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Research paper

Evolutionary trade-offs between drought resistance mechanisms across a precipitation gradient in a seasonally dry tropical oak (*Quercus oleoides*)

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In seasonally dry tropical forest regions, drought avoidance during the dry season coupled with high assimilation rates in the wet season is hypothesized to be an advantageous strategy for forest trees in regions with severe and long dry seasons. In contrast, where dry seasons are milder, drought tolerance coupled with a conservative resource-use strategy is expected to maximize carbon assimilation throughout the year. Tests of this hypothesis, particularly at the intraspecific level, have been seldom conducted. In this study, we tested the extent to which drought resistance mechanisms and rates of carbon assimilation have evolved under climates with varying dry season length and severity within *Quercus oleoides* Cham. and Schlect., a tropical dry forest species that is widely distributed in Central America. For this purpose, we conducted a greenhouse experiment where seedlings originating from five populations that vary in rainfall patterns were grown under different watering treatments. Our results revealed that populations from xeric climates with more severe dry seasons exhibited large mesophyllous leaves (with high specific leaf area, SLA), and leaf abscission in response to drought, consistent with a drought-avoidance strategy. In contrast, populations from more mesic climates with less severe dry seasons had small and thick sclerophyllous leaves with low SLA and reduced water potential at the turgor loss point (π_{tlp}), consistent with a drought-tolerance strategy. Mesic populations also showed high plasticity in π_{tlp} in response to water availability, indicating that osmotic adjustment to drought is an important component of this strategy. However, populations with mesophyllous leaves did not have higher maximum carbon assimilation rates under well-watered conditions. Furthermore, SLA was negatively associated with mass-based photosynthetic rates, contrary to expectations of the leaf economics spectrum, indicating that drought-resistance strategies are not necessarily tightly coupled with resource-use strategies. Overall, our study demonstrates the importance of considering intraspecific variation in analyses of the vulnerability of tropical trees to climate change.

Keywords: drought avoidance, drought tolerance, dry tropics, seasonally dry tropical ecosystems, trade-offs.

Introduction

Water availability is one of most critical selective abiotic factors in nature. It limits growth, survival and reproduction, and affects the distributions and adaptive evolution of species (Wright et al. 2001, Ordoñez et al. 2009). In recent years, an increasing number of drought-induced dieback events have been observed in

multiple ecosystems worldwide (Malhi and Wright 2004, Allen et al. 2010, Asner et al. 2016). These events have been associated with increases in the frequency, duration and severity of drought (Sheffield and Wood 2008, Allen et al. 2010, McDowell et al. 2011). Seedlings are particularly vulnerable to

water stress, and consequently, they are expected to be more susceptible to the effects of increasingly severe drought events than adult trees (Dale et al. 2001). Under changing climatic conditions, improving our understanding of how species have evolved in response to water limitation is of increased urgency because it informs predictions of the potential impact of climate change on plant communities (Engelbrecht et al. 2007, Bonan 2008, Feeley et al. 2011, Choat et al. 2012, Anderegg et al. 2016).

Drought avoidance and drought tolerance are considered the main strategies that plants use to resist drought (Levitt 1980, Ackerly 2004, Poorter and Markesteijn 2008, Tomlinson et al. 2013). In general, drought avoidance allows plants to maintain water potential by reducing water loss (e.g., reducing leaf transpiration surface area), increasing water uptake (e.g., having more investment in roots) or both (Levitt 1980, Lambers et al. 2008). Drought tolerance allows plants to maintain physiological function under low soil water potentials (Loewenstein and Pallardy 1998, Ackerly 2004, Bucci et al. 2005, Buckley 2005, Brodribb et al. 2014, Meinzer et al. 2014).

Some authors have suggested that these drought resistance strategies are aligned with the leaf economics spectrum (LES) (Reich 2014). The LES describes leaf economic variation at the global scale and is defined as the covariation of a suite of leaf morphological, physiological and biochemical traits along resource availability gradients (Reich et al. 1997, 2007, Wright et al. 2004, 2005). Six leaf traits are fundamental to this spectrum: leaf lifespan, specific leaf area (SLA), rates of photosynthesis and respiration, and concentrations of nitrogen and phosphorus (Reich et al. 1997, Wright et al. 2004). The LES represents a continuous range of strategies of leaf carbon and nutrient investment and return, between resource-acquisitive strategies characterized by leaves that require low investment and have high productivity but short leaf life spans, and resource-conservative strategies with high-investment, low-productivity leaves that last for much longer (Mason and Donovan 2015). Drought tolerance traits are generally expected to be associated with a resource-conservative strategy whereas drought avoidance traits are expected to be associated with a resource-acquisitive strategy (Reich et al. 2014). Consequently, increased drought tolerance is hypothesized to be associated with reduced carbon assimilation rates whereas drought avoidance is expected to be linked to increased growth potential (Wright et al. 2010, Reich et al. 2014). However, at small evolutionary scales these associations have not usually been tested (Brouillette et al. 2014, Mason and Donovan 2015, Niinemets 2015). This is particularly true for seasonally tropical biomes.

Seasonally dry tropical ecosystems are dominated by a mix of drought deciduous and evergreen tree species (Borchert et al. 2002, Givnish 2002, Bowman and Prior 2005, Klemens et al. 2011, Vico et al. 2015). Deciduousness has been related to drought avoidance. Deciduous species drop their leaves during

dry seasons in order to reduce water loss via transpiration and costs of maintaining leaves under unfavorable periods to fix carbon (Reich and Borchert 1984, Eamus and Prichard 1998). These species usually have leaves with high SLA (leaf area per unit leaf dry weight), low leaf thickness and short leaf life spans that reduce leaf construction costs and increase the investment in photosynthetic tissues per unit leaf mass (Sobrado 1986, Franco et al. 2005, Vico et al. 2015). Consequently, thinner mesophyllous leaves (with high SLA) are thought to sustain higher photosynthetic and growth rates under favorable conditions of water (Reich et al. 1997, Wright et al. 2004). These leaves are considered to be beneficial in terms of carbon, water and nutrient balance in seasonally dry tropical areas since they can maximize carbon uptake and nutrient use when water availability is not limiting and avoid water loss during the severe dry seasons (Cornelissen et al. 1996, Givnish 2002, Poorter and Markesteijn 2008).

