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## THE ECOPHYSIOLOGY OF XYLEM HYDRAULIC CONSTRAINTS BY “BASAL” VESSELS IN *CANELLA WINTERANA* (CANELLACEAE)

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Early angiosperms are hypothesized as constrained to wet environments where many of their defining characteristics evolved. A functional capability potentially enforcing the wet habitation of early angiosperms was possession of xylem with low hydraulic capability and drought intolerance. Recent studies indicate that a structural hallmark of these performances is long, steeply angled scalariform perforation plates in extant basal angiosperms. However, *Canella winterana* (Canellaceae), a magnoliid, appears to have radiated into much drier tropical dry forest habitats despite possessing a wood vasculature structurally similar to hypothesized ancestral wet-adapted system for angiosperms as a whole. Based on a field study of *Canella* wood hydraulic function, we present ecophysiological data that basal vessels do not necessarily mark low resistance to drought. We found that *Canella* wood was resistant to drought-induced cavitation. Leaf hydraulic capacity as well as leaf pressure-volume relations and leaf water potential minima found for *Canella* were comparable to some of the most drought-tolerant tropical dry forest angiosperms. Thus, our results suggest that vessels of an ancestral design can be exported to dry environments. We found that scalariform-plated vessels in *Canella* were associated with low hydraulic capacities at the xylem and whole shoot-scales as well as limited plasticity of the xylem to varying hydrodynamic demand. Consequently, our results support the hypothesis that evolution of simple perforation plates, not vessels per se, represented a critical adaptation for producing large hydraulic gains during angiosperm evolution across a broader range of environments.

*Keywords:* early angiosperm evolution, vessel evolution, drought tolerance, hydraulic constraint, plasticity.

### Introduction

The functional biology of the earliest angiosperms remains unclear, which constrains understanding of the ecology and environmental interactions that influenced early angiosperm success (Feild et al. 2009). However, recent evidence suggested that the way early angiosperms regulated water use in relation to the structure and function of xylem hydraulic transport influenced the early evolution of leaf photosynthetic capacity, regeneration niches, growth forms, and key aspects of flower function (Feild et al. 2009; Brodribb and Feild 2010; Carlquist 2010). The hypothesized mechanistic basis for how xylem hydraulic function bears on such diverse aspects of early angiosperm function is that the ancestral xylem of angiosperms functioned with low hydraulic throughput and feeble drought stress tolerance (Carlquist 1975; Sperry et al. 2007; Feild et al. 2009). How angiosperms transitioned out of their hypothesized ancestral “xerophobia,” which constrained ecological success, is unclear (Feild et al. 2009). However, a key mechanistic nexus that is critical for understanding these transi-

tions is how phases of early angiosperm radiation involved experimentation with xylem conduit designs that varied xylem hydraulic safety (hydraulic resilience to drought) versus efficiency (Sperry et al. 2007; Christman et al. 2009; Jansen et al. 2009; Lens et al. 2010).

A major hydraulic transition that unfolded with early angiosperm evolution was the origin of vessels from tracheids with scalariform pitting (Frost 1930; Bailey 1953; Carlquist 1975; Feild and Arens 2007; Feild et al. 2009). Selection for increased hydraulic capacity has long been viewed as the driving factor for vessel evolution (Carlquist 1975; Baas 1976, 1986; Zimmermann 1983). Evidence for the increased-flow hypothesis of vessel evolution emerged from reconstruction of the tracheid-to-vessel transition through anatomical work on extant basal taxa that apparently retain intermediate stages (Frost 1930; Bailey 1953; Carlquist 1975; Carlquist and Schneider 2002). Vessel origin begins with the loss of intertracheid pit membranes resulting in large perforations at the ends of cells, forming perforation plates. Vessel elements then evolved wider lumens, became more circular in shape and shorter in length. Also, perforation plates became shorter and less inclined and slowly transitioned from plates with dozens of narrowly spaced bars (scalariform plates) to form a simple plate with a single endwall pore (Frost 1930; Bailey

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1953). The selective vector of the “Baileyan” trend has been interpreted as progressively minimizing flow resistance (Bailey 1953; Carlquist 1975; Zimmermann 1983). Angiosperm vessels that retain transitional features between tracheids and the large-diameter vessel elements with simple perforation plates that define most dominant angiosperm lineages have been referred to as “basal vessels” for the sake of comparison (Sperry et al. 2007).

Recent xylem hydraulic measurements made across a phylogenetic gradient of basal vessel designs indicated that scalariform perforation plates impose large resistances to flow (Sperry et al. 2007; Christman and Sperry 2010). Apparently of less importance, at least during the early steps of vessel evolution, was the shift toward increasing conduit diameter in scalariform-plated vessels. To achieve a major increase in vessel flux capacity, scalariform plates must become consolidated into a simple plate (Christman and Sperry 2010). Evidence has also been found that basal vessels exhibited high vulnerability to drought-induced cavitation (Sperry et al. 2007). A hypothesized reason why basal vessels succumb easily to water-stress-induced cavitation is that they possess highly porous pit membranes and/or large amounts of pit area per vessel—variables that increase the likelihood of air-seeding under tension (Sperry et al. 2007).

The implication of these dual limitations of basal vessels is that extant basal angiosperms should persist in permissive habitats where maximizing water transport is of negligible competitive significance (Feild and Arens 2007; Christman and Sperry 2010). Indeed, the vast majority of extant basal angiosperm clades are restricted to some of the least evaporative terrestrial habitats known, such as tropical montane cloud forests (Carlquist 1975; Feild et al. 2009). But how the hydraulic properties of basal vessels scale up to limit whole-plant water balance and possibly determine ecological distribution remains unclear. To make such a link, analysis of how basal vessel function is embedded in the rest of a plant’s water balance mechanisms under field conditions is necessary. Such an analysis will shed new light on whether early angiosperm vessel form underpinned shifts in plant hydraulic function and ecological distribution (Hudson et al. 2010).

Of particular interest is the handful of exceptional magnoliid and basal eudicot lineages that possess basal vessels but that appear to have radiated into much drier habitats (Wilson 1960, 1986; Friis 1989). One of these exceptional species is *Canella winterana*, a member of the early-diverging magnoliid lineage Canellales that includes Canellaceae (five genera and ~35 species) as well as the vesselless angiosperm lineage Winteraceae (Salazar and Nixon 2008). Based on the current understanding of *Canella* wood, its vessels nest within the basal vessel morphospace and possess vessel elements with relatively long scalariform perforation plates with narrowly spaced bars, and tracheids are also present (Wilson 1960). Recent measurements of *Canella* wood found that hydraulic flow was highly vulnerable to water stress, with a 50% percent loss of stem xylem hydraulic conductivity at  $-0.45$  MPa and complete loss of hydraulic conductance at  $-2.0$  MPa (Sperry et al. 2007). Indeed, *C. winterana* was reported as the most vulnerable species in a survey of basal vessel-bearing angiosperms, which came predominantly from tropical cloud forest habitats (Hacke et al. 2007). Yet unlike the vast majority of

taxa with basal vessels, *Canella* occurs in tropical dry forests that experience much lower rainfall and higher evaporative demand—conditions where high capacity and drought-resilient water transport functions are of ecological importance (Wilson 1986; Brodribb et al. 2002, 2003; Feild et al. 2009).

