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Publication Date

2009

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On the contribution of leaf surface wetness, leaf size and leaf longevity to variation in leaf water and carbon balance

by

Kevin Allan Simonin

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Integrative Biology

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Todd E. Dawson, Chair
Professor Wayne P. Sousa
Professor Dennis D. Baldocchi

Fall 2009

Abstract

On the contribution of leaf surface wetness, leaf size and leaf longevity to variation in leaf water and carbon balance

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It is widely recognized that many aspects of plant form and function are coupled to variation in water availability. This is because plant persistence is ultimately dependent upon the process of carbon fixation and it is physically impossible for a plant to transport CO_2 to the sites of photosynthetic metabolism inside the leaf without, at the same time, loosing water to the surrounding atmosphere. How the efficiency of water use changes as leaves differ in size and longevity, and where leaves acquire their water from, are often times not well defined. In general, the water lost to the atmosphere by leaves is thought to originate from the soil via uptake by roots. However, previous research has shown that water deposited on leaf surfaces is often available for use via direct foliar uptake. Using field observations and a greenhouse experiment I show that leaf water interception can represent an overlooked water source for leaves that temporarily, but significantly, decouples leaf-level water and carbon relations from variation in soil water availability (Chapter 1). Additionally, within a particular environment water loss per unit leaf area is expected to increase with leaf size. Recent research suggests the construction cost of a leaf also increases with size and/or longevity. If leaves have maximized the ability to transport water to surfaces for energy and gas exchange in order to maximize CO_2 uptake from the atmosphere, then vascular network efficiency (Leaf hydraulic conductance) should be size invariant. Using a survey of 60 angiosperm species I show that leaf hydraulic conductance is maximized for a given surface area (Chapter 2). By extension, if the lifetime return (carbon gain) on dry-mass invested in leaf area (construction cost plus maintenance respiration per unit leaf area) is maximized, then leaf hydraulic conductance per unit leaf dry mass should scale isometrically with leaf lifespan. Using plants from a common garden and previously published values of leaf lifespan and leaf hydraulic conductance for species inhabiting a broad range of vegetation types and climate, I explored the relationship between leaf longevity and leaf hydraulic conductance per unit leaf mass. I observed a negative correlation between leaf hydraulic conductance per unit leaf mass and leaf lifespan. Further, the slope of the relationship describing the covariation between leaf hydraulic conductance per unit mass and leaf lifespan is not significantly different from one. Isometric scaling (slope = 1) provides strong support for a

constant net carbon gain per leaf despite significant variation in leaf size, longevity and environment. Therefore, variation in gross primary productivity is a function of the number of leaves a plant maintains over a given unit of time (Chapter 3).

I dedicate my thesis to my family and friends

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Acknowledgements

The writing of a dissertation takes patience and confidence, and when you can't find that in yourself it takes family and friends. Thank you all! It also takes a good supply of high quality coffee beans, thank you Sweet Maria's.

The six years I spent as a graduate student in the Dawson lab was truly a fantastic experience. My advisor, Todd Dawson, provided me with all the tools a plant ecophysiology student could need and somehow, Todd always new where everything was, and its history of use. I hope some of your organization skills rubbed off on me, I could use it. You gave me the freedom to explore my own ideas, and at the same time provided me with guidance when it came to formalizing my research questions. Thank you for your patience and all the time you put into reading and editing my writing. Thank you for all your enthusiasm and support.

Many faculty contributed to the work presented here. I would like to thank Mary Power and David Ackerly for your constructive criticisms. I owe many thanks to my dissertation committee Wayne Sousa and Dennis Baldocchi for taking time to work with me on the development of my ideas and for critically thinking about how my research fits into the broader field of plant ecophysiology and community ecology.

I couldn't have asked for a better group of lab mates, I learned a lot from all of you. Thank you Tasha Hausmann for your encouragement and the time you spent helping me with my writing. I enjoyed our early mornings in the lab before anyone arrived. Thank you Lou Santiago for introducing me to many fun places in San Francisco and Berkeley (which by the way Ryan Davis is also thankful for). You are an amazing collaborator and friend. Thank you Anthony Ambrose for giving me the opportunity to look at a forest from a new perspective, a view from the top of a redwood tree. Truly amazing! Thank you Michal Shuldman for your honesty and humor. Whenever I needed a smile I could count on you. Thank you Ansgar Kahmen, you taught me a lot about stable isotopes and boosted my confidence. Jarmilla Pittermann, thank you for coming to the Dawson lab. You are a wonderful collaborator, I learned a lot from you! Thank you Kevin Tu for introducing me to all the fun you can have with biophysical models. Thank you Stefania Mambelli for all the help with my isotope analyses. Thank you Greg Goldsmith for all the science chats over beer at Jupiter and over long rides in the Berkeley Hills. You helped maintain confidence in my research questions and provided valuable feedback on my writing. Adam Roddy, you have been an incredible addition to the lab. Thanks for your help in the final stages of my dissertation.