As the dry season becomes shorter and less severe in seasonally dry tropical ecosystems, there is usually an increasing abundance of species with longer leaf life spans that retain their leaves throughout the dry season (Oertli et al. 1990, Niinemets 2001, Read and Sanson 2003, Wright et al. 2005, Poorter et al. 2009, Markesteijn et al. 2011). This strategy requires greater carbon and nutrient investment in sclerophyllous leaf tissue (low SLA, high leaf thickness and high lignin concentration), which is thought to limit maximum photosynthetic rates but allow leaves to maintain function much longer. In fact, evergreen leaves are thought to be beneficial for carbon and nutrient balance when the dry season is short and mild because they allow carbon assimilation throughout the entire year including during the dry season. These leaves usually have low turnover rates, smaller size and lower surface-to-volume ratios, reducing water loss and increasing desiccation tolerance and water-use efficiency (WUE), particularly in the dry season (Parkhurst and Loucks 1972, Fetcher 1981, Niinemets 2001, Ackerly et al. 2002, Preston and Ackerly 2003, Read and Sanson 2003, Pickup et al. 2005, Wright et al. 2007, Markesteijn et al. 2011). These species with a conservative resource-use also possess traits that allow them to be functionally active at low soil water potentials such as adaptations that reduce xylem cavitation (e.g., narrow vessels with resistant pit membranes, high stem wood density) and traits that maintain leaf turgor (Brodribb et al. 2003). In particular, the water potential at the turgor loss point (π_{tlp}), or the wilting point, is considered an integrative trait that accurately captures leaf and plant drought tolerance (Niinemets 2001, Brodribb et al. 2003, Lenz et al. 2006, Baltzer et al. 2008). This trait is a measure of the water potential at which leaves lose turgor and wilt; it also indicates the minimum soil water potential at which plants can access soil water (Lambers et al. 2008). In general, plants decrease π_{tlp} by accumulating solutes and decreasing osmotic potential, which can be defined as the water potential produced by the cell solute concentration at full

hydration (Bartlett et al. 2012a). The turgor loss point (π_{tip}) is considered to be negatively associated with drought tolerance such that lower (more negative) values allow plants to maintain higher stomatal conductance, hydraulic conductance and assimilation rates under lower soil water potential. In a recent meta-analysis, Bartlett et al. (2012a) showed that π_{tip} varied across species in a manner expected based on biome, with plants from dry ecosystems showing lower π_{tip} than plants from moist ecosystems.

The attributes that confer the capacity to tolerate water stress are thought to limit growth potential under favorable conditions of water such that a trade-off between drought tolerance and growth rate has been hypothesized (Tilman 1988, Wright et al. 2010). Nevertheless, empirical evidence supporting this trade-off is still limited (Fernandez and Reynolds 2000). In this study, our goal was to determine the extent to which drought resistance mechanisms and carbon assimilation rates have evolved under tropical climates that differ in severity and length of the dry season within a species that is widely distributed in Central America, *Quercus oleoides*. Cham. and Schlect. We conducted a greenhouse experiment in which seedlings originating from five populations that vary in rainfall patterns were grown under different watering treatments. We asked the following questions:

- (i) Do morphological and physiological traits related to drought resistance and carbon assimilation respond plastically to water availability?
- (ii) Do populations with contrasting origins along a precipitation gradient differ in trait means?
- (iii) Do populations exhibit differences in phenotypic plasticity in response to water availability?
- (iv) Are drought-resistance strategies and resource-use strategies associated?

We hypothesized that, on average, low water availability would cause seedlings to: decrease carbon assimilation rates, growth rates, SLA, leaf size and water potential at the turgor loss point; reduce leaf surface area through leaf abscission; and increase WUE and leaf thickness. Second, we hypothesized that live oak populations originating from climates with less severe dry seasons would exhibit drought tolerance traits, including reduced turgor loss point and small, sclerophyllous leaves, particularly when exposed to dry conditions (Givnish 2002, Bowman and Prior 2005, Bartlett et al. 2012a, Vico et al. 2015). In contrast, we hypothesized that populations originating from climates with longer and more severe dry periods would have mesophyllous leaves and a greater propensity to abscise leaves in response to water stress (Borchert 1994, Condit 1998, Markesteijn et al. 2011). Finally, based on the expectation that drought-resistance strategies are associated with the LES across species (Reich et al. 2014), we hypothesized that within *Q. oleoides*, seedlings exhibiting drought avoidance would have traits associated with resource acquisition, including higher

carbon assimilation rates and relative growth, while those exhibiting drought tolerance would have traits associated with a resource-conservative strategy including limited growth potential.

Materials and methods

Study species

Quercus oleoides is a wind-pollinated, monoecious species that inhabits seasonally dry tropical forests of Central America. It usually forms mono-dominant stands and it is a key forest tree species for these ecosystems since it influences local hydrologic budgets and soil conservation (Boucher 1981). It is considered to have a sub-evergreen or brevi-deciduous leaf habit (Muller 1942). This species is a useful study system to explore evolution of drought strategies and growth rate for several reasons. First, it spans the largest gradient of dry-season aridity and wet-season rainfall within live oaks (*Quercus* section *Virentes*) (Cavender-Bares et al. 2015) and one of the highest precipitation ranges within the American oaks (M. Kaproth, personal communication). Second, previous studies have shown differences among populations in leaf morphology and photo-protective pigments associated with water availability (Cavender-Bares et al. 2011, 2015, Ramírez-Valiente et al. 2015), indicating genetic divergence associated with adaptation to different water availabilities.