How does *Canella winterana* manage what appear to be strong hydraulic constraints on whole-plant function in a tropical dry forest habitat? Are there other water balance mechanisms acting in the rest of the plant that mitigate the functional constraints of basal vessels? Are there ecological explanations responsible for what appears to be an uncoupling of wood hydraulic limits on ecological distribution? The goal of our study was to shed light on these questions by investigating how basal vessels present in *Canella winterana* related to stem hydraulic function and plant water balance in the field. We investigated several water relations variables, including diurnal changes in leaf water potential, stem hydraulic properties, leaf pressure-volume relations, and leaf hydraulic conductance, to understand how *Canella* accommodates high evaporative demand and presumably low leaf water potentials. In light of our results, we discuss how vessel evolution potentially influenced the early stages of angiosperm ecological radiation out of wet habitats.

## Material and Methods

### Study Species and Field Sites

*Canella winterana* is a small understory to subcanopy tropical dry forest tree (to 7 m tall and 20 cm dbh; Wilson 1986). Most individuals occur as understory trees of less than 10 cm dbh and 2–4 m tall (T. S. Feild, unpublished observations, 2000, 2010). *Canella* ranges broadly across the Caribbean region, with a northern limit in south Florida (Wilson 1986). *Canella winterana* occurs predominantly in dry tropical forests near coastlines that receive 800–1400 mm annual rainfall and have dry seasons of 2–5 mo with little to no rain (less than 40 mm  $\text{mo}^{-1}$ ).

We studied two populations of *Canella* from two field sites. The first population was in a subtropical hardwood hammock forest at Dagny Johnson Key Largo Hammock Botanical State Park northeast of Key Largo, Florida, at 2 m above sea level (25°10′32″N, 80°22′08″W). The substrate at this site was weathered limestone rock consisting of a smooth caprock pitted with solution holes filled with accumulated marl soil (Ross et al. 2001). Based on climate data from nearby Tavernier, mean annual temperature was 25.1°C and mean annual rainfall was 1178 mm, with 60%–80% of rain falling from May to October (Ross et al. 2001). To examine how *Canella* vessel element structure compared to common co-occurring species at the Key Largo site, we sampled the following taxa that were categorized as dominant canopy trees (*Bursera simaruba*, *Guapira discolor*, *Krugiodendron ferreum*, *Metopium toxiferum*, *Swietenia mahogni*), subdominant canopy and subcanopy trees (*Bourreria ovata*, *Eugenia axillaris*, *Eugenia foetida*, *Exostema caribaeum*, *Gyminda latifolia*, *Piscidia piscipula*, *Simarouba glauca*, *Zanthoxylum fagara*), and subcanopy-understory shrubs and small trees (*Ardisia escallonioides*, *Chiococca alba*, *Guaiacum sanctum*,

*Randia aculeata*; Ross et al. 2001). Fieldwork was conducted October 3–15, 2009.

The second population was from a coastal tropical dry forest in Hellshire Hills, Jamaica (17°54'N, 77°58'W). Mean annual temperature at the site is approximately 27°C. The long-term (30 yr) average annual rainfall at Great Salt Pond, an area within the Hellshire Hills, is 780 mm (McLaren and McDonald 2005). A small rainy season occurs in May–June, with the main rainy season occurring in October. This is usually followed by a longer dry period of 4–5-mo. In the study population, *Canella* occurred on white sand and within 50 m of the seashore. Plants were measured from mid May to early June 2010. During the measurement period, the rainy season had just begun, and the field site was rain-free for 12 days during our observations.

#### Water Status

Leaf water potential ( $\Psi_{\text{leaf}}$ ) was measured with a stainless steel pressure chamber (PMS-1000; Plant Moisture Status Instruments, Corvallis, OR) and a digital pressure gauge ( $\pm 0.01$  MPa; Ashcroft Scientific, Costa Mesa, CA). At the Hellshire Hills site, diurnal cycling of  $\Psi_{\text{leaf}}$  was determined by sampling two leafy shoots from three individual plants ( $n = 6$  per time point) at the following times on 2 days: 0400 (predawn), 0530, 0800 1000 1200, 1400, 1600, 1800, and 2000 hours. Shoots were double bagged in plastic for 5 min before measurements to reduce  $\Psi$  gradients across the leaf. During pressurization in the chamber, leaves were wrapped in a damp paper towel and aluminum foil to avoid desiccation by dry nitrogen gas during measurement. Exudation was observed with a  $\times 10$  hand lens and a light. At the Key Largo field site the same sampling procedures were used, except that measurements were available for 0430, 1200, and  $\sim 1800$  hours.

#### Leaf Pressure-Volume Relations

The  $\Psi$  isopleths ( $\Psi$  vs. relative water content [RWC]) of *Canella* leaves were determined by repeated measures (four to six observations per leaf) of mass and  $\Psi$  (Holbrook and Putz 1996). Fully expanded leaves were collected at maximum field hydration before sunrise and following nighttime rainfall. Leaves were then weighed using an electronic balance, and leaf  $\Psi$  was determined using a pressure chamber (as described above). Then, alternating measurements of mass and  $\Psi$  of leaves were made as the leaves were slowly desiccated to below the turgor loss (wilting) point. Fresh masses and dry mass values were used to calculate RWC (Holbrook and Putz 1996). The  $\Psi$  isopleths were determined on nine leaves from three individuals. We calculated the  $\Psi$  and RWC at the turgor loss point using accepted curve-fitting procedures with a data analysis program (Sigma-Plot, ver. 11; SPSS, Chicago, IL).

#### Leaf Hydraulic Conductance in Relation to $\Psi$

Leaf hydraulic conductance ( $K_{\text{leaf}}$ ;  $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ ) was determined from the relaxation kinetics of water uptake of leafy shoots attached to a flowmeter (Brodribb and Holbrook 2006). We first cut shoots with 80–200  $\text{cm}^2$  area from

plants at midmorning in the field (0930–1130 hours). These samples were double bagged in plastic and moved to a nearby field camp laboratory. After 5 min, the leaf water potentials ( $\Psi_{\text{leaf}}$ ) of two or three leaves over the shoot were determined. We measured  $\Psi_{\text{leaf}}$  with a pressure chamber (see above). Then, the shoot was attached to the flowmeter after recutting the end of the stem back underwater at least 2 cm from the first cut in air to remove embolized vessels from the first cut. The shoot was attached to a tube of filtered, degassed water. From the rehydration kinetics, two measurements of the suction pressure were taken: one (P1) at the peak suction (within 10–25 s after attachment) and a second point (P2) when suction decreased to 50% of the initial value. Once the second point was reached, the shoot was removed, and two or three leaves were measured for  $\Psi_{\text{leaf}}$ . Using  $\Psi_{\text{leaf}}$  and the values of P1 and P2 with leaf area of the shoot, we calculated  $K_{\text{leaf}}$  as described previously (Brodribb and Holbrook 2006).