Peter Cowan, working as a graduate student instructor with you for Todd's plant ecophysiology course was a highlight during my time at Berkeley.   I wrote most of this with you at cafe's around the bay area. Thank you for your support and for introducing me to Sweet Maria's.

Emily Limm, my dissertation would not have been the same without your help. I'd still be sitting at the base of a redwood tree trying to untangle a "spool of madness". Thanks for the many hours you spent helping me with my field work, you are an extraordinary collaborator and friend.

The Dawson lab had several lab technicians during my time at Berkeley. Thank you all for you help. You rock! I'd like to especially thank Vanessa Schmidt for being both a fabulous lab tech and friend. I wouldn't trade in any of our road trips to Sonoma and Big Basin.

Ryan Davis, I'm so glad you moved to San Francisco. You helped me step away from science and keep things in perspective. You are a good friend, thanks buddy!

All along the way my parents, Marty and Arlene, and my brother Michael have been there for support. Thanks for all your support, 35 years worth.

Thank you to my amazing wife, Anna, for so many things. You inspire so much in me and are always there to boost my confidence. You were there from the beginning, with my application to graduate school, and every step along the way. I can't imagine doing it over again without you.

Chapter 1: Fog interception by *Sequoia sempervirens* (D. Don) crowns decouples physiology from soil water deficit

ABSTRACT

Although crown wetting events can increase plant water status, leaf wetting is thought to negatively affect plant carbon balance by depressing photosynthesis and growth. We investigated the influence of crown fog interception on the water and carbon relations of juvenile and mature *Sequoia sempervirens* trees. Field observations of mature trees indicated that fog interception increased leaf water potential above that of leaves sheltered from fog. Further, observed increases in leaf water potential exceeded the maximum water potential predicted if soil water was the only available water source. Since field observations were limited to two mature trees we conducted a greenhouse experiment to investigate how fog interception influences plant water status and photosynthesis. Pre-dawn and midday branchlet water potential, leaf gas exchange and chlorophyll fluorescence were measured on *Sequoia sempervirens* saplings exposed to increasing soil water deficit, with and without overnight canopy fog interception. Sapling fog interception increased leaf water potential and photosynthesis above the control and soil water deficit treatments despite similar dark acclimated leaf chlorophyll fluorescence. The field observations and greenhouse experiment show that fog interception represents an overlooked flux into the soil-plant-atmosphere continuum that temporarily, but significantly, decouples leaf-level water and carbon relations from soil water availability.

INTRODUCTION

It is widely recognized that many aspects of plant form and function are significantly influenced by variation in soil water availability (Hsiao 1973; Stephenson 1990; Chaves 1991; Sperry et al. 1998). This is because soil water availability strongly constrains maximum leaf water potential (Ψ_L), gas exchange, turgor pressure, growth and plant distribution (Hsiao 1973; Running, Waring & Rydell 1975; Whitaker 1975; Stephenson 1990; Prior, Eamus & Duff 1997; Tyree and Zimmermann 2002). Further, as soil water is generally considered the only readily available water source for plants, water transport across the soil-plant-atmosphere continuum (SPAC) is considered unidirectional from soil to atmosphere *via* plant roots, stems, and leaves (Tyree and Zimmermann 2002). Thus over the lifetime of a plant a unidirectional SPAC framework predicts that water uptake by roots is equal to water loss from leaves with maximum Ψ_L approaching but not exceeding maximum soil water potential (Ψ_{Soil}) (Donovan, Linton & Richards 2001; Tyree and Zimmermann 2002; Donovan, Richards & Linton 2003).