Seed collection and population description

For this study, we collected acorns in early 2013 from 91 mother trees (10–33 per population) randomly selected within five populations of *Q. oleoides*. Mother trees were separated by more than 150 m, which is long enough to avoid familial structures (Deacon and Cavender-Bares 2015). The studied populations represented a wide range of the precipitation and soil moisture availabilities found within the species distribution (Table 1, Figure 1, Cavender-Bares et al. 2015). The length of the dry season ranges from ~3 months in the most mesic population (Rincón de la Vieja, Costa Rica) up to 5 months in the most xeric populations from Honduras (Las Tablas and Macuelizo), which occur in the driest region of the entire *Q. oleoides* range (Figure 1, see Cavender-Bares et al. 2015 for more information). Rincón is much wetter than all of the other populations and has the highest rainfall of anywhere in the range. Santa Elena (Costa Rica), which is in close proximity to Rincón but at lower elevation, gets much lower rainfall due to the rain shadow effect of the Cordillera de Guanacaste (Janzen and Hallwachs 2016). All Honduran populations (Las Tablas, Macuelizo and Sabana Grande) were also characterized by a 'little dry season' in the middle of the wet season. This is a two-month period (July and August) during which precipitation is lower than potential evapotranspiration (Figure 1).

In order to climatically characterize the studied populations, we recorded the geographical coordinates of each mother tree

Table 1. Location and climatic variables for the five studied populations.

Code	Site	<i>n</i>	Latitude	Longitude	Altitude	<i>P</i>	<i>T</i>	<i>I_m</i>
TA	Las Tablas	18	14° 00' 25" N	87° 02' 04" W	933	1014	22.2	-513.4
MZ	Macuelizo	12	13° 59' 51" N	87° 02' 41" W	1030	1017	21.7	-494.0
SG	Sabana Grande	10	13° 48' 54" N	87° 14' 55" W	1104	1185	21.2	-312.2
SE	Santa Elena	33	10° 53' 30" N	85° 34' 54" W	278	1776	24.9	172.3
RI	Rincón de la Vieja	18	10° 46' 42" N	85° 21' 36" W	779	2683	22.0	1164.3

n is the number of maternal trees sampled within each population. Altitude is given in meters above sea level. *P* is annual precipitation (mm), *T* is annual mean temperature and *I_m* is an index of moisture (see Materials and methods for details).

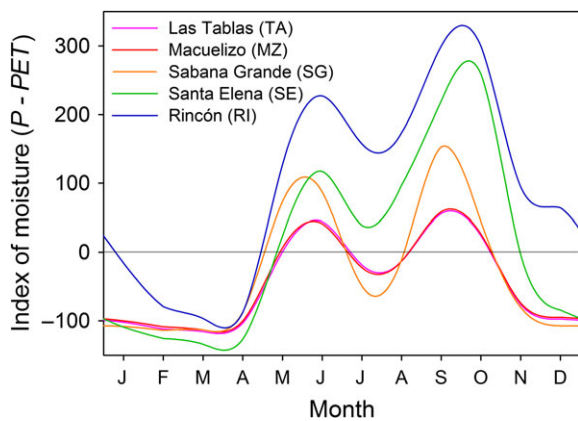


Figure 1. Monthly index of moisture (*I_m*) averaged across maternal families for the five studied populations (data from WorldClim; Hijmans et al. 2005). Index of moisture (*I_m*) was calculated as $I_m = P - PET$, where *P* is annual precipitation and *PET* potential evapotranspiration (see text for details). Values below zero indicate months of water deficit.

and used the WorldClim database to obtain monthly temperature and precipitation (Hijmans et al. 2005). We subsequently estimated an index of moisture, which takes into account precipitation and potential evapotranspiration at the location of each mother tree. This index of moisture (*I_m*) is calculated as $I_m = \sum (P_i - PET_i)$, where *P_i* is the monthly precipitation and *PET_i* monthly potential evapotranspiration. Potential evapotranspiration was estimated using monthly mean temperature and average day length per month following Thornthwaite (1948). This index reflects the annual water balance (negative values indicate water deficit and positive values indicate water surplus). It has been broadly used for spatio-temporal climatic characterization and global climate change studies (e.g., Sánchez-Salguero et al. 2010, Linares & Camarero 2012, Rigling et al. 2013, Trenberth et al. 2014). The index does not take into account soil properties or belowground water storage, which may impact water availability, but are not related to climate.

Common garden experiment

Acorns stored at 4 °C were synchronously sown in a greenhouse at the University of Minnesota in June 2013. A total of 12,160 acorns (11–96 per maternal family) were sown randomly in deepots (500 ml volume and 25 cm height approx.) with mix of

60% LC8 growing mix, 20% perlite and 20% vermiculite. Greenhouse temperatures were set at tropical conditions (28 °C (daytime average), 18 °C (nighttime average)). In October 2013, 1431 seedlings (6–25 per maternal family depending on availability) were randomly selected for transplantation into 6.2-l pots (40.6 cm height) with a 50% mix of sand and LC8 growing mix soil.

A common garden experiment was set up following a randomized block design with a total of seven blocks. Seedlings were grown for 5 months under well-watered conditions and maintained at tropical temperatures, as described above. Photoperiod was set to 12 h using halogen lamps between October 2013 and March 2014. At the end of this period, two watering treatments were implemented so that half of the plants per family were subjected at ~23–30% of soil moisture (well-watered treatment) whereas the other half was subjected to a reduction of soil moisture until reaching 9–13%, and then maintained at those levels throughout the experiment (dry treatment) (see Figure S1 available as Supplementary Data at *Tree Physiology Online*).

Soil moisture

We measured soil moisture in a subsample of plants established in the experiment using time domain reflectometry (TDR) throughout the experiment. A calibration curve between sensor values and volumetric soil water content (VWC) was constructed following previously described methods (Cavender-Bares and Holbrook 2001), $VWC = 2.6585 + 1.2036x$, $R^2 = 0.99$, $P < 0.0001$, where VWC is the volumetric water content and *x* is the relative water content measured using a Field Scout TDR 300 Digital soil moisture sensor calibrated (Spectrum® Technologies, Inc., Aurora, IL, USA). In the well-watered treatment, VWC was kept between 23% and 30% throughout the experiment by watering plants at field every day or every 2 days (see Figure S1 available as Supplementary Data at *Tree Physiology Online*). In the dry treatment, VWC was reduced from $24.35 \pm 0.13\%$ to $12.42 \pm 0.56\%$ in the first month and kept around 9–13% throughout the rest of the experiment by watering plants for ~30 s every 5 or 6 days (see Figure S1 available as Supplementary Data at *Tree Physiology Online*). We did not find differences among populations nor population by treatment interactions in VWC (data not shown).