#### Stem Hydraulic Properties and Xylem Anatomy

We determined stem hydraulic conductance ( $K$ , hydraulic flow rate for a given pressure, in  $\text{kg H}_2\text{O MPa}^{-1} \text{s}^{-1}$ ) with a portable flowmeter. The flowmeter consisted of tubing manifold and plastic Luer fittings, pressure transducer (0.001 MPa resolution; model 840081, SPER Scientific, Scottsdale, AZ), and a capillary tube (PEEK, Upchurch Scientific, Oak Harbor, WA) of known hydraulic conductance. The PEEK tube was placed series with the hydraulic head pressure and a stem, and the hydraulic conductance of the unknown stem segment was calculated using voltage divider theory. Hydraulic flow through a stem is calculated from the principle that hydraulic flow through an unknown resistor is proportional to pressure drop formed between the unknown and the known resistor at steady state flow. The hydraulic conductance of the capillary tube at range of head pressures was determined before fieldwork began, using an electronic balance in the laboratory ( $\pm 0.0001$  g resolution, Denver Instruments, Denver, CO) as described previously (Brodribb and Feild 2000). To maintain maximum hydraulic conductance of capillary tube, a solution of sodium hypochlorite (10% concentration) was perfused through the tube every 5 d to avoid microbial clogging.

Stem segments for measurements were cut from the branches while underwater. To examine intraspecific variation of stem hydraulic properties to light, we measured sun and shade branches of *C. winterana* from both field sites. Sun and shade environments were designated by field observations (Keeling and Phillips 2007). Sun shoots were taken as those that fully expanded under greater than 70% exposure to open sky. Shade shoots were taken as those that fully expanded in the forest understory, defined as less than 25% exposure to open sky. For each light environment for each field site, we sampled five individual plants and two branches from each individual for hydraulic measurements ( $n = 10$  branches per light habitat).

To avoid overestimating  $K$ , we determined the length of longest vessel in the wood of *Canella* branches by air injection at approximately 0.05 MPa using a 60-cc syringe and tubing. Branches were shortened with a double-edged razor blade in 1-cm steps until bubbles were observed emerging from the wood. The longest vessels were  $\sim 20$  cm long in

both populations. Therefore, all branches for hydraulic measurements were cut from three individual trees at both populations at lengths two to four times longer than the maximum vessel length. We severed branches from trees at predawn, and these branches were double bagged in wet plastic bags until measurement. We measured all stems within 2 h of sampling.

To determine  $K$ , stem segments that were 25 cm long and 3–5 mm in diameter were cut from branches while underwater. The ends of each segment were then shaved with a razor blade, and the stem was attached to the flowmeter while underwater. Hydraulic flux was calculated once a steady pressure gradient over the measured stem was observed (within 1 to 2 min of measurement). The hydraulic head pressure for determining stem hydraulic capacity was maintained at 0.09 MPa by using a plastic squirt bottle that was pressurized with air from a syringe. We used a hydraulic solution of known potassium chloride (15 mM) and calcium chloride (5 mM) ionic concentration to control for ionic-dependent effects on stem hydraulic conductance (van Ieperen 2007). In addition, the hydraulic solution was degassed by applying a vacuum generated using a 60-cc syringe.

We calculated stem hydraulic conductivity ( $K_H$ ) by multiplying  $K$  by the length of the measured stem segment. We then determined leaf area specific hydraulic conductivity ( $K_L$ ;  $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$ ), which expresses the ability of the stem vascular system to supply transpiring leaves with water as  $K_H$  divided by the leaf area ( $\text{LA}$ ,  $\text{m}^2$ ) distal to the cut stem end.  $\text{LA}$  was determined by scanning leaves at 300 dpi and digital image analysis using ImageJ (National Institutes of Health [NIH], Bethesda, MD). The hydraulic efficiency of stem xylem was expressed on a sapwood area ( $\text{SA}$ ) basis ( $K_S$ ) as  $K_H$  divided by  $\text{SA}$ . Stem xylem  $\text{SA}$  was determined on stem cross sections taken at the distal end. Stem sections were stained in safranin, photographed at  $\times 50$  using an upright microscope (CH-2, Olympus Optics) and a digital camera (D300S, Nikon), and  $\text{SA}$  was determined using ImageJ 1.40g freeware (NIH Image) with pith area subtracted out. For stems for the Key Largo population, xylem hydraulic efficiency was also expressed on a conduit area ( $\text{CA}$ ) basis ( $K_C$ ) as  $K_H$  divided  $\text{CA}$ .  $\text{CA}$  was determined on stem cross sections photographed at  $\times 400$ . The lumen areas of all vessels and tracheids (using published anatomical criteria for other *Canelleaceae*; Wilson 1960; Hudson et al. 2010) were measured using ImageJ. Two cross sections were analyzed per stem, and an average  $\text{CA}$  was calculated based on the stem  $\text{SA}$  (Hudson et al. 2010). Because we found that the samples taken *Canella* plants sampled at predawn did not contain any observable emboli or loss in stem  $K$  (see below), we took these values as approximating the maximum xylem hydraulic capabilities of *Canella* wood.

Vessel element length and perforation plate form were determined on macerated, safranin-stained wood preparations from stems of *Canella* and 17 co-occurring species from the Key Largo site (see above). Wood was macerated using heat, sodium hydroxide, and acetic acid (Hudson et al. 2010). Vessel element lengths were imaged on a microscope at  $\times 50$ , and 50 measurements were made per species. For vessel diameter, stem cross sections were used measurements per species. Three cross sections for three stems of each species were imaged at  $\times 200$ , and diameter was measured for 200 vessels for

each species. We also made observations on scalariform perforation plate characteristics on *Canella*. The following features were measured at  $\times 630$  and  $\times 100$ : plate angle, bar number, bar thickness, and slit width between bars as well as qualitative features of intervessel pits.