The degree to which whole plant water status approaches equilibrium with Ψ_{Soil} is influenced by several different mechanisms (Donovan et al. 2001, 2003). Nighttime transpiration (E_N) is one physiologically important mechanism that has previously been shown to prevent the overnight equilibration between Ψ_L and Ψ_{Soil} for many distantly related tree species (Sellin 1999, Donovan et al. 2001, Dawson et al. 2007). Heterogeneity of soil moisture within the rhizosphere is another factor that can impede nighttime equilibration through hydraulic redistribution between rhizosphere compartments with different Ψ_{Soil} (Brooks et al. 2006; Warren et al. 2007). Apoplastic solutes also contribute to pre-dawn disequilibrium between leaf and soil through extremely low osmotic potentials for a given turgor pressure (Donovan et al. 2001, 2003). Additionally, for tall trees like *S. sempervirens*, Ψ_L is expected to differ from Ψ_{Soil} even during periods of no E_N, due to the gravitational component $\rho Gh \sim -0.01$ MPa m^{-1}. Together these mechanisms are expected to constrain plant water content and maximum Ψ_L to an upper limit set by Ψ_{Soil} and soil to root hydraulic conductance (k_{S-Rt}) as described by the following unidirectional SPAC mass-balance model:

$$k_{S-Rt}\Delta\Psi_{Soil-Rt} = C_{Rt}(d\Psi_{Rt}/dt) + C_{St}(d\Psi_{St}/dt) + C_L(d\Psi_L/dt) + E_N \tag{1}$$

where $\Delta\Psi_{Soil-Rt}$ is the water potential difference from soil to root , C_{Rt}, C_{St} and C_L are root, stem-wood and leaf specific capacitances defined as the change in tissue water content per unit change in water potential, Ψ_{Rt}, Ψ_{St} and Ψ_L are root, stem-wood and leaf water potential, respectively, and E_N is nighttime transpiration (Dawson et al. 2007).

Whereas current use of the SPAC framework assumes mass balance between root water uptake and leaf water loss, several studies suggest that atmospheric water condensing on aboveground portions of plants can be utilized through direct uptake by leaves (Stone 1957; Rundel 1982; Yates and Hutley 1995; Boucher, Munson & Bernier 1995; Mune-Bosch, Nogues & Alegre 1999; Burgess and Dawson 2004; Hanba Mriya & Kimura 2004; Oliveira, Dawson & Burgess 2005; Breshears et al. 2008). This form of leaf and crown hydration alters the general perception of the SPAC by contributing to water transport from leaf to root, i.e. in the opposite direction of that normally considered by the SPAC model (Burgess and Dawson 2004). If plants

possess a mechanism to obtain water from sources other than soil (e.g., from water condensed on leaf surfaces) then Ψ_L and leaf water content are no longer constrained by Ψ_{Soil} and root water uptake (i.e. $k_{S-Rt}\Delta\Psi_{Soil-Rt}$; Eq. 1). Rearranging the SPAC model described above (Eq. 1) we can begin to evaluate how crown interception and subsequent foliar uptake can contribute to plant water status:

$$k_{Atm-L}\Delta\Psi_{Atm-L} = C_L(d\Psi_L/dt) + C_{St}(d\Psi_{St}/dt) + C_{Rt}(d\Psi_{Rt}/dt) - k_{S-Rt}\Delta\Psi_{Soil-Rt} \qquad (2)$$

where k_{Atm-L} is the efficiency of foliar uptake and $\Delta\Psi_{Atm-L}$ is the water potential gradient between the intercepted crown water and the leaf. A bi-directional (i.e. leaf to root) SPAC mass balance framework suggests Ψ_L is more directly related to rates of foliar uptake (i.e. $k_{Atm-L}\Delta\Psi_{Atm-L}$) when plant crowns are wet.

Although crown interception can positively influence plant water relations through direct foliar uptake, leaf wetting events are often viewed as having a negative effect on photosynthesis and eventually growth. This is because atmospheric water that condenses on leaf surfaces physically reduces the transport of CO_2 to the sites of carboxylation and may result in significant degradation of the photosynthetic enzyme Rubisco, depending upon the duration of canopy interception, the time of day, and the overall wettability of the leaf surface (Brewer and Smith 1994; Ishibashi and Terashima 1995; Hanba et al 2004). However, previous work has focused on the responses of plants grown in wet soils and did not evaluate the potential benefits of foliar uptake on leaf level gas exchange for plants exposed to soil water deficit. If crown interception and subsequent foliar uptake increases leaf water content and Ψ_L to values greater than those obtained through root water uptake the temporary cost of leaf wetting on CO_2 diffusion and Rubisco may be outweighed by greater leaf level gas exchange for a given level of soil water deficit or Ψ_{Soil}.