Growth rate

We measured stem height, basal diameter, number of leaves, and width and length of the largest leaf in all plants at the beginning and at the end of the experiment (i.e., 0 and 100 days after the start of the water treatments). We used these variables to estimate initial ($t = 0$ days) and final biomass ($t = 100$ days) with allometric equations developed from a subsample of plants harvested throughout the experiment ($n = 120$ plants, 12 plants per population within each treatment). A multiple regression was performed including total biomass as the dependent variable and height, diameter, number of leaves and leaf size of the largest leaf as independent variables ($n = 165$). The allometric equation obtained was: $M = 0.2376x_1 + 0.2063x_2 + 0.074x_3 + 0.2637x_4$, $R^2 = 0.92$, $P < 0.0001$, where M is plant biomass (g) x_1 is height (cm), x_2 is diameter (mm), x_3 is leaf lamina area and x_4 is number of leaves. Absolute growth rate (g day^{-1}) was calculated as $\text{AGR} = (M_{\text{final}} - M_{\text{initial}}) / (T_{\text{final}} - T_{\text{initial}})$, where M_{final} is the estimated biomass at the final of the experiment, M_{initial} is the estimated biomass at the beginning of the experiment, T_{final} is the date of the final measurement and T_{initial} is the starting date of the water treatment. Relative growth rate ($\text{g g}^{-1} \text{day}^{-1}$) was calculated as $\text{RGR} = [\log_e(M_{\text{final}}) - \log_e(M_{\text{initial}})] / (T_{\text{final}} - T_{\text{initial}})$ (see Cavender-Bares et al. 2004 for a similar procedure).

Leaf abscission

A few weeks after the beginning of the watering treatments, some plants started to drop leaves. We took measurements of growth including number of leaves at the middle of the experiment ($t = 50$ days). For further analyses, we calculated the difference between the number of leaves at the middle ($t = 50$ days) and the end ($t = 100$ days) of the experiment relative to the total number of leaves in $t = 50$. This trait is referred hereafter as 'Percentage of change in leaf number between April and June' and abbreviated as 'PCLN' (Table 2). Negative values for PCLN indicate leaf abscission and positive values indicate leaf flushing.

Gas exchange

Between Days 45 and 55 of the experiment, we measured gas exchange in two to five seedlings per maternal family within each treatment ($n = 695$ plants). Measurements were taken from 10:30 am to 2:00 pm on seven sunny days using an LI-COR 6400 (LI-COR, Lincoln, NE, USA). Leaves were exposed to a CO_2 concentration of 400 ppm, relative humidity around 45–65% and saturating light of $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$. Light levels sufficient for saturation were determined in a previous study (Cavender-Bares et al. 2007). Leaves were maintained in these specific conditions for 2 min, which allowed photosynthetic rate and stomatal conductance to stabilize. Photosynthetic rate and stomatal conductance were determined on both area and mass bases using mean SLA data for the same leaves (see details

Table 2. List of traits and their corresponding abbreviation and units.

Abbreviation	Trait	Units
AGR	Absolute growth rate	g day^{-1}
RGR	Relative growth rate	$\text{g g}^{-1} \text{day}^{-1}$
ψ_{pd}	Predawn water potential	MPa
π_{tip}	Water potential at the turgor loss point	MPa
PCLN	Percentage of change in leaf number between April and June	%
Leaf size	Leaf lamina area	cm^2
SLA	Specific leaf area	$\text{cm}^2 \text{g}^{-1}$
Thickness	Leaf lamina thickness	mm
A_{area}	Light-saturated photosynthetic capacity per unit leaf area	$\mu\text{mol CO}_2 \text{m}^2 \text{s}^{-1}$
A_{mass}	Light-saturated photosynthetic capacity per unit leaf mass	$\mu\text{mol CO}_2 \text{kg}^{-1} \text{s}^{-1}$
$g_{\text{s,area}}$	Stomatal conductance per unit leaf area	$\text{mol H}_2\text{O m}^2 \text{s}^{-1}$
$g_{\text{s,mass}}$	Stomatal conductance per unit leaf mass	$\text{mol H}_2\text{O kg}^{-1} \text{s}^{-1}$
WUEi	Intrinsic water use efficiency	$\mu\text{mol CO}_2 \text{mol H}_2\text{O}^{-1}$

below). Intrinsic water-use efficiency (WUEi) was also calculated by dividing photosynthetic rate and stomatal conductance.

We also measured soil moisture in every single plant selected for physiological measurements to control for potential differences in water availability within treatments that could affect gas exchange measurements. See statistical analyses.

Leaf morphology

The youngest fully expanded leaf was collected between Days 45 and 55 and included the one selected for physiological measurements. Thickness was measured using a micrometer and then leaves were scanned and dried at 60°C for 7 days to estimate leaf area and dry leaf mass. We calculated SLA as leaf area/leaf mass.

Predawn leaf water potential and water potential at the turgor loss point

At the end of the experiment ($t \approx 100$), we randomly sampled 456 plants (two to three plants per maternal family within treatment) to measure the predawn leaf water potential. We used a Scholander pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA).

We also estimated the water potential at turgor loss point (π_{tip}) following a modified protocol from Bartlett et al. (2012b). This protocol is based on a previously established linear relationship with the osmotic potential at full hydration (π_o) measured in an osmometer. For this purpose, we randomly selected one or two plants from 70 maternal families within each treatment ($n = 222$). To get full hydration of leaves, instead of rehydrating shoots by placing them in water overnight as in Bartlett et al. (2012b), we watered plants to field capacity three times with an interval of 2 h between each watering during the evening

previous to collection. Cutting shoots could have killed the sampled plants. We conducted initial tests with 82 plants collected from both treatments that we rehydrated using this method. All had predawn leaf water potential values between -0.03 and 0.00 MPa, indicating that plants were fully hydrated. Leaves for water potential at the turgor loss point were collected at predawn and placed in humidified zipper double-bags. They were kept at 4°C in a dark room until measurements (always less than 12 h after collection). A 1-cm^2 leaf disc was extracted from each leaf avoiding secondary veins and submerged in liquid nitrogen at least 2 min. Osmolality was measured with a VAPRO 5600 vapor pressure osmometer (Wescor, Logan, UT, USA). Solute concentration (mmol kg^{-1}) values were converted to pressure–volume curve osmotic potential and turgor loss point (hereafter referred as ‘estimated turgor loss point’, π_{tlp}) following equations in Bartlett et al. (2012b).

