapping (Carter & Smith 1985; Smith & Hughes 2009; Granado-Yela *et al*. 2011). To assess the daily silhouette area of the leaf blade (SAL_d) and the daily silhouette to area ratio ($STAR_d$) we calculated the integral of the leaf projection from sunrise to sunset for the day of the measurements every two minutes. In addition, we estimated SAL_t and $STAR_t$ for five day periods (T1 to T5) in which T3= solar noon, T1=sunrise, T5= sunset and T2 and T4 intermediate situations between T1-T3 and T3-T5 respectively. The calculations considered both leaf angles (L_{azi} was corrected for magnetic declination at each location), the location of the populations (geographic coordinates) and the sun position at each location. The used equation is sensitive to determine whether the exposure occurs in the adaxial or abaxial surface of the leaf (indicated by positive and negative signs, respectively). Values around or below 0 indicate that maximum potential interception occurs during a certain period of the day.

Single trait analysis

We assessed the intraspecific variability through a hierarchical structure in leaf traits at the population (FVpop), individual (FV_{BI}) and within individual levels (FV_{WI}) across five spatial scales; 1 - among leaves within crown layers; 2- among

crown layers within crown sectors; 3- among crown sectors within a tree; 4- among trees within a population, 5- among populations within a season and to one temporal scale; 6- among seasons. To assess the scaling of the variance of the selected traits the aforementioned nested structure was used a random intercept in linear mixed models as a baseline for comparisons. We conducted analyses on all traits including the six scales and to the set of traits of each season independently. Variances were estimated by maximizing the restricted log-likelihood (*REML*). Leaf traits were *log* transformed except for leaf angles which were transformed to radians to improve normality. Due to missing data the lowest *n* considered for leaf traits was 2454 leaves from four and five individuals on each population respectively.

Multiple traits

To account for the expression of trait syndromes at the individual level which ultimately influences plant fitness (Reich *et al.* 2003; Wilson & Nussey 2010) we conducted multivariate analyses on leaf traits (Albert *et al*. 2010b; Boucher *et al*. 2013). Details of *PCA* can be found in supplementary materials (Table S1, S2, S3). First, we performed a *PCA* to describe the relationship between the studied leaf traits. Secondly, we run *PCA-between* at the different scales to obtain the inertia of each scale in leaf traits. All *PCA* were conducted on scaled variables. All analyses were performed using R (R development Core Team 2007) using multiple packages (*nlme, ape, ade4, ggplot2, plyr*).

3. RESULTS

Seasonal

Results of three-way ANOVAs testing for the effects of season, crown sector and crown layer on leaf traits are shown in table 1. The season factor showed a significant effect in all traits except for *SLA, Lindex* and *Lazi*. The interaction among season, crown sector and crown layer was only significant for *Lazi* and marginally non-significant for $STAR_d$ (Table 1). Despite differences in the majority of leaf traits between seasons the partition of the variance at the season scale was absent or below 10% except for SAL_d and $STAR_d$ (~25%; Fig. 1). Likewise, the variance between populations was below 10% or absent for all traits

except for leaf width (FVpop ~25%). The variance at the tree scale was c. 20% for leaf area, leaf length and *SLA* whereas was higher for leaf width (FV_{BI} 25%) and *Lindex* (FV_{BI} above 40%). The variance within individuals (FV_{WI}) constituted the scale with the highest variance for all traits. FV_{WI} was ~50% of the total variance for leaf width and *Lindex* and above 70% for the remaining traits. The variance partitioning at each scale did not differed between seasons except for *SLA*, leaf tilt, SAL_d and $STAR_d$ (Fig. 2). The variance for these traits at the tree scale was higher for the set of traits measured during summer than for the winter ones.

The two leading axes of the *PCA* explained 28.9% and 25.3% of the variance for the set of traits respectively. The first axis (*PCA*1) showed a correlation above 80% to leaf area, weight, width and to a lesser extent to leaf length (67%). The second axis (*PCA2*) was correlated to $STAR_d$ and SAL_d (above 80%) and to leaf tilt (73%; Table S1, S2). The inertia of the *PCA-between* seasons revealed that the *season* scale accounted for 5% of the variance for the set of traits. The *tree* scale showed the highest variability (inertia *PCA-between trees* ~20%) whereas within individuals the variability was below 5% (Table 2). Independent analyses on each season showed that the variance partitioning was similar between *summer* and *winter* traits being FV_{WI} for winter traits slightly higher than for the traits measured in summer (Table 2).

Diurnal

The variance in the patterns of irradiance entailed differences up to 25 % between maximum irradiance periods (T3) at each season for $STAR_t$. Differences in leaf angles between seasons revealed and increase in $STAR_t$ during winter compared to the potential $STAR_t$ achieved through display of summer leaf angles during winter season. Likewise, summer $STAR_t$ estimated with winter leaf angles was higher than the actual $STAR_t$ due to leaf angles measured during summer (T2 to T4, Fig. 3). The partition of the variance for $STAR_t$ and SAL_t was mainly distributed within individuals (FV_{WI} ~100%) except during T3 in summer (FV_{BI} ~10%). The variability among crown sector and layers was up to 12.5% at each scale for SAL_t and $STAR_t$ in summer (Fig. 4). However, the variance partitioning for $STAR_t$ during the different day periods at the crown layer scale was lower in winter than

in summer. The variance partitioning across scales within individuals showed a daily pattern from T1 to T5 in which the variance decrease among crown sector and crown layers towards midday (T3) and increased again during the afternoon (T3 to T5). This trend was more prominent during winter (Fig. 4).

4. DISCUSSION

Our results showed that despite differences in leaf traits between summer and winter measurements, the variability at the season scale was low or negligible for most traits except for SAL_d and $STAR_d$ (~25%) coherent with the variation in the sun path trajectory. The variability between seasons for the set of traits was below 5% and was even lower (~2%) when SAL_d and $STAR_d$ were excluded from the analyses (Table S4). However, we found two general patterns in the partition of the variance across spatial scales among leaf traits. On one hand, leaf traits related to leaf shape and structure (*Lindex*, *SLA* and its constituents) showed above 50% of FV_{WI} and idiosyncratic levels of FVpop and FV_{BI}. On the other hand, leaf traits related to leaf's spatial position and light interception (leaf angles, $STAR_d$ and SAL_d) showed above 70% of FV_{WI} and low or negligible variation between populations and among individuals. The high levels of variation found in light-related traits among and within individuals challenges the general assumption of negligible intraspecific variance across spatial scales. However, the magnitude of the temporal variation for light-related foliar traits in the chosen species was low and did not show a different structure between seasons except for light interception traits directly related to sun's apparent trajectory.

In this sense, our results showed a non-negligible contribution of the variability in foliar traits due to differences among and within individuals of the same population (Albert 2010a; Violle & Jiang 2009; Boucher *et al.* 2013). Despite low annual variation for leaf traits and moderate daily variability for SAL_t and $STAR_t$ among day periods, the patterns of the variance partitioning at spatial scales may be critical in the response of the individuals to the environment, in their response within communities and in the assembly of communities as a whole. In fact, recent evidences point out that such variability is relevant for many ecological and evolutionary processes (Booth & Grime 2003; Boege & Dirzo 2004; Crutsinger *et*

al. 2006; Lecerf & Chauvet 2008). The variation at the tree scales may be genetic but species are expected to differ in how individuals respond in many dimensions (Clark 2010). The variability detected across scales could be due to the existence of different genotypes and/or to distinct phenotypic responses of the genotypes. Nonetheless, leaf traits sampled at each population likely differ genotypically, but survey samples over the two chosen periods belong to the same genotypes. Hence, most temporal variation in leaf traits may be attributable to phenotypic plasticity, while genotypic differences obviously participate in the spatial variation above the individual level.