We chose the California Coast Redwood (*Sequoia sempervirens* D. Don) for testing how well Ψ_L and leaf level gas exchange are coupled to variation in soil water availability when exposed to crown fog interception. The current distribution of *S. sempervirens* is largely constrained to areas characterized by the regular occurrence of summer time coastal fog (Marotz and Lahey 1975; Dawson 1998). Fog is considered to play an important role in the ecology and hydrology of *S. sempervirens* forests because fog frequency is greatest in the summer when drying soils coincide with otherwise low humidity conditions (Means 1927; Byers 1953; Oberlander 1956; Dawson 1998; Burgess and Dawson 2004; Ewing et al. 2009). The presence of fog during this annual summer time drought has previously been shown to: (1) reduce transpiration by decreasing the vapor pressure deficit (VPD) between leaves and the atmosphere (Burgess and Dawson 2004), (2) increase soil water content through fog drip from canopy to soil, also known as occult precipitation (Azevedo & Morgan 1974; Dawson 1998, Ewing et al. 2009), and (3) contribute to whole plant hydration through direct uptake of fog water deposited on leaf surfaces (Burgess and Dawson 2004). However, the overall effect of fog interception on *S. sempervirens* photosynthesis has not been measured, nor has the influence of fog interception on water potential gradients been explored.

Because fog water deposited on *S. sempervirens* canopies is available for water use through

direct foliar uptake (Burgess and Dawson 2004), we predicted that overnight canopy fog interception would: 1) increase crown water status above that maintained by nighttime rehydration *via* root water uptake, resulting in a decoupling between Ψ_L and Ψ_{Soil} and 2) minimize the negative effect of increasing soil water deficit on leaf level gas exchange.

MATERIALS AND METHODS
Field observations

Field observations were made at The Grove of the Old Trees, an 11 ha ridge-top parcel (300 m altitude) of old growth redwood forest in Sonoma County, California (38° 24' N 122° 59' W) approximately 8 km from the Pacific ocean. Within canopy variation in branchlet water potential was measured on two trees of similar height, but contrasting exposure to the marine fog; one, 70 m-tall tree (Tree 1) grew near the forest edge, while the other 63 m-tall tree (Tree 2) grew in the interior of the stand. Previous research at The Grove of Old Trees has demonstrated a decrease in annual occult precipitation from the forest edge to interior due to less fog interception by interior tree crowns (Ewing et al. 2009). On 10 August 2005, diurnal trends in branchlet xylem water potential (Ψ_L) were measured at three heights in the two trees on the southwest side of the crown (Tree 1 at 40, 53.8 and 60.3 m and Tree 2 at 32.9, 55.5 and 67.5 m). For the purposes of the data presented here, we define branchlet as a leafy shoot comprised of one small diameter photosynthetic stem with either needle- or scale-like leaves attached in such a manner that it is difficult to separate stem from leaf tissue. The measurements were made every 2 hours over the course of a 14-hour period, beginning pre-dawn (0540 h). Branchlets were cut and immediately sealed in plastic bags placed in the dark and transported to the base of the tree for determination of Ψ_L using a Scholander pressure chamber (Model 1000, PMS Instruments, Corvallis, OR). Balancing pressure was recorded when xylem exudates reached the cut stem surface as verified by a dissecting scope at 25X magnification. Previous studies on conifers have reported similar Ψ_L between measurements made immediately after excision and by this procedure (Kaufman and Thor 1982; Kolb et al. 1998).

Unexpectedly, during our field observations a moist front of marine air carrying fog entered the forest at approximately 0600 h. It initially penetrated the forest at the ground level and was only a few meters thick but then pushed upwards through the forest canopy and into the tree crowns until much of the forest canopy was enveloped in fog. The fog then dissipated shortly after 1200 h. At ~0700 h in the lower crown (32.9 m) and ~0800 h in the mid and upper crown (55.5 and 67.5 m) of the exposed 70m tall tree, branchlets intercepted fog water resulting in a thin film of water being deposited on the branchlet surface that lasted until ~1300 h. Although fog reached the crown of the interior 63 m tall tree (Tree 2), presumably increasing relative humidity and lowering temperature, branchlets on the interior tree never intercepted fog water and thus the branchlets on this tree remained dry. Therefore, the two trees we measured were exposed to very different fog treatments. We continued with our water potential measurements during this period despite the fog but these measurements were made on branchlets that were thoroughly dried using paper towels after the fog-water was shaken off the entire branchlet prior to using the Scholander pressure chamber (following the methods of Burgess and Dawson 2004). Based on these unexpected, but informative field observations we initiated the detailed greenhouse investigation outlined below.