Statistical analyses

Linear mixed models were used for statistical analyses of growth and functional traits. The estimated initial biomass of the plants was included in the models as a covariate because of its potential influence on leaf traits and growth rate and also to minimize potential maternal effects (Ramírez-Valiente et al. 2010, 2011, Koehler et al. 2012). Mixed models included population, water treatment, population by treatment and block as fixed factors, and maternal family nested within population as a random factor. For gas exchange traits, date of measurement was also included as a fixed factor and time as a covariate. In addition, we included VWC of the soil, measured the same day as the instantaneous measurements of gas exchange, as a covariate in the models, given its influence on gas exchange and the potential variation among pots within treatments. The statistical significance of the random factor was assessed using likelihood ratio tests (Littell et al. 2006).

In order to test whether traits were associated with climate of the origin, when differences among populations were found, we performed linear regressions between the index of moisture and traits using population means. We also tested how multivariate trait variation was associated with climate. For this purpose, (i) principal component analyses (PCA) were performed to summarize multidimensional trait variation. PCAs were carried out within each treatment (i.e., two PCAs in total) using maternal family means. Only traits that showed significant or marginally significant ($P < 0.10$) differences among populations within treatments according to mixed models and post-hoc Tukey's tests were included in the analyses (see Results section). (ii) PCA factor scores were extracted for each maternal family within each treatment and used to perform one-way ANOVAs (one ANOVA per PCA factor and treatment) and obtain their population means. (iii) Linear regressions were performed between PCA factors for populations and the index of moisture (see Craine and Lee 2003 for a similar procedure).

To test for relationships between plasticity of the traits and climate, when a significant interaction of population by treatment was found in the mixed models, we performed linear regressions between I_m and plasticity. We calculated plasticity of a given population as the difference of trait means between environments (represented hereafter as ‘ Δ ’ as used in Bartlett et al. 2014). We used this metric instead of other published plasticity indices because it better reflects the reaction norms (Valladares et al. 2006). We did not use principal factor analysis for plasticity because only a limited number of traits showed significant population by treatment interaction.

Results

Predawn leaf water potential (ψ_{pd}), growth rates and most functional traits differed between treatments, indicating that they exhibit phenotypic plasticity to water availability (Tables 3 and 4). In response to water limitation (lower values of ψ_{pd}), *Q. oleoides* seedlings reduced leaf size, light-saturated photosynthetic rates (A_{area} , A_{mass}), stomatal conductance ($g_{\text{s,area}}$, $g_{\text{s,mass}}$), absolute and relative growth rates (AGR, RGR) and increased WUEi (Table 3, Figure 2, see Figure S2 available as Supplementary Data at *Tree Physiology* Online).

Populations originating from across a precipitation gradient showed differences in RGR, leaf size, SLA, thickness and A_{mass} among treatments, indicating genetically based differences in these traits (Tables 3 and 4). In addition, AGR, estimated π_{tlp} and PCLN exhibited population by treatment interactions, indicating that populations differed in their levels of plasticity for these traits (Tables 3 and 4). On average, our results showed that populations from the most xeric climates tended to exhibit larger and thinner leaves with high SLA, higher leaf abscission in response to drought, but higher estimated π_{tlp} (i.e., lower drought tolerance under dry conditions) in comparison with more mesic populations (Figure 2, see Figure S3 available as Supplementary Data at *Tree Physiology* Online). Associations between index of moisture and growth rates (AGR and RGR) were significant but had small slopes. We obtained similar results in multivariate analyses (see Appendix S1 and Figure S4 available as Supplementary Data at *Tree Physiology* Online) as well as for climate-trait regressions using maternal family means (see Figure S5 available as Supplementary Data at *Tree Physiology* Online).

Plasticity (‘ Δ ’) of the estimated turgor loss point (π_{tlp}) was positively associated with I_m ($R^2 = 0.95$, $P = 0.005$, Figure 3), indicating that populations inhabiting more xeric climates have a canalized response (low phenotypic plasticity) in their tolerance to drought.

Finally, contrary to expectations, resource-conservative trait values such as low SLA, low estimated π_{tlp} and high leaf thickness were not associated with lower carbon assimilation or growth rates (see Table S1 available as Supplementary Data at

Table 3. Results of the mixed models for ψ_{pd} , AGR, RGR, π_{tlp} , PCLN, leaf size, SLA and leaf thickness. Fixed effects: treatment, population, population by treatment ($P \times T$), block. Random effects: family within population. Covariates: initial size.

Trait		Treatment	Population	$P \times T$	Family	Block	Initial size
ψ_{pd}	F or χ^2	227.27	1.16	2.03	0.00	3.26	17.42
	P	<0.0001	0.3298	0.0891	0.9909	0.0039	<0.0001
AGR	F or χ^2	712.02	4.40	3.52	33.98	20.61	37.52
	P	<0.0001	0.0016	0.0073	<0.0001	<0.0001	<0.0001
RGR	F or χ^2	681.98	4.09	0.73	25.96	21.02	154.03
	P	<0.0001	0.0027	0.5703	<0.0001	<0.0001	<0.0001
π_{tlp}	F or χ^2	43.77	0.58	2.77	1.11	1.01	11.83
	P	<0.0001	0.6791	0.0304	0.2926	0.4215	0.0008
PCLN	F or χ^2	532.80	3.58	3.49	7.46	10.71	49.94
	P	<0.0001	0.0066	0.0076	0.0063	<0.0001	<0.0001
Leaf size	F or χ^2	63.60	28.61	0.86	32.97	5.57	123.42
	P	<0.0001	<0.0001	0.4899	<0.0001	<0.0001	<0.0001
SLA	F or χ^2	1.14	13.80	0.71	62.57	24.79	84.10
	P	0.2859	<0.0001	0.5856	<0.0001	<0.0001	<0.0001
Thickness	F or χ^2	0.58	26.05	0.69	22.28	7.67	8.02
	P	0.4451	<0.0001	0.6002	<0.0001	<0.0001	0.0047

Significant values are in bold type ($P < 0.05$).