Seasonal differences found in *winter* measurements compared to *summer* (increased leaf area, weight, width and length) could be induced by processes related to leaf maturation whereas leaf position (lower leaf tilt angles and leaf course oriented towards South during *winter*) and potential light interception (lower $STAR_t$ and SAL_t during winter) may be related to phenotypic adjustments and to the seasonal variance in the irradiance patterns. Although due to our design we cannot differentiate leaf development from the effects caused by the harvest conducted during summer not we can assure whether changes occurred from a certain period or gradually between seasons. Despite avoiding recollection of young leaves our results may reflect different levels of senescence among leaves and seasons. However, while there were not overall allometric differences in leaf shape and structure (*SLA*, *Lindex*) between seasons we detected a significant interaction among spatial scales within individuals for SAL_t and $STAR_t$ in agreement with seasonal phenotypic adjustments in leaf angles. Leaf angle adjustments appear to increase light interception in winter and reduce it during the summer period. Indeed, trees of Mediterranean regions are expected to cope with a wide range of environmental stresses over time; from periods of drought and high irradiance during summer to potential photoinhibitory temperatures during winter (Díaz-Espejo *et al*. 2007; Speerlich *et al*. 2014). Narrow leaves with low *SLA* and vertical leaf tilt angles at the outer crown may contribute to avoid the negative impact of direct irradiance at high sun elevation angles and during summer (Tenhunen *et al*. 1990; Takenaka 1994). The foliar attributes found in leaf shape and structure (smaller and thicker leaves at the outer crown compared

to inner leaves) agree with spatial specialization dependent on the light regime found in previous studies contrasting sun and shade leaves (Kull & Niinemets 1993, Terashima & Hikosaka 1995, Niinemets 2001). However, we found that foliar adjustments occur at finer spatial scales within individuals as shown by the interaction between crown sectors and crown layers.

During summer drought, maximal carbon fixation in Mediterranean sclerophylls occurs during periods of relatively low incident light intensity and relatively low temperature early and late within the day (Tenhunen *et al*. 1990). The steeper leaf inclinations displayed during summer not only reduce the potential intercepting surface compared to winter angles during T2 to T4 but may also lower leaf temperatures and transpiration rates (Comstock & Mahall 1985). In fact, leaf lifespan of evergreen sclerophylls may take advantage of avoidance of irreversible damage through protective processes, such as non-photochemical energy dissipation of excessive excitation energy, even at the expense of carbon gain losses (Werner *et al*. 1999). The observed leaf angle display may constitute a form of structural photoprotection to cope with high irradiance stress in poor and adverse habitats (Valladares & Pugnaire 1999). Likewise, *winter* leaf angles may rise leaf temperatures to counteract photoinhibition due to low winter temperatures (Díaz-Espejo *et al*. 2007; Sperlich *et al*. 2014). SAL_t and $STAR_t$ attributes observed across spatial scales within individuals agree with previous studies that had shown evidences of complementarity in the productive processes such as light interception, carbon fixation and carbon accumulation/export in individual *Olea europaea* trees (García-verdugo *et al*. 2010; Granado-Yela *et al*. 2011; Rubio de Casas *et al*. 2011). Indeed, the ability to adjust phenotypically in response to environmental heterogeneity within individuals may constitute a mechanism by which plants increase performance (Picotte *et al*. 2007).

5. CONCLUSIONS

In the present study we have shown that the magnitude and structure of the functional variation in leaf traits did not change substantially over temporal scales in agreement with previous studies (Garnier *et al*. 2001) except for the potential exposure to direct irradiance due to the annual variation of the sun path trajectory.

However, the variation in light gradients across the tree crown within days and between seasons may play a crucial role in determining leaf attributes of plant species (Anten & Hirose, 1999; Niinemets 2007; Niinemets *et al*. 2014) as shown by the spatial variation found within individuals. Concluding the above, although within-canopy gradients may foster species-specific variation in foliar traits (Valladares & Niinemets 2008, Sperlich *et al*. 2014) the variability found among individuals and within individuals can constitute an important part of the trait variance in Mediterranean evergreens linked to responses to abiotic stress. We believe that establishing a link between intraspecific variation in light-related foliar traits to within canopy gradients will enhance our understanding of the expression of leaf attributes across tree crowns and its functional value.

ACKNOWLEDGEMENTS

We wish to thank MD Jiménez, A López-Pintor, A Vázquez for their expertise and help during the design and field sampling. This research was funded by the Spanish Ministry of Science and Education (project CGL2009-10392), through a FPI grant to A.G.E. (BES-2010-032767). We are also indebted to the Madrid Regional Govt. (project REMEDINAL-2, S2009/AMB-1783).

6. TABLES AND FIGURES

Table 1. Three way ANOVA testing the effect of Season, Crown sector, crown layer and the interaction on each leaf trait. Fitted models had the form: Leaf trait ~ Season * Crown sector *Crown layer + Pop(Tree) where tree was nested in population as a random term.

	Larea				*Lweight*			
	df.	Sum Sq.	Mean Sq.	*F* -value	df.	Sum Sq.	Mean Sq.	*F* -value
Season	1	1.55	1.55	20.7 ***	1	0.83	0.83	8.91 **
C.sector	3	0.37	0.12	1.63	3	1.79	0.60	6.41 ***
C.layer	2	0.17	0.08	1.12	2	19.18	9.59	102.99 ***
Season:C.sector	3	0.20	0.07	0.87	3	0.57	0.19	2.05
Season:C.layer	2	0.07	0.03	0.45	2	0.39	0.20	2.11
C.sector:C.layer	6	1.37	0.23	3.04 **	6	2.17	0.36	3.89 ***
Season:C.sector:C.layer	6	0.31	0.05	0.69	6	0.44	0.07	0.78
Residuals	2487	186.79	0.08		2477	230.68	0.09	

	Llength				*Lwidth*			
	df.	Sum Sq.	Mean Sq.	*F* -value	df.	Sum Sq.	Mean Sq.	*F* -value
Season	1	7.01	7.01	241.86 ***	1	5.66	5.66	146.74 ***
C.sector	3	0.03	0.01	0.34	3	0.22	0.08	1.94
C.layer	2	1.83	0.92	31.59 ***	2	1.75	0.88	22.75 ***
Season:C.sector	3	0.17	0.06	2	3	0.00	0.00	0.03
Season:C.layer	2	0.01	0.01	0.24	2	0.05	0.02	0.61
C.sector:C.layer	6	0.62	0.10	3.58 **	6	0.28	0.05	1.2
Season:C.sector:C.layer	6	0.10	0.02	0.6	6	0.16	0.03	0.71
Residuals	2454	71.10	0.03		2454	94.59	0.04	

	SLA				*Lindex*			
	df.	Sum Sq.	Mean Sq.	*F* -value	df.	Sum Sq.	Mean Sq.	*F* -value
Season	1	0.08	0.08	2.38	1	0.07	0.07	1.3
C.sector	3	0.65	0.22	6.47 ***	3	0.35	0.12	2.13 .
C.layer	2	20.32	10.16	303.13 ***	2	7.01	3.51	63.1 ***
Season:C.sector	3	0.66	0.22	6.57 ***	3	0.16	0.05	0.97
Season:C.layer	2	0.16	0.08	2.35 .	2	0.02	0.01	0.18
C.sector:C.layer	6	0.45	0.08	2.26 *	6	0.27	0.05	0.82
Season:C.sector:C.layer	6	0.10	0.02	0.5	6	0.24	0.04	0.73
Residuals	2476	83.01	0.03		2454	136.38	0.06	

	Ltilt				*Lcourse*			
	df.	Sum Sq.	Mean Sq.	*F* -value	df.	Sum Sq.	Mean Sq.	*F* -value
Season	1	3.00	3.04	22.99 ***	1	9.00	8.62	3.35 .
C.sector	3	4.60	1.52	11.5 ***	3	385.00	128.46	49.87 ***
C.layer	2	37.30	18.64	141.2 ***	2	19.00	9.33	3.62 *
Season:C.sector	3	1.80	0.61	4.59 **	3	80.00	26.52	10.29 ***
Season:C.layer	2	4.70	2.35	17.83 ***	2	12.00	5.87	2.28
C.sector:C.layer	6	2.60	0.44	3.31 **	6	185.00	30.75	11.94 ***
Season:C.sector:C.layer	6	1.30	0.21	1.59	6	62.00	10.40	4.04 ***
Residuals	2493	329.10	0.13		2492	6419.00	2.58	

	STAR				*SAL*			
	df.	Sum Sq.	Mean Sq.	*F* -value	df.	Sum Sq.	Mean Sq.	*F* -value
Season	1	7.90	7.90	450.45 ***	1	57.70	57.70	344.6 ***
C.sector	3	2.30	0.77	43.71 ***	3	19.80	6.61	39.46 ***
C.layer	2	0.99	0.50	28.37 ***	2	8.50	4.23	25.24 ***
Season:C.sector	3	0.99	0.33	18.84 ***	3	8.10	2.70	16.11 ***
Season:C.layer	2	0.03	0.02	0.99	2	0.10	0.06	0.35
C.sector:C.layer	6	0.47	0.08	4.46 ***	6	3.90	0.65	3.88 ***
Season:C.sector:C.layer	6	0.20	0.03	1.9 .	6	1.10	0.18	1.07
Residuals	2492	43.70	0.02		2486	416.20	0.17	

Table 2. Variance at each scale for the set of traits (Inertia *PCA-between*) in both seasons and in each season separately.