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Greenhouse Experiment

Study plants and experimental design

We measured the effect of experimental crown fog interception on leaf level carbon and water relations of redwood saplings in a greenhouse. Twelve, 1.6 m tall *S. sempervirens* saplings of the Santa Cruz variety were obtained from a local nursery. Saplings were repotted in 10 L pots and allowed to equilibrate to ambient greenhouse conditions for three weeks before the start of the treatment period. Relative humidity (*RH*) in the greenhouse ranged from 36 to 70% and air temperature (*T*$_a$) ranged from 15 to 23°C. Greenhouse *RH* and *T*$_a$ reflected August conditions at The Grove of Old Trees when fog was not present (30.2 to 87.4% RH and 11.6 to 29.4°C). The influence of fog on redwood leaf carbon and water relations was investigated over a 32-day treatment period (7 March to 7 April 2006). Saplings were randomly assigned to one of three treatment groups: 1) well-watered (control), 2) water withheld from soil (dry-down), and 3) water withheld from soil, plus crown interception of fog water (fog). After the experiment began, trees in the control treatment were watered daily to saturation (between 1900 and 2000 h) while the other treatments received no soil water additions. We rotated pots every three days throughout the experiment to remove potential microclimate effects.

Fog was generated for the fog treatment nightly between 2000 and 0800 h by an ultrasonic water atomizer (Chaoneng Electronics, Nanhai, Guangdong, China) placed in a water reservoir, similar to the method of Burgess and Dawson (2004). Fog was generated and contained inside a clear polyvinyl chloride (PVC) chamber that was large enough to accommodate one 2 m tall *S. sempervirens* sapling. Two small electric waterproof computer fans (Adda AQ series, Brea, California, USA) were used to circulate the fog throughout each chamber. After each nightly fog event the sides of the PVC chamber were removed and trees were taken out of the chamber. Extreme care was taken to prevent fog water from reaching the soil through fog drip by placing PVC lids on each pot and sealing the tree bole to the lid with a waterproof putty (Terrastat IX, Henkel Technologies, Germany). Using these methods, fog exposure was confined to the canopy. This meant that during fog events, the only means of fog water use was through above ground structures.

Water potential

We measured Ψ_L at pre-dawn (between 0400 and 0600 h) and midday (between 1100 and 1400 h) on two branchlets from each tree. Pre-dawn water potential (Ψ_{Pd}) measurements were taken 1 and 4 days before treatment to insure that trees in all treatment groups were at similar levels of canopy water status at the start of the experiment. All water potential measurements were made 4, 7, 14 and 21 days after the start of the treatment. Midday water potential (Ψ_{Md}) was measured on branchlets after gas exchange measurements were taken.

Soil water availability

In order to evaluate the water potential components along the soil-plant-atmosphere continuum (SPAC) among the treatment plants, we estimated Ψ_{Soil} by measuring Ψ_{Pd} and volumetric soil water content on treatment days 14, 21, and 28. Soil water content was measured using a handheld soil moisture sensor (Hydrosense, Campbell Scientific, Logan, Utah, USA).

The relationship between Ψ_{Pd} and volumetric soil water content provides an estimate of Ψ_{Soil} in the root zone, assuming no mechanisms of pre-dawn disequilibrium, such as nighttime transpiration or heterogeneity in soil water content, are observed (See Introduction for further discussion). Equilibration between Ψ_{Pd} and Ψ_{Soil} is a common assumption when nighttime transpiration is minimal or absent, and is widely used for calculating whole plant and leaf specific hydraulic conductance (Irvine et al. 1998; Hubbard, Bond & Ryan 1999; Ryan et al. 2000; Fischer, Kolb & Dewald 2002; Phillips et al. 2003; Simonin et al. 2006). We did not detect nighttime transpiration or heterogeneity in soil water content. Therefore, we assumed overnight equilibration between Ψ_{Pd} and Ψ_{Soil} and used volumetric soil water content measurements and Ψ_{Pd} to generate a soil water-release curve. Soil water-release curves typically use a power function to describe variation in soil water potential with soil water content (Campbell 1998). The power function describing variation between Ψ_{Pd} and soil water content for the control and dry-down treatment trees was used to model pre-dawn Ψ_{Soil} for all treatment groups ($y = -77.9x^{-1.8}$; $r^2 = 0.83$; $p < 0.01$).