Table 4. Results of the mixed models for gas exchange traits: A_{area} , A_{mass} , $g_{s,area}$, $g_{s,mass}$, WUE. Fixed effects: treatment, population, population by treatment ($P \times T$), block, date. Random effects: family within population. Covariates: time, volumetric water content within treatment (VWC), initial size.

		Treatment	Population	$P \times T$	Family	Block	Date	Time	VWC	Initial size
A_{area}	F or χ^2	27.27	3.84	2.22	5.37	1.86	5.91	1.21	3.67	20.04
	P	<0.0001	0.0044	0.0654	0.0205	0.0856	<0.0001	0.2715	0.0262	<0.0001
A_{mass}	F or χ^2	30.55	2.42	1.36	0.99	1.27	6.78	0.96	3.85	30.43
	P	<0.0001	0.0479	0.2454	0.3204	0.2699	<0.0001	0.3269	0.3269	<0.0001
$g_{s,area}$	F or χ^2	35.82	1.22	2.34	1.75	1.37	2.24	0.02	5.57	27.98
	P	<0.0001	0.3011	0.0541	0.1857	0.2230	0.0380	0.8805	0.0041	<0.0001
$g_{s,mass}$	F or χ^2	29.53	0.77	1.92	0.11	0.69	1.73	0.19	4.32	38.37
	P	<0.0001	0.5429	0.1058	0.4566	0.6579	0.1121	0.6647	0.0139	<0.0001
WUE	F or χ^2	13.23	0.95	1.66	0	1.03	12.36	0.04	2.61	30.68
	P	0.0003	0.4354	0.1577	1	0.4030	<0.0001	0.8414	0.0748	<0.0001

Significant values are in bold type ($P < 0.05$).

Tree Physiology Online). In fact, SLA was negatively (and leaf thickness was positively) associated with photosynthetic rates (A_{area} and A_{mass}) and growth rates (AGR and RGR) (see Table S1 available as Supplementary Data at *Tree Physiology* Online).

Discussion

Our common garden study reveals differences in leaf morphology and physiology among populations of the tropical live oak, *Q. oleoides*, originating from contrasting precipitation regimes in dry tropical forests of Mesoamerica. Populations from climates with the most severe and prolonged dry seasons exhibited thin, mesophyllous leaves with high SLA, large leaf size and leaf abscission in response to drought, as expected. Populations from climates with less severe and shorter dry seasons had small and thick sclerophyllous leaves with low SLA and decreased the

estimated water potential at the turgor loss point (π_{tlp}) when grown under dry conditions. However, contrary to expectations based on the LES at global scale, *Q. oleoides* seedlings with lower SLA and higher leaf thickness exhibited higher photosynthetic rates (on both area and mass bases) and growth rates. Our study also provides the first evidence of intraspecific genetic differences in plasticity of π_{tlp} and suggests that differential adaptation to climatic regimes that vary in dry season length and severity has resulted in population divergence in functional traits related to drought resistance and resource acquisition within this species.

Climate predicts population-level variation in traits related to drought-resistance

In the dry treatment, populations differed significantly in the estimated turgor loss point (π_{tlp}), leaf morphology, leaf abscission and to some extent in relative growth rates (Figure 2B–G). Variation in individual and multivariate traits showed strong