#### Vulnerability of Stem Hydraulic Conductance to Water Potential

Changes in stem hydraulic conductance to water potential ( $\Psi$ ) were measured on detached leafy branches that were air dried to a range of  $\Psi$  values (Brodribb et al. 2003). Three branches that were four to six times the length of longest vessel (see above) were severed at predawn (0430 hours) from three different plants at the Hellshire Hills field site. Branches were bagged in plastic to prevent water loss until measurements. Branches were then air dried under field conditions in the shade while  $\Psi_{\text{leaf}}$  was measured periodically as described above. Once a desired  $\Psi$  value was reached, the branches were rebagged for 10 min. Then, the  $\Psi$  of two to three leaves from a selected shoot for hydraulic conductance measurement were determined. These  $\Psi$  values were averaged and taken as approximating the  $\Psi$  of the stem xylem (Sperry et al. 1988). Afterward, a small stem length,  $\sim 1$  cm long, was cut from the branch while underwater. Hydraulic conductance was then measured on the stem segment at low head pressure (0.005–0.009 MPa) to avoid pushing out possible emboli because at such short stem lengths essentially all vessels were cut open at both ends in *C. winterana*. Once these conductance measurements were completed, we flushed the measured stem with (as above) at approximately 0.25 MPa with a syringe until all visible bubbles were purged from the wood. The percentage loss of hydraulic conductance (PLC) was calculated from the ratio of nonflushed to maximal (flushed) conductance (Sperry et al. 1988). PLC tests were conducted at approximately every  $-0.5$  MPa from  $-0.5$  to  $-6.0$  MPa. PLC measurements were plotted with  $\Psi$  to determine a vulnerability curve for *Canella* using an exponential sigmoid function to fit the data (Pammenter and Vander Willigen 1998) using SPSS 15.0 (SPSS, Chicago, IL).

#### Ecomorphic Traits of Leaves and Wood

Measurements of vein densities ( $\text{mm}$  of vein length per  $\text{mm}^2$ ) were made on sampled pieces of tissue ( $2 \times 3$  mm) from the middle portions of mature leaves ( $n = 5$  for each). Samples were fixed in 50% ethanol and then cleared using a standard procedure (Hudson et al. 2010). Veins were stained in safranin, mounted on slides, and measured using an upright microscope at  $\times 50$  (Axio-Imager, Carl Zeiss, New York). Digital images were captured with a digital camera (as above), and processed using ImageJ.

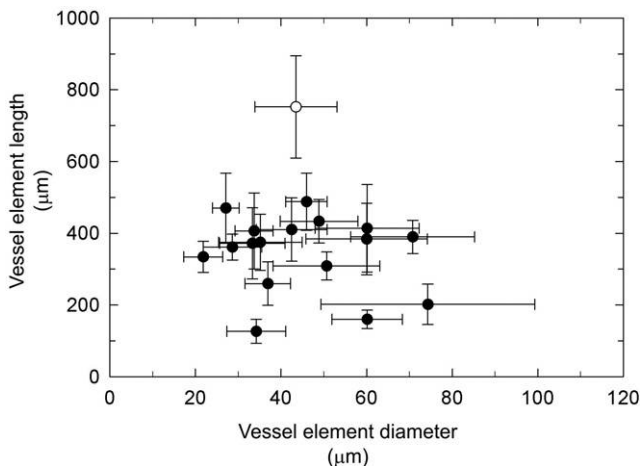
Stem wood density ( $\rho_{\text{wood}}$ ,  $\text{g cm}^{-3}$ ) was calculated as dry mass of stem wood divided by green wood volume using the volumetric displacement of water. We halved five 3-cm-long and 1-cm-thick pieces of wood and then removed the pith and the bark. Next, a syringe needle was used to submerge each sample in a 60-mL beaker on a portable balance ( $\pm 0.001$  g resolution, Denver Instruments), and the displacement of water was measured. We dried the samples in an oven at

100°C for 2 d and weighed them. We accounted for the effect of temperature on the density of water by normalizing values to 20°C. Leaf mass per area of leaves (LMA,  $\text{g m}^{-2}$ ) was determined by scanning a fresh area with a flatbed scanner at 300 dpi, and the samples were dried in an oven at 60°C for 2 d before weighing on an electronic balance (Denver Instruments).

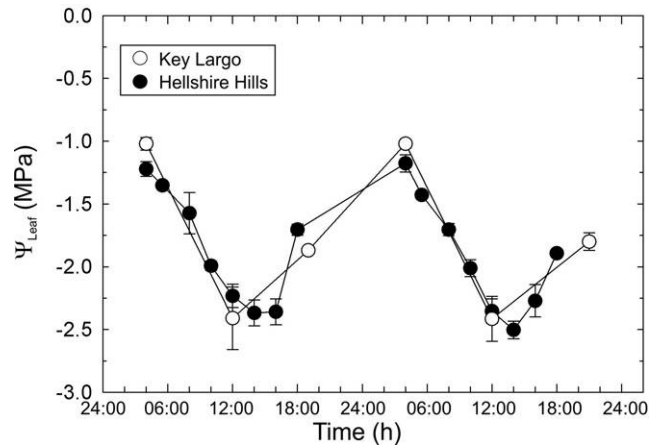
## Results

We found that the vessel elements of *Canella winterana* occupied a different position in the morphospace of vessel element length and vessel element diameter across diverse taxa in the Key Largo field site that represented a broad range of regeneration ecologies (fig. 1). Vessel elements of *Canella* (data shown  $\pm$ SD) averaged  $752 \pm 142 \mu\text{m}$ , which was 39%–85% longer than those of 18 co-occurring taxa. *Canella* was found to be the only species to possess vessel elements with scalariform perforation plates. Vessel elements of all other species sampled possessed simple perforation plates. The scalariform perforation plates of *Canella* were  $100 \pm 8 \mu\text{m}$  long and consisted on average of  $18 \pm 0.69$  bars. The bars of the scalariform plates were spaced on average  $4.95 \pm 0.17 \mu\text{m}$ , and the bars were  $3.23 \pm 0.06 \mu\text{m}$  thick. The mean perforation plate angle of *C. winterana* was  $22.5^\circ \pm 1^\circ$ . Intervessel pits of *C. winterana* were variable, including varying proportions of scalariform and circular bordered pits. Mean vessel element diameter of *Canella* ( $43.5 \pm 9.6 \mu\text{m}$ ), however, was comparable to the total mean of other taxa sampled (mean =  $44.9 \pm 15.7 \mu\text{m}$ ).

The  $\Psi_{\text{leaf}}$  values of *C. winterana* at predawn were similar at both field sites (mean =  $-1.1 \pm 0.02$  MPa at Hellshire Hills and  $-1.02 \pm 0.02$  MPa at Key Largo; fig. 2). In addition, midday to late afternoon minimum  $\Psi_{\text{leaf}}$  values did not differ between the two sites (mean =  $-2.4 \pm 0.05$  MPa at Hellshire Hills and  $-2.4 \pm 0.03$  MPa at Key Largo; fig. 2). At Hellshire Hills, no differences between flushed and un-



**Fig. 1** Variation in vessel element structure (length to diameter) of *Canella winterana* (open circle) as compared to co-occurring species (filled circles) that represented a range of regeneration ecologies in a subtropical hardwood hammock, Key Largo, Florida. Each point represents a mean of 50 measurements, with error bars denoting the standard deviation about the mean.