Leaf gas exchange and chlorophyll fluorescence

Maximum rates of midday net CO_2 assimilation (A_{area}) and stomatal conductance (g_s) per unit leaf area were measured between 1100 and 1400 h, 3 hours after the fog treatment when leaf surfaces were dry. The leaf-level gas exchange measurements were made on treatment days 4, 7, 14 and 21 using an infrared gas analyzer (6400, Li-Cor, Biosciences Inc., Lincoln Nebraska, USA). Three mature branchlets, from the same cohort per individual tree, were measured at 400 $\mu mol\ mol^{-1}$ CO_2 (slightly higher than ambient CO_2 concentration), with 1000 $\mu mol\ m^{-2}\ s^{-1}$ photosynthetic photon flux density (PFD) provided by a cool red-blue light source (6400-02B SI-710, Li-Cor, Inc.). The light level used in the gas-exchange cuvette was chosen based on the response of A_{area} to variation in light availability measured for understory saplings at The Grove of Old Trees field site during the spring of 2005 when soil water availability was not limiting. The light saturation point for understory tree saplings at our field site occurs between 0800 and 1100 $\mu mol\ m^{-2}\ s^{-1}$. Leaf temperature was allowed to vary naturally and ranged from 24 - 30 °C, with leaf-to-air vapor pressure deficits ranging from 1.87-3.28 kPa. Gas exchange measurements represent the maximum values that saplings in each treatment achieve given the light level, CO_2 concentration in the cuvette, and water status of the leaf. Leaf area was measured with a leaf area meter (LI-3100, Li-Cor, Inc.), in order to express midday maximum photosynthesis on a leaf area basis.

Chlorophyll fluorescence was measured between 0400 and 0600 h on 10 dark-acclimated leaves per tree on treatment days 4, 7, 14 and 21 with a pulse amplitude modulated fluorometer (MINI-PAM, H. Walz GmbH, Effeltrich, Germany). To maintain a constant distance and angle (60°) relative to the leaf plane, the fiber optic probe that delivered the measuring beam and saturating pulse was mounted above the leaf with a leaf clip holder (Model 2030-B, H. Walz GmbH).

Statistical Analysis

Greenhouse treatment comparisons were made using a repeated measures analysis of

variance to test for within-subject (days since water was withheld) and between-subject (water regime) effects on all tree response variables. Repeated measures analyses were performed using the JMP (version 5.0.1) statistical software package. Linear and non-linear regressions were used to test for correlation between tree response variables measured in the field (Sigma Plot, version 8).

RESULTS
Field observations

Diurnal variation in Ψ_L is expected to follow a sinusoidal pattern where Ψ_L begins at a maximum value at or near pre-dawn, declines steadily to a midday minimum, and then recovers in the afternoon. This sinusoidal pattern has been shown in many distantly related woody and herbaceous species (Jarvis 1976; Batten, McConchie & Lloyd 1994; Gallego et al. 1994; Prior et al. 1997, Sellin 1999). When immersed in fog, diurnal variation in *S. sempervirens* Ψ_L followed the opposite pattern than expected (Figure 1A and B) for these mature trees. As fog pushed up off of the ground through the tree crowns (bottom to top) Ψ_L approached, but did not exceed, Ψ_{Pd} for all three canopy heights (Figure 1A). When fog finally condensed on leaf surfaces of the exposed tree (~ 0700 h for the lower crown and ~ 0800 for mid and upper crown), Ψ_L increased above Ψ_{Pd} and stayed above Ψ_{Pd} across all three heights until the fog dissipated and leaf surfaces were dry (Figure 1B).

For both trees Ψ_{Pd} followed the expected gravitation water potential gradient of approximately -0.01 MPa m^{-1} across all three canopy heights (Figure 2A r^2 = 0.99, a = -0.011 ± 0.0004, y$_0$ = -0.336 ± 0.02). In accordance with a unidirectional SPAC model the vertical gradient in Ψ_L was below the $\Psi_{Soil} + \rho Gh$ gradient during periods of no fog (Figure 2A r^2 = 0.86, a = -0.029 ± 0.0059, y$_0$ = 0.273 ± 0.317; Figure 2C r^2 = 0.93, a = -0.025 ± 0.0035, y$_0$ = 0.079 ± 0.186) and approached, but did not exceed, the $\Psi_{Soil} + \rho Gh$ gradient during the foggy period when leaves were dry and the driving gradient for *E* was at a minimum (Figure 2B; r^2 = 0.53, a = -0.0046 ± 0.0043, y$_0$ = -0.797 ± 0.228). Contrary to a unidirectional SPAC model at mid-morning after leaves of the exposed tree were wetted by intercepted fog (1120 h), Ψ_L was well above the $\Psi_{Soil} + \rho Gh$ gradient, such that a positive Ψ_{Soil} was predicted from fitting a linear regression to Ψ_L versus height (Figure 2B; r^2 = 0.99, a = -0.127 ± 0.010, y$_0$ = -0.013 ± 0.0002).