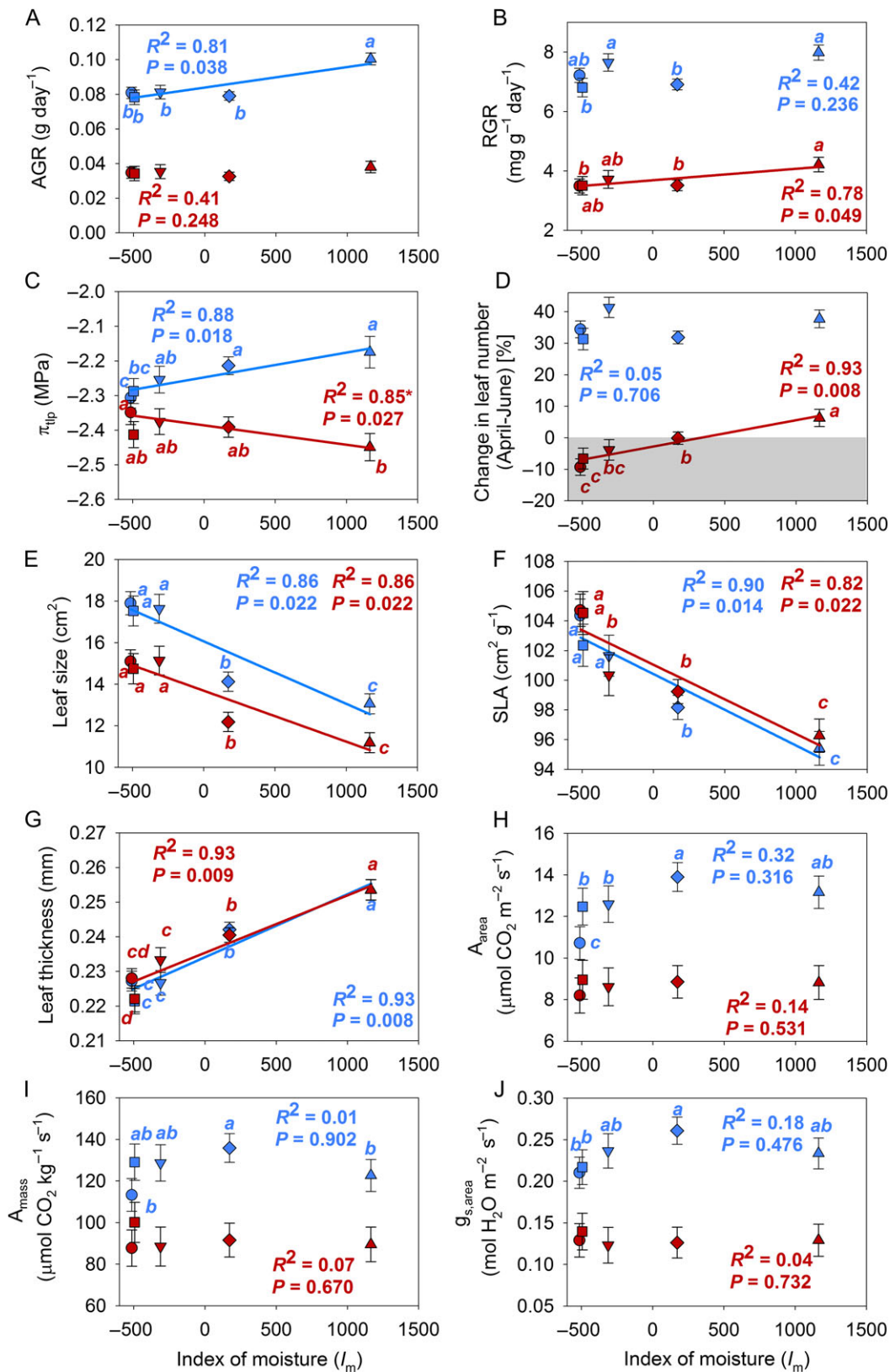


Figure 2. Relationship between the index of moisture (I_m) and AGR (A), RGR (B), π_{tip} (C), change in leaf number or PCLN (D), leaf size (E), SLA (F), thickness (G), A_{area} (H), A_{mass} (I) and $g_{\text{s,area}}$ (J). Points represent population means for well-watered (blue) and dry (red) treatments: Las Tablas (circles), Macuelizo (squares), Sabana Grande (down-triangles), Santa Elena (diamonds) and Rincón (up-triangles). Different letters indicate homogenous groups using Tukey's HSD tests for the well-watered and dry treatments, separately. The shaded area in (D) indicates negative values for the change in number of leaves between April and June, i.e., leaf abscission. *Regression coefficient (R^2) and significance level were obtained excluding the most extreme individual value in the experiment that was observed in a seedling from Macuelizo ($\pi_{\text{tip}} = -2.88$ MPa).

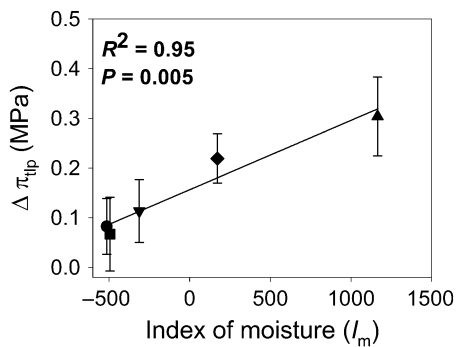


Figure 3. Plasticity across water treatments in turgor loss point ($\Delta \pi_{\text{tip}} = \pi_{\text{tip}} \text{ dry treatment} - \pi_{\text{tip}} \text{ well-watered treatment}$) in relation to index of moisture (I_m) of the population. Points represent population means \pm SE: Las Tablas (circle), Macuelizo (square), Sabana Grande (down-triangle), Santa Elena (diamond) and Rincón (up-triangle). Population means and standard errors were estimated by averaging $\Delta \pi_{\text{tip}}$ for family means within populations.

associations between local precipitation regimes, such that populations from more mesic sites exhibited smaller sclerophyllous leaves (low SLA and high thickness) (Figure 2E–G) and greater drought tolerance (lower π_{tip}) (Figure 2C) than populations from more xeric sites, which have larger mesophyllous leaves (high SLA and low thickness) and increased leaf abscission in response to drought.

These results contrast with the intraspecific variation observed in temperate oak species that inhabit seasonally dry ecosystems, which tend to exhibit smaller sclerophyllous leaves with longer life spans and higher drought tolerance under increasing summer drought (Gratani et al. 2003, Ramírez-Valiente et al. 2010, 2014, Niinemets 2015).

The association between drought resistance strategy and environment is frequently explained by cost–benefit ratio with respect to investment in acquisition of water, carbon and nutrients. Leaves may be costly for the plant under longer dry seasons because water loss via transpiration increases proportionally with leaf surface area (Kikuzawa 1991, Lambers et al. 2008). Since all populations showed similar values of stomatal conductance and WUE under dry conditions, foliage surface area might be the main mechanism leading population-level differentiation in total plant transpiration of *Q. oleoides*. Therefore, leaf senescence in response to drought may have been favored in more xeric soil conditions as a means of reducing water loss (Jonasson et al. 1997, Condit et al. 2000, Franklin 2005, Stevens et al. 2016). Nevertheless, other traits that improve water uptake such as root investment, root length, etc., should be measured in future studies to have a better understanding of the water balance in seedlings of *Q. oleoides*.

The observed leaf abscission in seedlings from xeric populations may have been selected for as a consequence of the high costs of leaves that can withstand excess of light and heat as the dry season becomes longer and more severe (Givnish 2002).

Under dry conditions plants need to protect leaves against the excess of absorbed light because of down-regulation of photosynthesis (Ishida et al. 2014). Photoprotection by controlled dissipation of excess excitation energy in the form of heat is known to be mediated via de-epoxidation reactions through the xanthophyll cycle (Demmig et al. 1987, Adams and Demmig-Adams 1994, Demmig-Adams and Adams 1996). The results derived from our previous study showed that *Q. oleoides* populations from mesic sites increased their de-epoxidation state (i.e., increased activity of xanthophyll cycle) in response to water shortage (Ramírez-Valiente et al. 2015). This mechanism, although it is energetically more efficient than repairing tissues from oxidative damage, incurs an energy cost during overnight epoxidation reactions (Raven 2011). Construction of leaves also represents important carbon and nutrient costs for the plant. More durable leaves, such as those with low SLA and high leaf thickness, are more costly to construct and need longer periods to pay back their investment (Williams et al. 1989, Eamus and Prichard 1998, Villar and Merino 2001, Villar et al. 2006). When the favorable season becomes shorter, the need to balance investment costs with photosynthetic returns may favor mesophyllous leaves, which are cheaper to construct (Eamus 1999, Villar and Merino 2001, McDowell et al. 2008).