**Fig. 2** Diurnal changes in leaf water potential ( $\Psi_{\text{Leaf}}$ , MPa) for *Canella winterana* at Hellshire Hills, Jamaica (filled circles), and Key Largo, Florida (open circles). Each point is the mean of two leaves from three individual trees with error bars denoting the standard deviation.

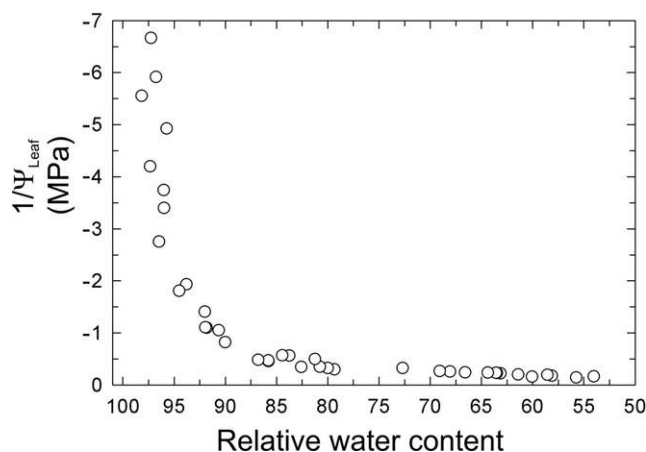
flushed stem conductance were observed throughout the day (data not shown). Thus, no native xylem embolism was detected in the field.

A plot of  $1/\Psi_{\text{leaf}}$  with leaf RWC revealed that leaves of *C. winterana* lost turgor at approximately  $-2.85$  MPa and 80.7 RWC at the Key Largo site (fig. 3) and at  $-3.0$  MPa and 77.4 RWC at the Hellshire Hills site (observations not shown). Maximum leaf hydraulic conductance ( $K_{\text{Leaf}}$ ) of *C. winterana*, when summed across both field sites, was  $\sim 6 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$  (fig. 4).  $K_{\text{Leaf}}$  declined with  $\Psi_{\text{leaf}}$ . We found that a sharp decline in  $K_{\text{Leaf}}$  to  $\sim 1.5 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$ , representing a 66% decrease, occurred at  $-2.5$  MPa. After this point,  $K_{\text{Leaf}}$  value was maintained across  $\Psi_{\text{leaf}}$  values as low as  $-4.5$  MPa. At  $-5.0$  MPa,  $K_{\text{Leaf}}$  became essentially zero. Across the tested stems, no percentage losses of stem hydraulic conductance were found in *C. winterana* stems over a  $\Psi$  range from  $-0.05$  to nearly  $-3.0$  MPa (fig. 5). At  $\Psi$  values less than  $-3.0$  MPa, PLC increased sharply with 50% loss of stem hydraulic conductance (i.e., the mean cavitation pressure [MCP]) lost at  $-4.9$  MPa and 100% loss at  $-5.5$  MPa (fig. 5).

We did not observe any significant differences for all stem hydraulic architecture properties measured, including  $K_L$ ,  $H_V$ ,  $K_S$ , and  $K_C$ , between sun-shade environments within both sites nor when means were compared across sites (fig. 6). Consistent with the lack of stem hydraulic adjustment of light environment, we did not observe significant changes in wood density or xylem CA in shade versus sun environments or xylem CA at both sites (table 1). In addition, we found very small amounts of plasticity in leaf  $D_V$  and LMA between sun versus shade light environments at both field sites (table 1).

## Discussion

We found that vessel elements of *Canella winterana* occupied an unusual position in the vessel element morphospace



**Fig. 3** Leaf pressure volume relations for *Canella winterana* leaves from the Key Largo field site. A plot of  $1/\Psi_{\text{Leaf}}$  presented to illustrate the turgor loss point. The response curve constructed based on nine leaves sampled from three individuals.

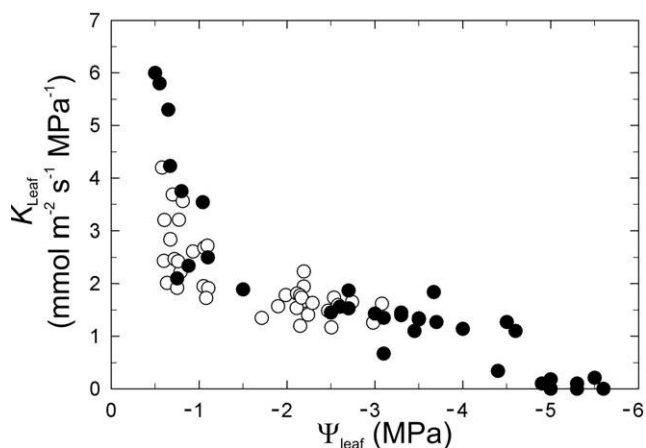
for tropical dry forest angiosperms. At the Key Largo field site, *Canella* was the only species out of 17 co-occurring and ecologically abundant species that possessed scalariform perforation plates and relatively long vessel elements (fig. 1). All other species, including taxa that occurred in the habitats of *C. winterana* underneath canopy dominants possessed simple-plated vessels. The pattern of vessel element form we found at Key Largo is consistent with the global view that taxa bearing scalariform-plated vessels are rare (1% to 5% of species) in arid and highly evaporative environments, such as tropical dry forests (Carlquist 1975; Baas 1976, 1986; Jansen et al. 2004; Wheeler et al. 2008).

Our anatomical measurements indicate that the vessels of *Canella* occupy a position of intermediate perforation plate specialization in the vessel element morphospace bounded by extant basal lineages such as Austrobaileyales, Chloranthales, as well as several lineages of the Magnoliidae (magnoliids), basal eudicots (i.e., Berberidopsidales), and derived eudicots with scalariform perforation plates that are restricted to wet habitats (Frost 1930; Bailey 1953; Carlquist 1975; Carlquist and Schneider 2002; Feild et al. 2009; Christman and Sperry 2010). In comparison to basal angiosperm species, the vessel elements of *Canella* (1) are up to ~30% shorter, (2) possess a lower number of bars per scalariform plate (an average 18 bar pits vs. up to 200 in some Chloranthaceae); (3) have less steep plate angles, and (4) have bars that are less thick and not as closely spaced (fig. 1; Wilson 1960; Carlquist and Schneider 2002; Christman and Sperry 2010). However in relation to some derived eudicots, the scalariform perforation plates of *Canella* are significantly less specialized than mesic forest and the uncommon dry-adapted derived eudicots with scalariform perforation plates, such as *Clethra*, *Davidia*, *Dillenia*, *Ilex*, and *Symplocos* (Baas 1976; Christman and Sperry 2010). Such derived taxa with scalariform perforation plates possess lower bar numbers and greater spaces between bars than *Canella*—features that when taken as whole reduce perforation plate hydraulic resistance, although not to values as low as simple perforation plates (Christman and Sperry