Scale	*PCA-Seasons*	*PCA-Summer*	*PCA-Winter*
Season	4.19	-	-
Location	6.18	6.65	6.52
Tree	18.39	22.25	19.41
Crown Sector	1.61	1.16	3.1
Crown Layer	4.56	4.15	5.7

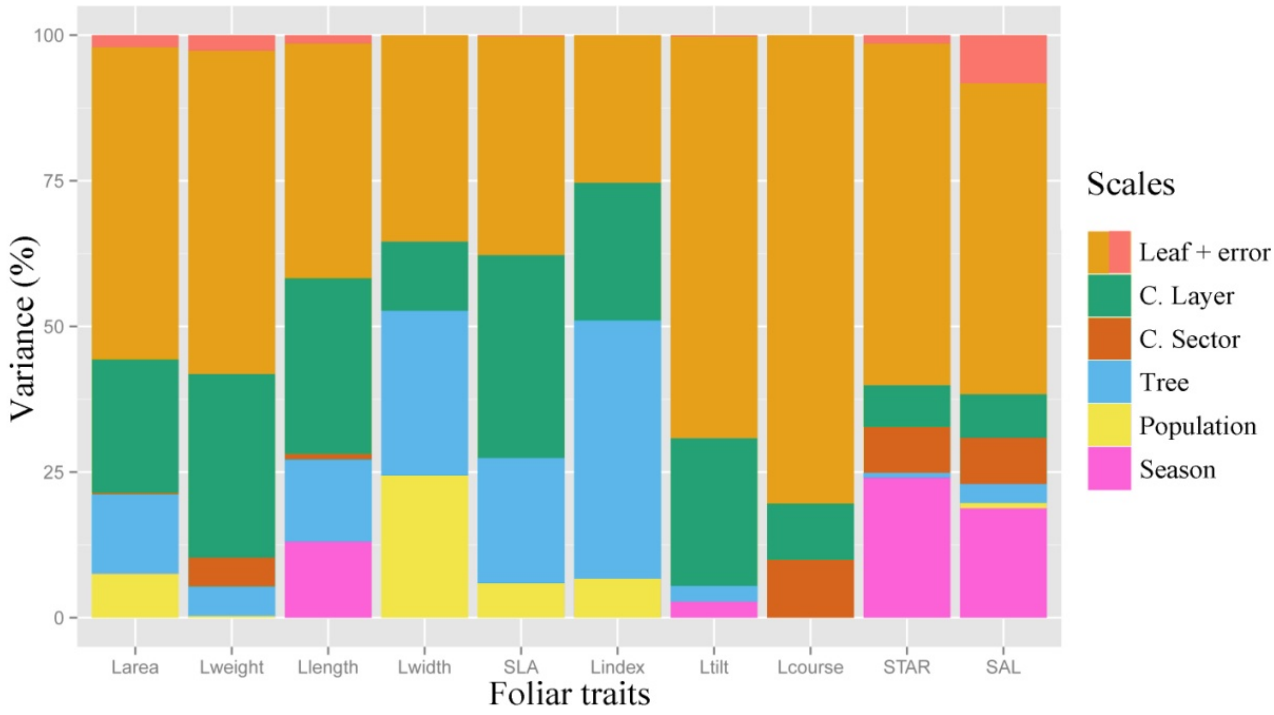

Figure 1. Variance partitioning across six nested scales for ten light-related foliar traits.

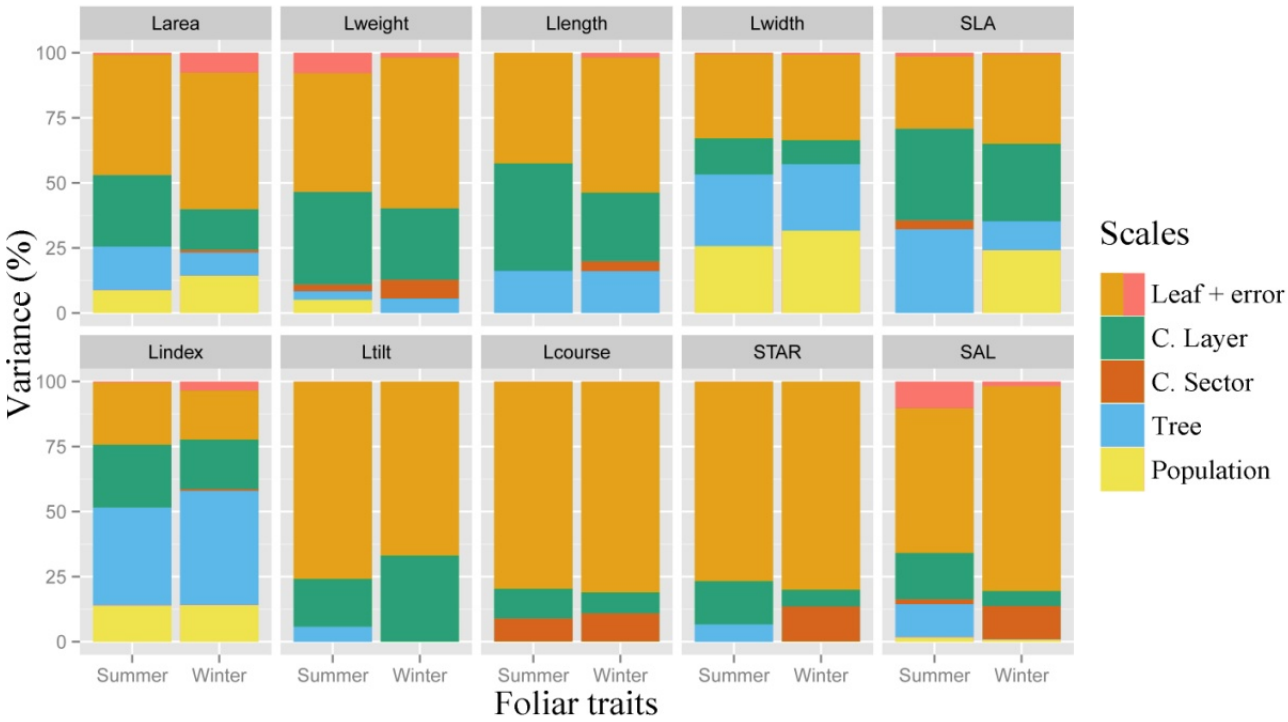

Figure 2. Variance partitioning across scales for *Summer* and *Winter* measurements.

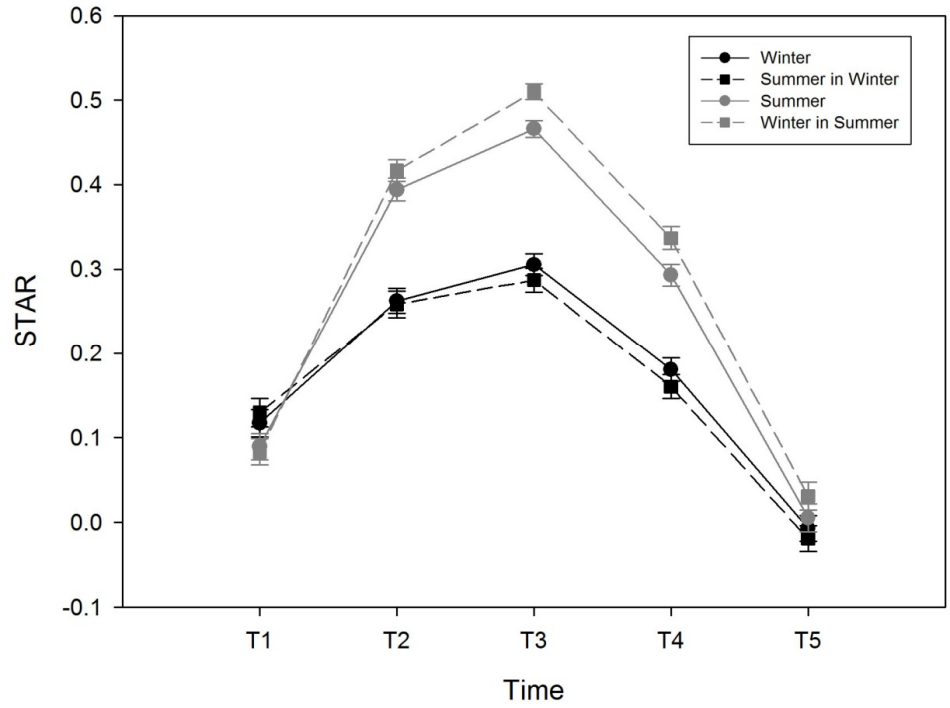

Figure 3. Differences in *STAR* between seasons along five equivalent day periods (T1 to T5). Solid lines show the actual *STAR* at each season (Black: *winter*; grey: *summer*), dashed lines show *STAR* calculated with the leaf angles from the opposite *season*. Mean ± SD.