Greenhouse experiment
Soil water availability and leaf water potential

We found no significant difference in Ψ_{Pd} between treatment groups before the start of the experimental dry-down (Table 1, Figure 3A). However, we observed a significant effect of both watering regime and the interaction of the watering regime with time on post-treatment Ψ_{Pd} and Ψ_{Md} (Table 1, Figure 3A and 3B). After withholding water for more than 4 days, trees in the control treatment had greater Ψ_{Pd} and Ψ_{Md} than trees in either of the drought or fog treatment groups. At 4 days post treatment, Ψ_{Pd} was highest for the fog treatment (-0.10 ± 0.08 MPa), followed by control (-0.21 ± 0.02 MPa), and drought (-0.23 ± 0.08 MPa) treatment groups. Control trees maintained Ψ_{Pd} around -0.20 MPa for the entire 28-day treatment period. Ψ_{Pd} of

trees in the fog treatment never dropped below -0.70 MPa, while trees in the drought treatment dropped below -1.1 MPa (Figure 3A). When Ψ_{Pd} was compared to soil water content, trees in the fog treatment maintained greater Ψ_{Pd} than trees in the drought and control treatments for a given soil water content (Figure 4A and 4B). When comparing the log values of Ψ_{Pd} and soil moisture, a distinct separation between the trees exposed to, and withheld from, overnight fog events occurred during periods of low soil moisture (Figure 4B).

Leaf gas exchange and chlorophyll fluorescence
    The watering regime and the interaction of watering regime with time had a significant effect on A_{area} (p = 0.015 and 0.007, respectively, Table 1) and a marginally significant effect on g_s (p = 0.07 and 0.075, Table 1). As expected, A_{area} was relatively constant for trees in the control treatment (4.06 ± 0.92 µmol m^{-2} s^{-1}, Figure 3C). Trees in the dry-down treatment showed a strong decline in A_{area} after water was withheld. Withholding water resulted in a 23% decline in A_{area} at day 7, a 72% decline at day 14, and a 67% decline at day 21 (Figure 3C). In contrast to the dry-down treatment, trees in the fog treatment maintained A_{area} similar to that of control, 4.39 ± 0.86 µmol m^{-2} s^{-1}, up to 14 days post-treatment. After day 14 A_{area} decreased by 63% to 1.89 ± 0.51 µmol m^{-2} s^{-1} (Figure 3C).
    Variation in g_s showed a similar pattern to variation in A_{area} across all three treatment groups. Trees in the control treatment maintained relatively stable g_s at 0.03 ± 0.004 mol m^{-2} s^{-1}, whereas trees in the dry-down treatment showed a strong decline in g_s after water was withheld (Figure 3D). Withholding water resulted in a 43% decline in g_s at day 7, a 61% decline at day 14 and a 70% decline at day 21 (Figure 3D). The pattern of g_s for trees in the fog treatment was similar to the control treatment up to day 14. But after 21 days, g_s for the fog trees decreased by 65%.
    Maximum mid-day leaf gas exchange was highly correlated with changes in water availability as assessed by Ψ_{Pd} for both the dry-down and fog water regimes (Figure 5A and 5B; A_{area} r^2 = 0.72 p < 0.0001; g_s r^2 = 0.63; p < 0.0001). Both A_{area} and g_s were sensitive to changes in Ψ_{Pd} between -0.2 and -0.6 MPa for trees in the dry-down and fog water regimes. Any further drop in Ψ_{Pd}, between -0.6 and -1.5 MPa, had a minor effect on A_{area} and g_s (Figure 5A and 5B). Although A_{area} and g_s were strongly correlated to variation in Ψ_{Pd} the sensitivity of A_{area} and g_s to variation in Ψ_{Md} was less pronounced (A_{area} r^2 = 0.28 g_s r^2 = 0.31; data not shown). The weaker correlation between leaf gas exchange and Ψ_{Md} can be attributed to the large variation in Ψ_{Md} for a given Ψ_{Pd}. For example Ψ_{Md} varied between -0.50 and -1.28 MPa when Ψ_{Pd} was greater than -0.24 and less than -0.15 MPa (Figure 3). This suggests that for *Sequoia sempervirens* saplings the Ψ_L associated with the onset of stomatal closure is strongly influenced by both water availability, as assessed by our Ψ_{Pd} measurements, and demand, as assessed by Ψ_{Md}. Taken together, the strong decline in g_s on day 21, for trees in the fog treatment, was associated with either Ψ_{Pd} less than -0.38 MPa and/or Ψ_{Md} less than -1.3 MPa (Figure 3 and 5).
    Additionally, we observed no effect of water regime or the interaction of water regime with time on leaf chlorophyll fluorescence (p = 0.810 and 0.924, respectively) and therefore no correlation between chlorophyll fluorescence and variation in Ψ_{Pd}. The lack of change between treatments in chlorophyll fluorescence and the strong correlation between A_{area} and g_s across all