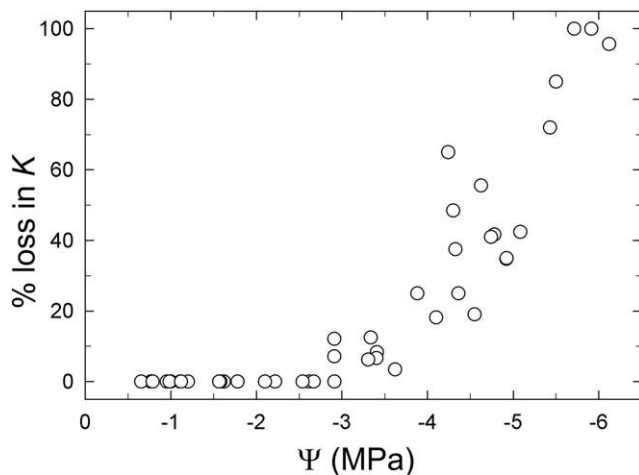
2010). Thus, while the perforation plates of *C. winterana* are not of the least specialized form, our results confirm that *Canella* possesses unusual perforation plate design in relation to its dry environments (Sperry et al. 2007; Christman and Sperry 2010).

We found that *C. winterana* possessed drought-resistant xylem. No stem PLC was observed for xylem tensions less than  $-3.0$  MPa during air-drying vulnerability tests on cut branches. In the field, no PLC was measured in branches sampled from morning to late afternoon, when  $\Psi_{\text{Leaf}}$  values decreased to approximately  $-2.5$  MPa. Thus, we found no evidence for native embolism in *C. winterana*. On experimentally manipulated branches, PLC increased sharply with decreasing  $\Psi$  values less than  $-3.0$  MPa, with 50% of stem hydraulic conductance lost at  $-4.9$  MPa (MCP) and complete stem hydraulic failure at  $-5.5$  MPa (fig. 5). As compared to diverse of tropical dry forest angiosperms measured worldwide, xylem vulnerabilities of *Canella* wood to decreasing  $\Psi$  fell in the upper portion of range (MCP of stem  $K_H$  for 15 species averaging  $-3.2$  MPa and ranging from  $-0.8$  to  $-7$  MPa; Brodrribb and Holbrook 2003; Brodrribb et al. 2003; Choat et al. 2005; Ishida et al. 2010). We believe that the differences between our stem cavitation responses and earlier findings that *C. winterana* functioned with highly vulnerable wood are explained by measurement errors. This is because previous measurements of *Canella* involved stems with significant proportion of cut-open (endwall-less) vessels (J. S. Sperry, personal communication, 2010). When refilled and spun on a centrifuge to test flow responses to tension, open vessels can cavitate prematurely and inflate estimates of water-stress-induced stem xylem vulnerability (Choat et al. 2010; Cochard et al. 2010).

Coupled with drought-resilient wood hydraulics, *Canella* leaf hydraulic function was resistant low  $\Psi$ -induced dysfunction. First, leaf pressure-volume relations demonstrated that the turgor loss point of *C. winterana* leaves occurred



**Fig. 4** Response of leaf hydraulic conductance ( $K_{\text{leaf}}$ ,  $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ ) to decreasing  $\Psi_{\text{Leaf}}$  (MPa) in *Canella winterana* at Hellshire Hills, Jamaica (filled circles), and Key Largo, Florida (open circles). Each point represents a leaf shoot from one individual sampled branch. Sampled branches came from a total of five individual trees sampled at both field sites.



**Fig. 5** Percentage loss in stem xylem hydraulic conductance (PLC, %) in relation to  $\Psi$  (MPa) for stem segments of *Canella winterana* from Hellshire Hills, Jamaica. Each point represents a stem segment from one individual sampled branch. The vulnerability curve was based on branches from a total of five individual trees.

at approximately  $-3.0$  MPa when averaged across both field study sites (fig. 3). In the field, diurnal  $\Psi_{\text{Leaf}}$  minima at Key Largo and Hellshire Hills populations came close to the turgor loss point, averaging approximately  $-2.5$  MPa. In addition, we found that  $K_{\text{Leaf}}$ , although decreasing significantly up to the turgor loss point, remained at approximately 30% of maximum conductance to  $\Psi_{\text{Leaf}}$  values low as  $-4.0$  MPa (fig. 4). These data suggest that the leaf xylem remained conductive and therefore capable of hydrating mesophyll cells to prevent cell death to well past the turgor loss point. These results are consistent with field observations that *Canella* is evergreen and has leaves with a long life span ( $>1$  yr), even during peak dry season conditions when nearly 30% other co-occurring species are leafless (Wilson 1986; A. Sharma, personal field observations, Hellshire Hills, 2009). *Canella*'s leaf water balance properties in relation to  $\Psi$  would fall near the upper end of the drought-tolerance range observed previously (turgor loss points and 50% loss of  $K_{\text{Leaf}}$  varying from  $-2.5$  to  $-4.0$  MPa; Brodribb and Holbrook 2003, 2006).