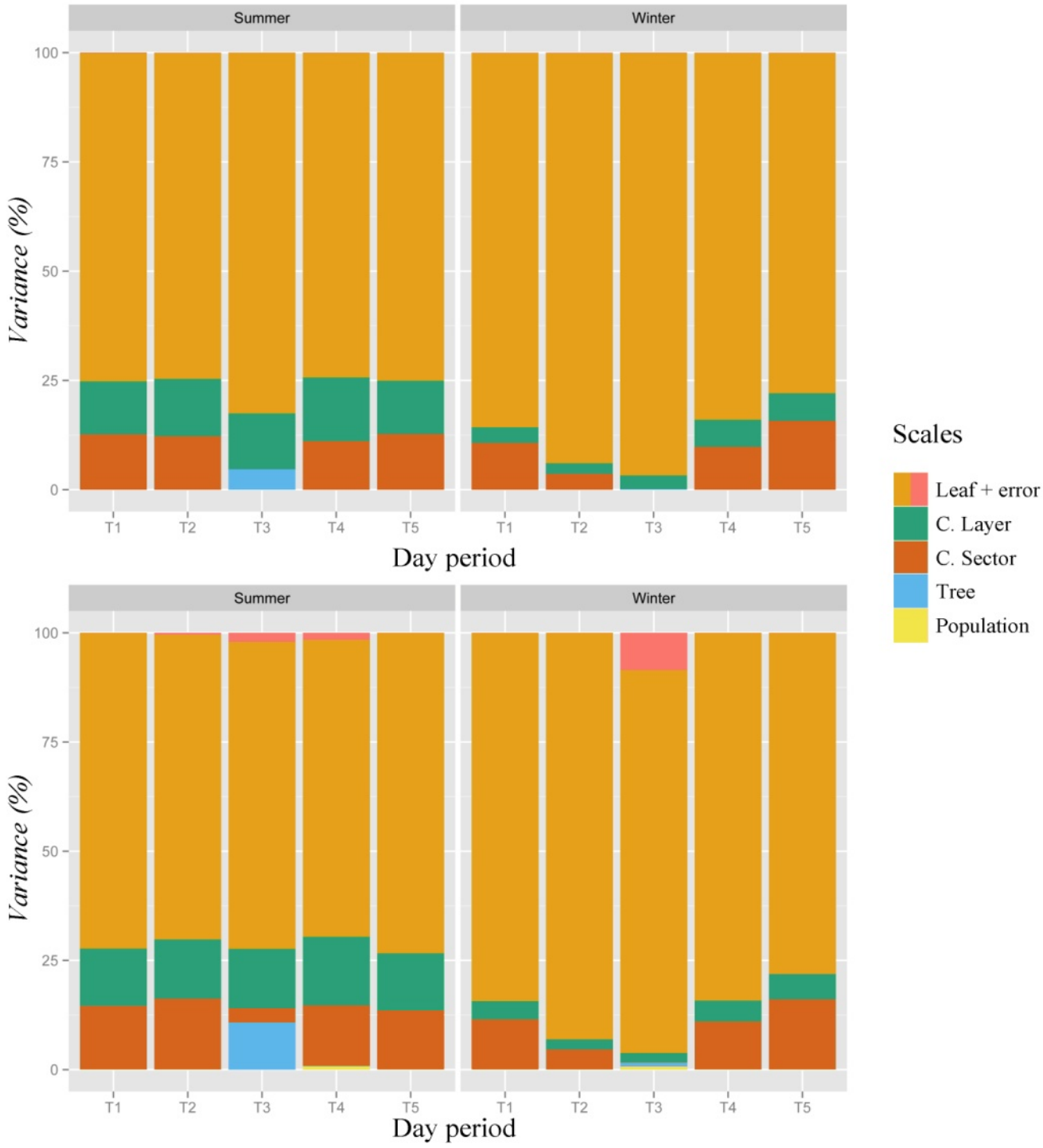

Figure 4. Variance partitioning for $STAR_t$ (upper pannels) and SAL_t (lower pannels) across scales at five periods during the day (T1-T5) in each season.

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8. SUPPLEMENTARY MATERIALS

Table S1. Correlation between the two leading axes of the PCA to leaf traits.

	PCA1	PCA2
Larea	-0.97	0.08
Llength	-0.67	0.42
Lweight	-0.86	0.22
Lwidth	-0.80	-0.14
SLA	0.05	-0.23
Lindex	0.18	0.43
Ltilt	0.11	0.73
Lazi	-0.05	0.00
STAR	-0.07	-0.89
SAL	-0.27	-0.84

Table S3. Correlation *PCA* axes of each season to leaf traits.

	Winter		**Summer**	
	PCA1	*PCA2*	*PCA1*	*PCA2*
Larea	0.97	0.05	-0.78	-0.57
Llength	0.68	0.40	-0.42	-0.68
Lwidth	0.79	-0.25	-0.72	-0.24
Lweight	0.87	0.24	-0.62	-0.66
SLA	-0.07	-0.30	-0.06	0.26
Lindex	-0.18	0.50	0.28	-0.30
Ltilt	-0.01	0.69	0.62	-0.70
Lazi	0.08	-0.05	0.01	-0.02
STAR	0.10	-0.84	-0.63	0.71
SAL	0.20	-0.82	-0.88	0.38

Table S4. *PCA* for both seasons without SAL_d and $STAR_d$.

Scale	*PCA-Seasons (%)*
Season	2.10
Location	7.34
Tree	22.09
Crown Sector	1.07
Crown Layer	5.32

DISCUSIÓN Y CONCLUSIONES GENERALES

1. DISCUSIÓN

Los capítulos de de la presente memoria doctoral constituyen distintas etapas de la caracterización de rasgos funcionales foliares relacionados con la captura de luz en *Olea europaea* L., de su variabilidad a través de múltiples escalas espaciales y temporales y de su relación con variables ambientales a escala global.

En el Capítulo I se presenta una metodología, basada en dispositivos móviles, que permite determinar eficazmente la posición de poblaciones foliares en condiciones de campo y estimar la exposición potencial en un momento y lugar dado de las mismas. También se muestra cómo esta metodología es capaz de evitar los principales inconvenientes de los métodos empleados hasta la fecha para determinar la posición de las hojas de la copa de un árbol. Además, la versatilidad de la misma, su bajo coste y su accesibilidad, permiten su empleo generalizado para la caracterización de la posición de cualquier superficie plana, tanto por investigadores como por educadores y estudiantes. En el Capítulo II se detalla como la posición foliar, junto a la forma y estructura de la hojas, dan lugar a patrones complementarios respecto a la captación potencial de luz en distintas posiciones de la copa de *Olea europaea* L., lo que podría suponer una ventaja en términos de optimización de la captura de luz a escala de individuo. En el Capítulo III se ponen de manifiesto la distribución de la variación de caracteres funcionales foliares relacionados con la captura de luz a través de múltiples escalas espaciales y temporales. En el Capítulo IV se muestra que la variación temporal en los caracteres funcionales foliares entre estaciones es baja o inexistente en las poblaciones consideradas, excepto para los rasgos directamente relacionados con la trayectoria solar. En este capítulo se destaca cómo pequeños ajustes en la posición foliar entre estaciones conducen a reducir la exposición potencial a la radiación durante el mediodía en el periodo de verano y a maximizar la exposición potencial durante el mediodía en el periodo de invierno, pudiendo disminuir el impacto negativo de la fotoinhibición durante esos periodos.

Caracteres funcionales foliares; estimación de la posición foliar y captura de luz

La captura de luz, el intercambio gaseoso y la fijación de carbono constituyen la principal función de las estructuras foliares de la copa de un árbol. A nivel de hoja, la posición foliar (orientación e inclinación) determinan, junto con la trayectoria solar en una localización geográfica dada, el límite teórico o potencial de exposición a la radiación accesible para dicha superficie. A nivel de copa, la posición foliar y la exposición potencial a la radiación es relevante para muchos procesos operando a nivel de individuo (Givnish 1987; Smith *et al*. 1997; Falster & Westoby 2003; Pearcy *et al*. 2005; Granado-Yela *et al*. 2011). De hecho, la posición foliar junto con otras características foliares son factores importantes en la regulación de la fijación de carbono y el uso de los recursos hídricos (Ehleringer & Werk 1986). A pesar de contar con la limitación de no considerar el auto sombreo de las propias estructuras del árbol (otras hojas, ramas, etc.) u otras estructuras adyacentes, los resultados obtenidos en el Capítulo I sitúan el método presentado como una alternativa que supera a los métodos frecuentemente utilizados para medir, en condiciones de campo, los ángulos foliares de una porción representativa de la copa de un árbol y estimar la exposición potencial de dichas hojas en un momento del día y del año. Por lo tanto consideramos que el método constituye un avance en la adquisición directa de datos basado en una herramienta económica, portátil, versátil y que se encuentra ampliamente distribuida a nivel global (Capítulo I).