Negative association between photosynthetic rates and SLA

An important perspective in the ecophysiological literature is that species and populations from tropical climates with severe dry seasons, which often exhibit mesophyllous leaves (high SLA, low leaf thickness and short leaf life spans), are expected to have high photosynthetic rates given the shorter time available to repay construction costs (Eamus and Prichard 1998, Eamus 1999, Reich et al. 2014).

In our study, populations from climates with longer and more severe dry seasons had mesophyllous leaves but did not have greater growth or photosynthetic capacity under well-watered conditions (Figure 2A, B, H, I). Furthermore, SLA was negatively related to photosynthesis (on both area and mass bases) and growth rates (AGR and RGR) in the well-watered treatment (see Table S1 available as Supplementary Data at *Tree Physiology Online*), contrary to expectations based on the LES (Reich et al. 1997, Wright et al. 2004). Differences between global trends and intraspecific variation have been reported for some species (Martin et al. 2007, Grady et al. 2013, Brouillette et al. 2014). Interestingly, negative associations between SLA and photosynthetic rates have been reported in other evergreen oaks from temperate and Mediterranean ecosystems (Niinemets 2015, Harayama et al. 2016). Leaves with high SLA and low leaf thickness are expected to have higher photosynthetic rates because of their higher investment in photosynthetic tissues per unit of leaf mass (Reich et al. 1997, Wright et al. 2004). Niinemets (2015) suggested that the negative relationship between SLA and area-based photosynthetic rate found for *Q. ilex* could rise

from the increased thickness of mesophyll and accumulation of photosynthetic biomass per unit leaf area in this species. Harayama et al. (2016) suggested that high investment of photosynthetic nitrogen was the cause for the high photosynthetic rates observed in temperate evergreen oaks with low SLA. In our study, we found that leaf thickness was positively associated with area- and mass-based photosynthesis (see Table S1 available as Supplementary Data at *Tree Physiology* Online). These results suggest that high photosynthetic rates in leaves with low SLA could be the result of higher mesophyll conductance (Niinemets 1999, Garnier et al. 2001, Niinemets 2015). In addition, in a recent review for Mediterranean species, Flexas et al. (2014) suggested that the relatively high photosynthetic rates in species with low SLA and thicker leaves could be the result of higher carboxylation velocity. Further research on leaf anatomy and photochemistry is needed to fully understand the mechanisms causing the negative association between SLA and area- or mass-based photosynthetic rates.

From an evolutionary perspective, relationships among LES traits have been suggested to be influenced by biome- or species-specific constraints caused by evolution under particular environmental conditions (Wright et al. 2001, Heberling and Fridley 2012, Mason and Donovan 2015, Niinemets 2015). In *Q. oleoides*, the observed negative SLA- A_{mass} or SLA-RGR observed under well-watered conditions could be due to the fact that xeric populations (with high SLA, low leaf thickness and increased leaf abscission in response to drought) experience a short period of water stress (July–August) within the wet season during which, precipitation is lower than potential evapotranspiration (negative I_m , Figure 1). This ‘little dry season’ may constrain the evolution of increased A_{mass} and RGR under favorable conditions of water, particularly in seedlings, and promote leaf drought resistance to prevent damage during intermittent water shortage during the wet season (Choat et al. 2007). For example, Brodribb & Holbrook (2004) showed that the little dry season has consequences similar to the actual dry season in terms of reducing water potential, stomatal conductance and leaf hydraulic conductance. Consistent with this idea were the observations of a positive relationship between the estimated turgor loss point (π_{tlp}) and I_m in well-watered conditions and the lack of plasticity in π_{tlp} in response to water availability for xeric populations (Figure 3).

Finally, the intraspecific differentiation of phenotypic plasticity in π_{tlp} that we observed in this study for *Q. oleoides* contrasts results obtained in the meta-analyses by Bartlett et al. (2014). Bartlett et al. (2014) found that despite a wide prevalence of plasticity in π_{tlp} in response to water availability in nature, differences in the degree of plasticity among populations, species or biomes were minimal. To our knowledge, the significant differences in π_{tlp} plasticity among populations of *Q. oleoides* we found under different water treatments are the first evidence of intraspecific divergence in plasticity of π_{tlp} related to climate of origin.

Conclusions

Our results show intraspecific differences in traits related to growth and drought resistance in *Q. oleoides*. Consistent with multi-species studies in seasonally dry ecosystems, populations from more mesic climates had smaller and thicker sclerophyllous leaves with low SLA and decreased the estimated turgor loss point (π_{tlp}). In contrast, populations from more xeric climates exhibited thinner, larger mesophyllous leaves with high SLA, and increased leaf abscission in response to drought. However, contrary to our expectations, xeric populations did not have greater capacity to grow and photosynthesize under well-watered conditions. Furthermore, SLA was negatively related to photosynthesis (A_{area} and A_{mass}) and growth rates (AGR and RGR), contrary to expectations based on the LES.

These unexpected patterns could be the result of adaptation to a short period of water-stress within the wet season in populations with the most xeric origins, which may have constrained their A_{mass} and RGR despite higher SLA and lower thickness in these populations. Both the positive relationship between the estimated turgor loss point (π_{tlp}) and I_m under well-watered conditions and the lack of plasticity in π_{tlp} in response to water availability within xeric populations are consistent with this possibility. Overall, this study demonstrates the existence of genetically based population variation in traits related to drought resistance in *Q. oleoides* as a result of adaptation to different precipitation regimes. To our knowledge, we provide the first evidence for intraspecific divergence in π_{tlp} plasticity in relation to climate of origin.

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Supplementary Data

Supplementary Data for this article are available at *Tree Physiology* Online.

Conflict of interest

Nothing to declare.

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