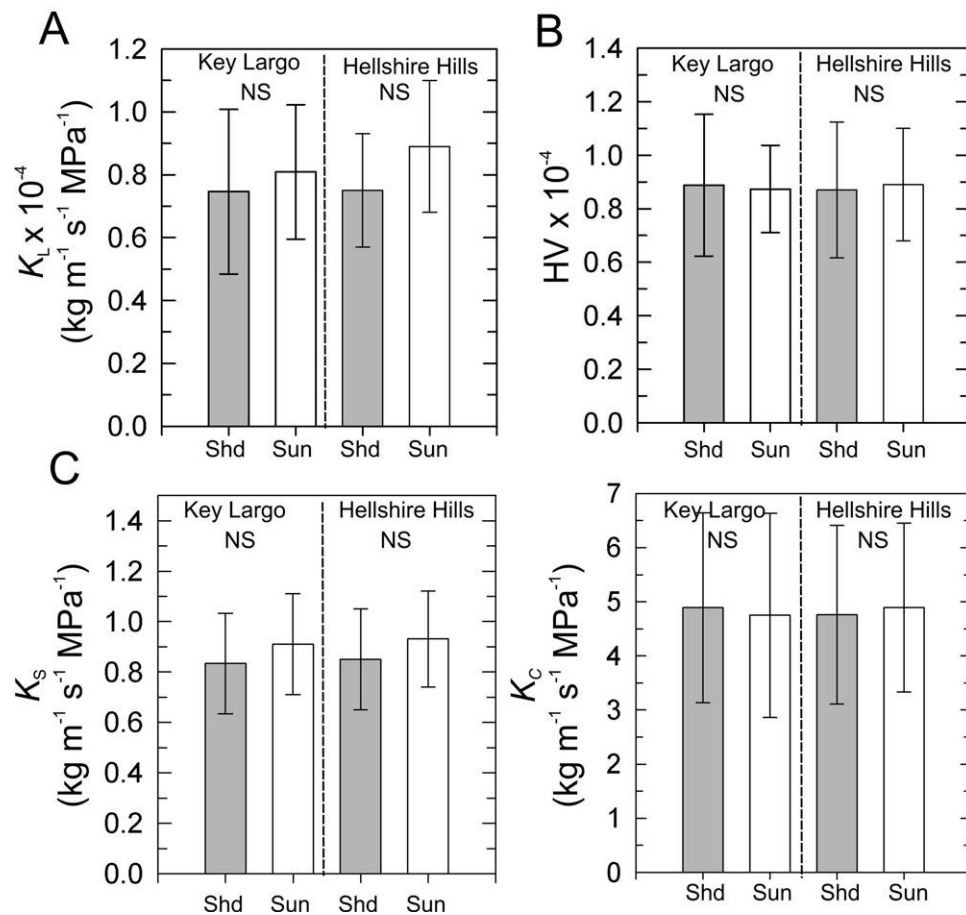
Overall, our results indicate that *Canella* manages drought stress well in its dry tropical forest habitats. We found no evidence that *Canella* ecologically sidestepped low  $\Psi_{\text{Leaf}}$  as a result of (1) occurring under forest canopy shade, (2) rooting in water-abundant "safe sites" such as near-freshwater-solution holes as present in the limestone substrates at the Key Largo site; or (3) having deep roots that tap more stable water supplies (Wilson 1986; Ross et al. 2001). The current of the phylogenetic distribution of habitat variation across Canellales and other related magnoliid clades suggests that the Canellaceae were ancestrally adapted to wet upland rainforest conditions (Feild et al. 2002, 2009). Thus, our data demonstrate that *Canella* represents a genuine ecological shift of basal vessel-bearing lineage into a much drier zone. To contextualize the drought tolerance properties of *Canella* with its relatives, we note that exposure leaves and stems of the vast majority of other Canellales to the  $\Psi_{\text{Leaf}}$  minima measured for *Canella winterana* during prolonged rain-free periods

would result in wilting and cessation of leaf carbon gain, severe xylem cavitation, or even shoot death (Hacke et al. 2007; Hudson et al. 2010).

We do not understand the structural basis for how the vessels of *Canella* are more resistant to  $\Psi$ -induced cavitation as compared to other basal angiosperms with long scalariform-plated vessels (Christman and Sperry 2010). Compared to other basal vessel designs, *Canella* vessels were reported to possess a relatively low surface area devoted to pit membranes, which may be associated with low xylem vulnerability to drought (Sperry et al. 2007; Christman and Sperry 2010). However, low vessel pit membrane area does not appear to account for the MCP performance of *Canella* wood since we found a lower MCP than would be predicted based on pit area as based on the relation demonstrated for other basal angiosperms (Sperry et al. 2007). Thus, other structural characteristics are involved in explaining the relatively high drought-induced cavitation resistance of *Canella* wood (Christman et al. 2009; Jansen et al. 2009; Lens et al. 2010). For example, the wood of *Canella* is dense (table 1). Dense wood may reduce the likelihood of air-seeding under tension (Jacobsen et al. 2005; Christman et al. 2009). We also note that vessel walls of *C. winterana* are thick, averaging nearly  $1 \mu\text{m}$  (T. S. Feild, unpublished observations, 2010). Such an observation suggests that vessel pit membranes are thick. Thick pit membranes are associated with smaller maximal porosities (i.e., rare pits) that can determine the threshold of air-seeding (Jansen et al. 2009).

Since our results demonstrate that basal vessels do not necessarily impose high vulnerability to drought-induced dysfunction, why then have so few basal vessel-bearing taxa radiated into zones of high evaporative demand? The perplexity of such an evolutionary pattern is heightened in view of evidence that a relatively larger number of derived eudicot clades that possess scalariform-plated vessels can occur in dry environments (e.g., *Symplocos*, *Ilex*; Baas 1976). In view of our findings on *Canella*'s stem hydraulic properties, we think that the key limitation of basal vessels is their low xylem hydraulic capacities (Sperry et al. 2007; Christman and Sperry 2010; Hudson et al. 2010). In support, xylem hydraulic capacities at the SA basis ( $K_S$ ) and LA basis ( $K_L$ ) of *C. winterana* were considerably lower (i.e., two to nine times) than those reported for most other dry tropical forest species (Brodribb et al. 2002; Choat et al. 2005). *Canella*'s  $K_L$  and  $K_S$  values were even lower than some vesselless Winteraceae (Hudson et al. 2010). High  $K_L$  means that leaf gas exchange capacity is high (Brodribb and Feild 2000; Brodribb et al. 2002). When high  $K_L$  is driven primarily by  $K_S$ , then high  $K_L$  can be economically cheap and realized by a low amount of wood allocation for a given amount of LA (Zimmermann 1983; Brodribb and Feild 2000; Brodribb et al. 2002). *Canella*, however, lacked either of these hydraulic capabilities. In fact, the  $K_L$  values for a given HV in *C. winterana* represented one of the lowest reported for any dry tropical forest species so far (Brodribb et al. 2002; Choat et al. 2005; Ishida et al. 2010). All else being equal in comparison to other dry forest angiosperm trees with simple-plated vessels, *Canella* operates with an expensive wood allocation because its wood is hydraulically resistive. Furthermore, *Canella* does not possess a high photosynthetic capacity to offset these carbon costs.





**Fig. 6** Comparison of stem hydraulic architectural characteristics of *Canella winterana* sampled from two field sites in relation to shade (gray bars) versus sun (open bars) light environments. The following stem architecture characteristics were measured: A, leaf area-specific hydraulic conductivity ( $K_L$ ,  $\text{kg m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ ); B, sapwood area to leaf area ratio (Huber value); C, stem hydraulic conductivity on a sapwood-area basis ( $K_S$ ,  $\text{kg m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ ); D, stem hydraulic conductivity on a xylem conduit area basis ( $K_C$ ,  $\text{kg m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ ). For each light environment at each field site, each bar represents a mean of five individual plants and two branches from each individual for hydraulic measurements ( $n = 10$  branches per light habitat) with error bars denoting the standard deviation. No statistical differences were observed between means across field sites (Mann-Whitney  $U$ -test).