Los resultados del Capítulo II y del Capítulo IV sugieren cierta preferencia en la distribución de los ángulos de orientación e inclinación foliares a lo largo de distintas partes de la copa similar a los encontrados entre las hojas más y menos expuestas de un individuo de *Olea europaea* subesp. *guanchica* por Granado-Yela *et al*. (2011). La preferencia de ciertas orientaciones e inclinaciones junto con la expresión coordinada de otros rasgos foliares dan lugar a patrones complementarios en la optimización de la captura de luz a lo largo del día, en la fijación de carbono y en la exportación de productos de la fotosíntesis entre distintas porciones del propio árbol. Los experimentos llevados a cabo en

individuos procedentes de estaquillas bajo distintos tratamientos de luz en invernadero, muestran como la complementariedad entre las porciones de la copa puede surgir como estrategia de optimización en la captura de luz en árboles que han superado cierto tamaño y pudiendo darse funciones complementarais respecto al ambiente que experimentan. La orientación e inclinación foliar en otras especies sometidas a ambientes de alta radiación supone ventajas para el uso de los recursos hídricos y la captura de carbono comparada con otras orientaciones posibles (Jurik *et al*. 1990), incluso estando relacionada con la reproducción de la planta a través de un efecto positivo sobre la producción del número de estructuras florales (Werk & Ehleringer 1986). Los patrones de captación de la radiación en las distintas posiciones de la copa revelan una estructura complementaria a lo largo del día (Capítulo II y IV) y entre dos situaciones del año (Capítulo IV) entre los distintos sectores de la misma y diferencias entre las capas más interna y la externa en cuanto a la captación de luz. La reducción de la superficie expuesta a la radiación en la capa más externa de hojas también sugiere mecanismos de fotoprotección estructural observado en ambientes sometidos a alta intensidad y a otros factores de estrés como la disponibilidad hídrica y la temperatura. Estos hallazgos ponen de manifiesto una elevada variabilidad de los caracteres estudiados a nivel de individuo en Olea europaea la cual puede tener implicaciones directas en la captura de luz y en última estancia sobre los componentes de la eficacia biológica.

Variabilidad de los caracteres funcionales foliares a través de escalas espaciales y temporales.

"One limitation for theoreticians is the scarcity of empirical data regarding the causes, patterns, and consequences of within-population ecological variance"

-Bolnick *et al*. (2011)

La magnitud y la estructura de la variabilidad de los caracteres funcionales foliares de *Olea europaea* L. a distintas escalas espaciales (Capítulo III y IV) y temporales (Capítulo IV) es más compleja de lo esperado mostrando patrones distintos para distintos rasgos foliares. A pesar de que el paradigma de la ecología

funcional está basado en la asunción de que la variación en los caracteres funcionales dentro de una misma especie es anecdótica comparada con la variabilidad entre especies (Garnier *et al*. 2001; Roche *et al*. 2004) la evidencia reciente pone de manifiesto la relevancia de la variabilidad de los caracteres funcionales en muchos procesos ecológicos y evolutivos (Booth & Grime 2003; Boege & Dirzo 2004; Crutsinger et al. 2006; Lecerf & Chauvet 2008). Los altos niveles de variación en los atributos de los caracteres funcionales foliares relacionados con la captura de luz en ambos capítulos se oponen a aceptar la premisa de que, a distintas escalas espaciales y temporales, la variabilidad es despreciable dentro de una misma especie. La variación de este grupo de caracteres dentro de los individuos puede constituir una característica fundamental de la función de la planta que permite a los individuos superar filtros abióticos como el exceso de radiación, temperaturas extremas o escasa disponibilidad hídrica (Capítulos II, III y IV). Ciertamente, las plantas son capaces de adaptarse morfológica y fisiológicamente a cambios ambientales sutiles que se producen a escalas menores que la del individuo (de Kroon *et al*. 2005; Esteso-Martínez *et al*. 2006; Niinemets 2010) lo que puede dar lugar a interacciones entre unidades funcionales diferenciadas y potencialmente optimizar la adquisición de recursos (de Kroon *et al*. 2005; Kawamura 2010; Granado-Yela *et al*. 2011).

La expresión de los atributos foliares relacionados con la captura de luz en *Olea europaea* L. es variable dentro de la copa de un mismo árbol dando lugar a patrones complementarios en la captura potencial de luz entre porciones de la misma a lo largo del día (Capítulo II, IV) y del año (Capítulo IV). Además, los atributos foliares en *Olea europaea* L. están relacionados a lo largo de un extenso gradiente latitudinal con la incidencia de la radiación solar, y la distribución anual de la temperatura y de las precipitaciones. Finalmente los resultados obtenidos en la presente tesis constituyen un valioso aporte para modelos basados en rasgos funcionales dada la escasez de datos empíricos sobre la variabilidad intraespecífica a distintas escalas.

"*To predict the consequences of trait variation, ecologists need to: (1) Identify the genetic and phenotypic causes of ecological variation, and (2) quantify ecological*

variation across multiple species, trophic levels, or entire communities. Ideally, such information could guide theoreticians in developing biologically realistic models that can be parameterized with empirical data to yield testable quantitative predictions"

-Bolnick *et al*. (2011)

A pesar de la extensa caracterización de los caracteres funcionales foliares relacionados con la captura de luz en *Olea europaea* L., la copa de un árbol es una estructura multifuncional compleja que está sometida a otras presiones selectivas como las del desarrollo, las biomecánicas, las hidráulicas y las relacionadas con las limitaciones de la distribución de recursos (Pearcy *et al.* 2005). Un mayor conocimiento de la función de la copa de un árbol requiere estudios adicionales que determinen hasta qué punto las copas modifican el ambiente qué experimentan para optimizar la eficacia fotosintética a nivel de árbol, como por ejemplo investigar la calidad y cantidad de radiación que penetra en el interior de la copa a través de las distintas posiciones de la misma. El gradiente lumínico dentro de la copa a lo largo del día y los patrones anuales pueden promover la variabilidad dentro de una misma especie en los caracteres funcionales foliares (Anten & Hirose 1999; Niinemets 2007; Valladares & Niinemets 2008; Niinemets *et al*. 2014; Sperlich *et al*. 2014). A raíz de la estructura y magnitud de la variabilidad observada a distintas escalas espaciales dentro de los individuos considero que establecer una relación entre la variación en los caracteres funcionales foliares y los gradientes de luz dentro de la copa, pueden suponer un claro avance sobre nuestro conocimiento sobre de la expresión de los atributos foliares en distintas porciones de la copa de un árbol y de su valor funcional. Más aún, las consecuencias potenciales de la variabilidad fenotípica a través de la copa de un árbol continúan sin ser evaluadas en términos de su efecto sobre los componentes de la eficacia biológica.

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3. CONCLUSIONES GENERALES

A continuación se desarrollan las conclusiones generales que pueden extraerse a partir de los resultados de la presente tesis doctoral

1.- El uso de *Smartphones* equipados con sensores capaces de registrar la posición relativa del mismo (al menos un magnetómetro, acelerómetro de 3 ejes y/o giróscopo) permite realizar una caracterización espacial detallada de los ángulos foliares en condiciones de campo con numerosas ventajas frente a las herramientas frecuentemente utilizadas (métodos tradicionales y digitalizadores *3D*).

2.- La metodología presentada es accesible, asequible, versátil y permite realizar estimaciones detalladas de la exposición potencial instantánea de las superficies fotosintéticas de un árbol mediante medidas directas susceptibles de extrapolarse a nivel de copa.

3.- Las diferencias en los caracteres foliares entre las distintas porciones de la copa de *Olea europaea* L. apoyan ajustes a escala intraindividual orientadas a minimizar los efectos adversos de la exposición a alta radiación. La variación intra individual entre sectores y capas en caracteres foliares relacionados con el ambiente lumínico puede tener consecuencias funcionales a nivel de copa en el contexto espacio temporal en el que se desarrollan.

4.- Los ángulos foliares presentes en los distintos sectores y capas permiten reducir la exposición potencial instantánea a la radiación de las partes más expuestas de la copa (capa externa, sector orientado hacia el sur) durante los periodos de máxima radiación (verano/ mediodía). Los patrones de exposición potencial de las hojas junto con los caracteres funcionales expresados apoyan la optimización a nivel de individuo de la captación de luz en los momentos más favorables del día para cada porción de la copa.

5.- La variabilidad a escala de árbol, población y entre poblaciones de los caracteres funcionales foliares relacionados con la forma y la estructura de las hojas forma una parte importante de la variabilidad a través de las escalas

espaciales estudiadas. Por el contrario, la mayor parte de variabilidad de los caracteres foliares relacionados con la posición de la hoja está estructurada dentro de los individuos. La variabilidad de los caracteres funcionales foliares entre individuos de *Olea europaea* L. y dentro de los mismos no debería obviarse.

6.- La variación de los caracteres funcionales foliares en *Olea europaea* L. está altamente relacionada con las diferencias anuales en radiación, temperatura y precipitación a escala global (estacionalidad de las temperaturas y las precipitaciones).

7.- La variabilidad a escala temporal en los caracteres funcionales foliares de *Olea europaea* L. muestra escasas diferencias entre los periodos de máxima y mínima elevación solar (verano vs. invierno en la latitud considerada) excepto las esperadas en la captación potencial de luz durante un día debido a la variación en la trayectoria solar aparente.

8.- Pequeños ajustes en la posición foliar entre verano e invierno permiten a *Olea europaea* L. una reducción de la exposición potencial durante el verano y aumentarla durante el invierno comparado con mantener los mismos ángulos para ambos periodos. Este efecto es mayor en el periodo de máxima elevación solar a lo largo del día.

SUMMARY

TITLE: The crown concert: variability for foliar functional traits in Olea europaea

INTRODUCTION

Due to the difficulty of identifying functionality *per se* and with the aim of categorizing functional traits by their relative importance at the organism scale, functional traits have been defined as any morpho-physiologic characteristic with an indirect impact on growth, reproduction and/or survival from the cellular scale to the individual scale. For the study of vascular plants, functional traits are an indirect link between plant performance and the experienced environment. Indeed, functional traits are useful to disentangle relevant questions in ecology. At the organ scale, functional traits such as the structural or biochemical characteristics of the leaves, can link leaf's photosynthetic activity to photosynthetic performance at different scales.

One of the assumptions early adopted in functional ecological theory implies that functional trait variability within species is negligible compared to functional trait variability among species. However, many evidences emerge showing that intra-specific functional trait variability plays a crucial role on ecosystem functioning and community assembly processes, specifically in those communities dominated by one or a few species. Intra-specific functional trait variability (FV) is the distinct expression of functional trait attributes in the individuals of a given species. FV arises from genetic diversity and from the phenotypic response at diverse spatio-temporal scales. In an evolutionary context FV is crucial to speciation and natural selection processes that should be quantified in terms of curve –response relationships among functional traits and the environment, as well as including the interaction across scales.

Long-lived woody plants respond to environmental heterogeneity through the expression of diverse attributes (phenotypic syndrome) that had an effect on whole-plant performance. Variability in the expression of structural and morphological leaf attributes can have profound implications on light caption and be the product of environmental filters at distinct spatio-temporal scales. The ability to adjust the foliar syndrome to environmental limitations at local scales

enables optimization in the light interception and efficient light use. On isolated woody plants, light interception is highly dependant on the number, shape, structure, size, distribution and dispersion (clumping) of the leaves. These characteristics are influenced by the tree crown itself and by the light gradient that generates.

Despite the importance of quantifying functional variance across scales and its implication in population ecology and evolutionary dynamics, the characterization of functional variation for foliar traits and its relationship to environmental sources of variation in natural conditions remain poorly addressed, specifically in woody plants. Nonetheless, the study of the relationships among functional traits and the quantification of their impact should be conducted in absolute terms (magnitude) and relative (structure) in relation to the sources of variation for a group of traits that define the phenotypic syndrome of the plant.

MAIN GOALS

The main aim of the present thesis is to offer a detailed characterization of the FV for light-related foliar traits such as leaf shape, leaf structure and potential light interception across scales in the evergreen tree *Olea europaea* L. We explore the implications of leaf angles in potential light interception at the individual level across crown positions and related the variability for foliar traits to environmental variables along a wide latitudinal gradient (40º N – 21ºS). To accomplish the main objective the following specific goals were set.

- To develop a methodology capable of measuring foliar angles in a tree crown to which estimate potential light interception at the leaf and crown scale in a given time and location. Moreover, it should incorporate a tool that overcomes the main drawbacks of the current used methodologies in the field. In Chapter I of the present thesis a methodology was developed to accomplish this specific objective.

- To evaluate the functional implications on light interception patterns across the tree crown in *Olea europaea* L. during high irradiance periods in the field. In Chapter II the counterbalance patterns of light interception across the tree crown scales and the distinct expression of foliar traits within individuals are explored.

- To characterize FV for foliar light-related traits in *Olea europaea* L. across global spatial scales (among populations, within populations, among individuals within a population and within individuals). Chapter III is dedicated to such characterization in a wide latitudinal gradient covering both hemispheres and establishing a relationship between FV and the main environmental variables related to shape and structure of the leaves.

- To estimate the magnitude and structure of temporal variation in light-related foliar traits in *Olea europaea* L. between seasons (*summer* vs. *winter*) in two Mediterranean populations. In Chapter IV the magnitude and structure of temporal variation in foliar traits was determined between two contrasting seasons in terms of irradiance, temperature and precipitation.

MAIN RESULTS

The methodology to simplify data acquisition in plant canopies with a cell phone (Chapter I) successfully passed calibration and validation processes to conduct leaf angle measurements in the field and to estimate the potential light interception of the leaves and the tree crown. The spatial display of the leaves in addition to leaf's shape and structure enable counterbalancing patterns in the light capture strategy across the tree crown in *Olea europaea* L. (Chapter II). Light-related functional foliar traits shown two patterns in the distribution of the variance across spatial scales (Chapter III) characterized by the absence or presence of variability at global scales. The magnitude and structure of the variability for the studied traits at distinct spatial (Chapter III) and temporal (Chapter IV) scales appears more complex than previously thought. The structure of the variability across spatial scales for the studied leaf traits showed low differences between seasons except for those traits related to the apparent sun path trajectory (Chapter IV).

CONCLUSIONS

1.- Smartphones equipped with accelerometers and magnetometers can be used to characterise leaf angles within the tree crown with a simple gesture. This tool can outperform common same-purpose devices in the field.

2.- The methodology presented is accessible, affordable, versatile and allows instant detailed estimates of the potential light interception across the tree crown via direct measurements of leaf angles.

3.- Differences in leaf functional traits among distinct crown portions in *Olea europaea* L. support intra-individual adjustments oriented towards minimizing the negative effects of direct irradiation. Intra-individual variation in light-related leaf functional traits across the tree crown can have profound consequences in the light interception patterns.

4.- The expression of distinct leaf angles among crown sectors and layers can reduce the light interception of the most exposed parts of the tree crown (outer layer, facing south sector) during periods of maximum radiation (summer/ noon). In addition to the attributes expressed across the tree crown, the patterns of potential light interception support optimization in the light capture strategy at the tree scale.

5.- The variability among trees, within populations and among populations was an important part of the variability across spatial scales for leaf traits related to leaf form and structure. By contrast, most of the variability for leaf traits related to the position of the leaves was structured within individuals. The variability of leaf functional traits within and among individuals of *Olea europaea* L. should not be overlooked.

6. The variability found for leaf functional traits in *Olea europaea* L. is highly related to the annual differences in radiation, temperature and precipitation on a global scale (seasonality). Most variable populations in terms of precipitation and temperature along the year have more elongated leaves and lower SLA than populations where temperatures and precipitation are less variable.

7.- Variability in leaf functional traits of *Olea europaea* L. shows few differences between maximum and minimum periods of solar elevation angles (summer vs. winter) except in those expected by variation in the sun's apparent path in the potential light interception.

8.- Small adjustments in the spatial position of the leaves between summer and winter periods enables a reduction of the potential exposure during summer and an increase during winter compared to maintaining the same leaf angles for both seasons (either summer or winter leaf angles) in the studied populations of *Olea europaea* L.

RESUMEN

TÍTULO: Copa y concierto: variabilidad en caracteres funcionales foliares en Olea europaea

INTRODUCCIÓN

Debido a la dificultad de identificar funcionalidad *per se*, y con el objetivo de sintetizar y categorizar los caracteres basándose en su importancia relativa a nivel de organismo, se define carácter funcional como cualquier característica morfológica, fisiológica o fenológica, de la célula al individuo, con un impacto indirecto en la eficacia biológica a través de sus efectos sobre los componentes de la misma. Desde un punto de vista de las plantas vasculares, los caracteres funcionales constituyen una aproximación indirecta entre la eficacia biológica y el ambiente que experimentan, útiles para desentrañar cuestiones relevantes en varias áreas de la ecología.