Maximum  $K_{\text{Leaf}}$  as well as leaf vein density ( $D_V$ , which is a major determinant of hydraulic flow in the leaf; Brodribb et al. 2007) of *C. winterana* were lower than previously reported ranges of tropical dry forest trees (table 1; fig. 4;  $K_{\text{Leaf}}$

from 8 to 20  $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$  and  $D_V$  8–16.5  $\text{mm mm}^{-2}$ ; Brodribb and Holbrook 2003, 2006; Brodribb et al. 2007).  $D_V$  values for the 17 co-occurring taxa at Key Largo were all greater than *Canella* (table 1; mean = 9.6  $\text{mm mm}^{-2}$ ).

**Table 1**

**Comparison of Leaf Ecomorphic Characteristics, Wood Density, and Xylem Conduit Area of *Canella winterana* Sampled from Two Field Sites and Sun-Shade Habitats**

Trait	Key Largo, Florida		Hellshire Hills, Jamaica	
	Sun	Shade	Sun	Shade
$D_V$	5.23 ± .23 <sup>A</sup>	5.20 ± .29 <sup>A</sup>	5.34 ± .15 <sup>A</sup>	5.22 ± .18 <sup>A</sup>
LMA	97.1 ± 9.6 <sup>A</sup>	92.5 ± 10.8 <sup>A</sup>	100.5 ± 7.6 <sup>B</sup>	96.1 ± 8.2 <sup>A</sup>
$\rho_{\text{wood}}$	.768 ± .02 <sup>A</sup>	.753 ± .03 <sup>A</sup>	.768 ± .05 <sup>A</sup>	.765 ± .04 <sup>A</sup>
$C_A$	$4.66 \times 10^{-7} \pm 1.71 \times 10^{-7A}$	$4.74 \times 10^{-7} \pm 2.23 \times 10^{-7A}$	$4.62 \times 10^{-7} \pm 1.81 \times 10^{-7A}$	$4.69 \times 10^{-7} \pm 2.01 \times 10^{-7A}$

Note. Abbreviations and units for the traits are:  $D_V$  = leaf vein density ( $\text{mm mm}^{-2}$ ); LMA = leaf mass per area ( $\text{g m}^{-2}$ );  $\rho_{\text{wood}}$  = wood density ( $\text{g cm}^{-3}$ );  $C_A$  = xylem conduit area ( $\text{m}^2$ ). Data are means ± standard deviations. Means with a different letter are statistically different at  $P < 0.01$  (Mann-Whitney  $U$ -test).

Consistent with high xylem hydraulic constraints, *C. winterana* occupies some of the least evaporative sites possible in dry tropical forests (primarily under canopy cover). *Canella* also occurs as a small tree, which means water most likely flows over relatively short hydraulic path length from root to leaf compared to other taller dry tropical forest trees (Wilson 1986; Brodribb et al. 2002, 2003; Choat et al. 2005; Ishida et al. 2010).

Thus far none of the other derived eudicots with scalariform-plated vessels and that also in dry zones (Baas 1976) have been investigated to the structural-functional detail as here for *C. winterana* (Sperry et al. 2007; Christman and Sperry 2010). Scalariform perforation plates in such dry-adapted eudicots, however, are likely to be fairly resistive (but not to the extent of those in *Canella*) in limiting vessel-specific hydraulic capacity (Christman and Sperry 2010). Based on our results, we would expect that similar explanations of structural compensation, by pit membrane characters, for example, may operate in these taxa to explain their ecological adaptation in drier regions (Jansen et al. 2009). Nevertheless, such occurrences are in the extreme minority—angiosperms with simple perforation plates are ecologically dominant in dry zones (Carlquist 1975; Baas 1976, 1986; Jansen et al. 2004; Wheeler et al. 2008). Again, such is likely the case because inefficient vessels limit the ability to reduce the costs of wood allocation for a given amount of shoot photosynthetic capacity (Brodribb and Feild 2000). It may also be the case that scalariform perforation plates can be exported to dry environments provided the taxa possessing them are more slow growing and hydraulically conservative than their wet-adapted relatives. However, future work will need to be conducted to test whether these other taxa experience drought stress in the field versus occurring in localized wet pockets in otherwise dry regions.

Our data suggest that a further limitation of vasculatures plumbed by scalariform-plated vessels is that they enforce a low plasticity of hydraulic properties to varying hydrodynamic demand. We found no evidence that *Canella* increased stem hydraulic capacity ( $K_L$ , HV,  $K_S$ ,  $K_C$ ) or xylem CA in sun-acclimated versus shade-acclimated branches (fig. 6). Interestingly, leaf xylem hydraulic properties (as quantified by  $K_{Leaf}$  and  $D_V$ ), which may be a function of analogously resistive scalariform-plated vessels present in the primary xylem (Bierhorst 1960), also exhibited essentially zero plasticity with light environment (table 1). It is surprising that  $K_S$  and particularly  $K_C$  did not increase with greater evaporative loading, because in most vessel-bearing angiosperms studied, the addition of just a few more vessels can greatly increase wood hydraulic conductance (Zimmermann 1983). Indeed, *Canella* vessel diameters are fairly wide (up to 55  $\mu\text{m}$ , a value 20%–80% wider than the widest tracheids of its vesselless sis-

ter group Winteraceae; Feild et al. 2002; Hacke et al. 2007; Hudson et al. 2010), which adds further to the conundrum of why stem hydraulic properties are apparently inflexible with light environment.

However, we believe that the lack of hydraulic acclimation provides evidence that the high resistance of scalariform perforation plates scale up to impose limitations on shoot xylem hydraulic plasticity. Our results support previous findings that changes in conduit diameter are of limited effectiveness in increasing xylem hydraulic capacity when resistive scalariform perforation plates are present (Christman and Sperry 2010). Thus, in addition to bringing about substantial increases in xylem hydraulic capacity (Sperry et al. 2007; Christman and Sperry 2010), we suggest that the evolution of simple perforation plates, which has occurred several times during angiosperm diversification (Baas and Wheeler 1996), may represent a critical feature in the evolution of plasticity in xylem hydraulic function in angiosperms.

To conclude, recent studies using living basal angiosperms to test structure-function hypotheses of xylem hydraulics have opened new and exciting avenues toward the understanding the hydraulic nexus of early vessel evolution (Sperry et al. 2007; Christman and Sperry 2010). Our results show that it is possible for at least one basal angiosperm with scalariform-plated vessels to shift into a much drier environment than other related taxa can occupy through modification of the intrinsic drought resistance of the wood. By analyzing how basal vessels scale up to determine shoot-hydraulic function and are integrated with how the rest of the plant regulates water balance in relation to habitat conditions, we believe that the key limitation of basal vessels resides in the low hydraulic flows that they furnish. Our results provide further support for the hypothesis that vessels did not spur immediate gains in xylem hydraulic capacity with their origin. Major changes in xylem hydraulic capacity would have to wait until the origin of simple perforation plates (Sperry et al. 2007; Christman and Sperry 2010; Hudson et al. 2010).

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