Una de las premisas adoptadas en la teoría ecológica implica que la variabilidad en la expresión de caracteres funcionales es despreciable entre individuos conespecíficos comparada con la variabilidad existente entre especies. Sin embargo, la evidencia acumulada destaca la variabilidad intraespecífica en el funcionamiento de los ecosistemas y en los procesos de ensamblaje y dinámica de comunidades, especialmente en aquellas dominadas por una o pocas especies. La variabilidad funcional intraespecifica (VF) es la expresión diferencial en los valores de los caracteres funcionales de los individuos de una especie dada. En un contexto evolutivo la VF es esencial para los procesos de selección natural y especiación que debería cuantificarse tanto en términos de curvas-respuesta entre caracteres funcionales y el ambiente (habitualmente no pueden extrapolarse de relaciones interespecíficas) como abarcando la interacción entre las diferentes escalas en las que se estructura.

Las plantas leñosas responden a la heterogeneidad ambiental a través de la expresión de variedad de fenotipos y la prevalencia de un conjunto determinado de caracteres (síndrome fenotípico) que afectan a la eficacia biológica de todo el individuo. La capacidad de ajustar la expresión del síndrome foliar en función de las limitaciones ambientales a escala local permite alterar las limitaciones que surgen en la captación y uso de la luz. La captura de luz está determinada tanto

por patrones de radiación solar a escala local, variables climáticas (nubosidad, transmisividad de la atmósfera), gradientes ambientales en el interior de la copa, como por la forma, estructura y disposición de los elementos que la componen. En plantas de porte arbóreo, relativamente aisladas de estructuras o árboles vecinos, la eficiencia de la captura de luz es altamente dependiente del número, forma, tamaño, disposición, distribución y dispersión de las estructuras foliares. A pesar de la importancia de la partición de la VF a través de las distintas escalas y de sus implicaciones en la ecología de poblaciones y dinámica evolutiva existe una escasa caracterización de la VF y de la relación con fuentes de variación ambientales en condiciones naturales, especialmente en especies de porte arbóreo. Sin embargo, la búsqueda de asociaciones entre rasgos funcionales, la cuantificación de su impacto y la relación con el ambiente constituyen un campo fructífero en áreas diversas de la ecología que deberían realizarse en términos absolutos (magnitud) y relativos (estructura) para el conjunto de caracteres que definen el síndrome fenotípico en relación a las posibles fuentes de variación.

OBJETIVOS

- Desarrollar una metodología capaz de capturar la variabilidad respecto a los ángulos foliares presentes en el dosel mediante los que estimar la exposición potencial a nivel de copa en un momento concreto de una localidad geográfica dada. Además esta metodología debe incorporar una herramienta que supere las principales desventajas de los métodos frecuentemente utilizados en condiciones de campo. El capítulo II de la presente memoria se dedica a desarrollar una metodología capaz de abarcar este objetivo específico.

- Evaluar las implicaciones funcionales en cuanto a la captura de luz diurna durante el período de máxima radiación en las distintas parte de la copa de *Olea europaea* L. en ambientes mediterráneos. Concretamente en el capítulo III se analizan las posibles relaciones de complementariedad espacial y temporal de los caracteres foliares y en los patrones de intercepción de luz a nivel de individuo en distintos momentos del día.

- Caracterizar la partición de la variabilidad en los caracteres foliares de *Olea europaea* L. a través de múltiples escalas espaciales (análisis de la variabilidad

entre las distintas poblaciones, dentro las poblaciones, entre individuos y dentro de los individuos) a nivel planetario. En el capítulo IV se aborda esta caracterización en un extenso gradiente latitudinal que abarca ambos hemisferios y se establecen asociaciones con las principales variables ambientales relacionadas con la estructura y forma de las superficies foliares.

- Estimar la presencia y magnitud de la variabilidad temporal en un conjunto de caracteres foliares relacionados con la captura de luz. Relación con los patrones de radiación diurno de dos situaciones estacionales de contraste (Invierno /Verano) en ambientes Mediterráneos. En el capítulo V se establece una aproximación de la presencia y magnitud de la variabilidad temporal de los caracteres foliares en *Olea europaea* L.

RESULTADOS

La metodología presentada, basada en dispositivos móviles, permite determinar eficazmente la posición de poblaciones foliares en condiciones de campo y estimar la exposición potencial en un momento y lugar dado de las mismas (Capítulo I). La disposición espacial de las superficies foliares permite patrones complementarios en la captación de luz en las distintas posiciones de la copa de *Olea europaea* L. (Capítulo II). Los caracteres funcionales foliares relacionados con la captura de luz muestran dos patrones en la estructura de la variabilidad a través de distintas escalas espaciales caracterizados por la presencia o ausencia de diferencias entre poblaciones (Capítulo II). La magnitud y la estructura de la variabilidad de los caracteres funcionales a través de escalas espaciales (Capítulo III) y temporales (Capítulo IV) es más compleja de la esperada inicialmente. La estructura de la variabilidad a través de escalas espaciales para los caracteres foliares estudiados han mostrado una variación escasa entre estaciones excepto para aquellos rasgos relacionados con el movimiento solar aparente (Capítulo IV).

CONCLUSIONES

1.- El uso de *Smartphones* equipados con sensores capaces de registrar la posición relativa del mismo (al menos un magnetómetro, acelerómetro de 3 ejes y/o giróscopo) permite realizar una caracterización espacial detallada de los ángulos foliares en condiciones de campo con numerosas ventajas frente a las

herramientas frecuentemente utilizadas (métodos tradicionales y digitalizadores *3D*).

2.- La metodología presentada es accesible, asequible, versátil y permite realizar estimaciones detalladas de la exposición potencial instantánea de las superficies fotosintéticas de un árbol mediante medidas directas susceptibles de extrapolarse a nivel de copa.

3.- Las diferencias en los caracteres foliares entre las distintas porciones de la copa de *Olea europaea* L. apoyan ajustes a escala intraindividual orientadas a minimizar los efectos adversos de la exposición a alta radiación. La variación intra individual entre sectores y capas en caracteres foliares relacionados con el ambiente lumínico puede tener consecuencias funcionales a nivel de copa en el contexto espacio temporal en el que se desarrollan.

4.- Los ángulos foliares presentes en los distintos sectores y capas permiten reducir la exposición potencial instantánea a la radiación de las partes más expuestas de la copa (capa externa, sector orientado hacia el sur) durante los periodos de máxima radiación (verano/ mediodía). Los patrones de exposición potencial de las hojas junto con los caracteres funcionales expresados apoyan la optimización a nivel de individuo de la captación de luz en los momentos más favorables del día para cada porción de la copa.

5.- La variabilidad a escala de árbol, población y entre poblaciones de los caracteres funcionales foliares relacionados con la forma y la estructura de las hojas forma una parte importante de la variabilidad a través de las escalas espaciales estudiadas. Por el contrario, la mayor parte de variabilidad de los caracteres foliares relacionados con la posición de la hoja está estructurada dentro de los individuos. La variabilidad de los caracteres funcionales foliares entre individuos de *Olea europaea* L. y dentro de los mismos no debería obviarse.

6.- La variación de los caracteres funcionales foliares en *Olea europaea* L. está altamente relacionada con las diferencias anuales en radiación, temperatura y

precipitación a escala global (estacionalidad de las temperaturas y las precipitaciones).

7.- La variabilidad a escala temporal en los caracteres funcionales foliares de *Olea europaea* L. muestra escasas diferencias entre los periodos de máxima y mínima elevación solar (verano vs. invierno en la latitud considerada) excepto las esperadas en la captación potencial de luz durante un día debido a la variación en la trayectoria solar aparente.

8.- Pequeños ajustes en la posición foliar entre verano e invierno permiten a *Olea europaea* L. una reducción de la exposición potencial durante el verano y aumentarla durante el invierno comparado con mantener los mismos ángulos para ambos periodos. Este efecto es mayor en el periodo de máxima elevación solar a lo largo del día.

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AGRADECIMIENTOS

Querido lector;

Muchas gracias por haber llegado hasta aquí de una manera u otra. Esta tesis doctoral no hubiera sido posible sin el apoyo que he recibido de mis directores, mis compañeros y mis familiares y amigos. Es más, me resulta difícil desligar semejante logro de la ayuda ofrecida, a lo largo de estos años, por una cantidad ingente de personas y el acceso a un buen número de programas de formación y financiación. Sin embargo, los resultados plasmados en esta memoria son puramente académicos y no recogen, ni de lejos, la influencia de personas admirables con las que he tenido la oportunidad de interactuar durante este aprendizaje. Permíteme tratar de reflejar el privilegio que supone haber compartido esta experiencia con ellos y el gustazo que me produce poder dedicarles unas palabras, abusando de la aliteración, sin orden ni concierto.

Adrián G. Escribano-Rocafort

Este trabajo no sería posible si mis progenitores no fueran mi *Big Bang* particular. Tampoco lo sería sin todas esas otras figuras que se han preocupado de criarme, darme una educación y confiar en mí; a diario, de vez en cuando, constantemente. Son ellos a los primeros a los que me siento agradecido; a mis abuelos, a sus hermanos, a mis tíos, a esas personas que sin saberlo se convirtieron en un modelo a seguir, a mis muchos padres y madres.

Por otra parte no tendría la oportunidad de defender esta tesis y tratar de "volar alto" en el mundo académico sin la ayuda de mis directores. Me siento muy afortunado y honrado por contar con su apoyo y por empujarme a compartir esta obra con el lector casual y el especializado.

Mi admiración y agradecimiento a Juan Antonio Delgado y a Adrián Escudero por ser pilares en los que apoyarme ante las vicisitudes de la vida, por mantener mi entusiasmo, por ser pacientes, comprensivos, y por motivarme en esta etapa final. Gracias a los dos por ofrecerme vuestro apoyo incondicional, por estar siempre dispuestos a escuchar mis inquietudes con una sonrisa (aunque vengan acompañadas de un archivo infernal o en gráficas de mil colores) y por ofrecerme

la oportunidad de aprender con vosotros. Gracias por ser como sois, sin vuestra dedicación hubiera sido imposible concluir esta tesis.

Esta tesis tampoco puede concebirse sin la dedicación, esfuerzo y pasión de Luis Balaguer. Tanto por impulsar el proyecto y mi carrera investigadora como por su implicación como director durante la mayor parte de la misma. No quiero dejar pasar la oportunidad de plasmar lo agradecido que me siento y la importancia e influencia que ha tenido en mi vida. Por cautivarme en sus clases de restauración de la cubierta vegetal, por querer que viéramos procesos, por ensañarnos a ser proactivos, por trasmitir ilusión, por exigirme, por formar un cenobio multifuncional a base de personas excepcionales, por querer "*algo rompedor*", por transmitirme su aprobación después de verme salir de un marrón en el que me ha metido él, por apostar por mí, por ser franco y directo, por fomentar que siga subiéndome a los árboles, por mandarme a sitios remotos, por plantearme retos, por sacarle la brillantez al mundo. Y por ser el germen de tantas preguntas y la puerta al fascinante mundo de la ecología funcional y la restauración ecológica, por permitirme hacer pequeños paréntesis para explorar otras formas de trabajar, otras hipótesis, otro instrumental, otras herramientas. Por darme la oportunidad de convertir mis sueños en experiencias vitales.

Pero sería un error pensar que el apoyo recibido acaba aquí. Afortunadamente para mí, toda mi labor se gestó bajo el amparo emocional y técnico de todos los compañeros con los que he compartido inquietudes en la universidad. Me siento agradecido a todos aquellos que ayudaron a perfilar este proyecto; José Serrano, Esther Pérez, Mariló Jiménez, Antonio López, Vicente Muñoz, Gabriel Dorado, Rafa Rubio y Carlos García, han hecho posible, a través de reuniones sesudas en el principio de los tiempos y de trabajos propios, que tengamos una historia relevante que contar sobre la variabilidad de caracteres funcionales foliares en árboles y su relación con el ambiente… ¡a escala planetaria!

Otra parte del apoyo fundamental e imprescindible ha sido el ofrecido por todas aquellas personas con las que he compartido campañas de campo épicas, de manera habitual y no tan habitual, por las inusuales zonas de muestreo que he tenido la oportunidad de explorar. Todos ellos han hecho posible la toma de datos

y muestras de los que se nutre este trabajo; a Simon Pichillou y Marta (Enormes!) por su asistencia en la isla de La Reunión, a Mark y a su familia (Betty, Shiva, Tasiana *Asante sana*!) por su increíble hospitalidad, su asistencia y ayuda en Kenia, y a Mamen por prestarnos su tiempo y sus buenos consejos en la isla de Menorca. Para los habituales de las reuniones y del trabajo de campo me faltan palabras para transmitirles la suerte que considero que he tenido al compartir con ellos una de esas experiencias que se tienen una sola vez en la vida; Peri, Agus, Ana y Katty han sido unos compañeros excepcionales del día a día y de las aventuras que fueron las campañas de campo. El anecdotario de vivencias y situaciones -producto de nuestra empatía, de irte a buscar árboles con la determinación de muestrearlos allende los mares y de una inconsciencia similar a nuestras ganas por obtener datos- es ingente y constituye uno de los recuerdos que mejor atesoro. Gracias a ellos las miserias de trabajar en campo se han transformado en vagas reflexiones que me resultan extrañamente ajenas: ¿Estuve al borde de la desesperación y de la lipotimia?, ¿Muestreábamos en lo alto de una escalera con una mano ocupada en aferrarnos a la vida?, ¿De verdad estábamos en el hábitat idóneo de tantas serpientes venenosas?, ¿Era yo el que se planteaba dedicarse a otra cosa?, ¿Fui yo el que propuso reducir las escaleras que llevábamos de un lado a otro a sus partículas más elementales?,…especialmente cuando ha sobrevivido testimonio gráfico.

También ha sido crítico el apoyo moral y logístico que he recibido en actividades tan determinantes en nuestra especie como lo son el aprender a base de mirar cómo se hace, el solicitar ayuda o el socializar compartiendo bebestibles fermentados. a Miguel Ángel Casado, Ignacio Mola, Rocío Torres, Sandra Magro, Juanma Arenas, Carlos García, Irene Cordero, Juanjo, Elena Granda, Ana Lázaro, Mario Mairal, Adán, Javi, Kike, Quique, Víctor y Richard, por convertir este singular camino en una gesta que sabe compartida y en una experiencia enriquecedora.

Agradezco a los departamentos de Biología Vegetal I y Ecología de la UCM, por brindarme la oportunidad de desarrollar la tesis bajo su amparo en distintas etapas de la misma, por permitirme el uso de los laboratorios y el instrumental y por

darme la oportunidad de desarrollar las primeras colaboraciones docentes de las que guardo un grato recuerdo. A Carmen Prada, José María Gabriel y Galán, Gisela Oliván, Beatriz Pías y Mónica López gracias por enseñarme a enseñar y por permitirme aplicar la metodología que hemos desarrollado en clases prácticas.

Al personal de la UCM tanto de la facultad de Biología como de los vicerrectorados de Investigación, Estudiantes y de Posgrado y Formación Continua, por su diligencia burocrática, paciencia infinita y predisposición a no masajearse las sienes con las yemas de los dedos cada vez que les he expuesto mis problemas…o he llegado sin leerme la convocatoria…o con el documento fuera de plazo.

A todos los profesores que trataron de enseñarme a anticipar consecuencias, a esforzarme y a tener mis propios estándares, a todos los que me transmitieron su entusiasmo por las distintas ramas del conocimiento humano: Ana Isabel, Violeta, Julio, Paco, Félix, Jesús, Godelieve, Javier y Elena, gracias por vuestra labor. A mis familiares y amigos: a María del Carmen, a Sonsoles, a los del cole y allegados, a la biologada, a Freiji, Mel, Mapaches, Viole, Nuno, Itziar, Pedro, María, Bea, Mario, Miguel, Juan, Gonso, Gonsa, Isaac, Canario, Nachete, Pedrito, Mon y Adri, gracias por aceptar que me quiero dedicar a esta profesión, por no cuestionar mis sacrificios, por aguantarme todas esas veces que solo he sabido hablaros de hojas, de ángulos, de acebuches, de escalas, de gráficas sin contexto, de luz y de evolución, gracias por estar ahí y por vuestro apoyo incondicional.

A los evaluadores que han mejorado este trabajo con sus sugerencias y comentarios. A todas esas personas que deberían estar aquí incluidas e inexcusablemente olvido.

Y a mi abuela, Mercedes de las Casas, por su amor incondicional.
Gracias a todos,

"*Transportándonos a sus hombros,*
gigantes corrientes
de la lluvia a la escarcha"

"…but you can stop and think about it, to really get the pleasure about the complexity, the inconcibable nature of Nature"

- Richard Feynman