three treatment groups ($r^2 = 0.86$; $p < 0.0001$; Figure 6) suggests the relationship between A_{area} and changes in Ψ_{Pd} was largely attributed to variation in g_s, and not the result of damage to photosystem II. When leaf level gas exchange was plotted against modeled Ψ_{Soil} a large amount of variation was observed between fog and dry-down treatment responses. Both A_{area} and g_s for trees in the fog treatment were less sensitive to variation in modeled Ψ_{Soil} when compared to trees in the dry-down treatment (Figure 5C and 5D; A_{area} $r^2 = 0.44$; $p = 0.04$, g_S $r^2 = 0.51$ $p = 0.03$).

DISCUSSION
Fog Interception and Leaf Water Relations
Our field observations showed that daytime crown fog interception resulted in Ψ_L that exceeded the predicted maximum Ψ_L if soil water was the only readily available water source (Figure 2B). Interestingly, we found that tree crown fog interception resulted in complete compensation for the negative effect of gravity with increasing height (i.e. $\Psi_{Soil} + \rho Gh$) on Ψ_L for leaves in the lower crown (32.9 m), with only partial compensation observed at mid (55.5 m) and upper (67.5 m) crown positions. Furthermore, fog interception by lower crown foliage maintained Ψ_L above that predicted by soil water availability throughout the entire day (Figure 2B). If fog condensation on leaves and stems had simply eliminated transpiration, a unidirectional SPAC model would predict equilibration between Ψ_L and Ψ_{Soil} and a vertical Ψ_L gradient with y-intercept (y_0) or $\Psi_{Soil} < 0$. In contrast, the vertical Ψ_L gradient observed in the field predicted a $\Psi_{Soil} > 0$ when the crown was wet (Figure 2; $y_0 = 0.127 \pm 0.010$). Interestingly, when foliage at each height in the exposed tree was covered in a film of water, the water potential gradient was steeper than the gravitational gradient of -0.01 MPa m^{-1}. The lower slope observed during crown wetting events could come about in response to within canopy variation in the capacity for foliar uptake (i.e. $k_{Atm-L}\Delta\Psi_{Atm-L}$; From equation 2) and/or the time course of water potential changes in leaves and stems, what we call their rehydration kinetics (e.g. $C_L(d\Psi_L/dt) \neq C_{St}(d\Psi_{St}/dt)$). Differences in the timing of canopy exposure to fog could also contribute to the observed slope of -0.013 MPa m^{-1}. Additionally, using the mass-balance framework described in Eq 2, we can see that the impact of foliar uptake on whole plant water relations is ultimately dependent on both the rate of foliar uptake (i.e. $k_{Atm-L}\Delta\Psi_{Atm-L}$), and the rate of root water loss to the soil (i.e. $k_{Rt-S}\Delta\Psi_{Rt-Soil}$). It is also likely that water uptake by roots and leaves could have occurred at the same time. Concurrent water uptake by the crown and the roots is most likely to occur when transpiration is interrupted by a crown wetting event, similar to the conditions seen during the field observations. Under this scenario stem water potential (Ψ_{St}) would be initially less than Ψ_L and Ψ_{Rt} as both leaves and roots would have direct access to water. Thus from a mass balance perspective (Equation 2) the impact of foliar uptake on plant water status and the $\Psi_{Soil} + \rho Gh$ is a function of both leaf and root water relations.

Although the field observations of crown fog interception and Ψ_L were limited to two mature redwood trees, the results from the greenhouse experiment provided further and more definitive evidence that fog interception can result in a decoupling between maximum Ψ_L and Ψ_{Soil}. We found that crown fog interception increased Ψ_{Pd} above that maintained by nighttime

rehydration *via* root water uptake only (Figure 4A and 4B). Taken together, the field observations and results from the greenhouse experiment indicate that leaf and stem water absorption alter the generalized unidirectional model of the SPAC, as foliar uptake provides an additional water source that can decouple plant crown water relations from soil water availability to the point where Ψ_L can exceed $\Psi_{Soil} + \rho Gh$. In light of our results, we propose that the SPAC model is best viewed as a true "continuum" among all potential water sources, not simply unidirectional.

Increased leaf water content associated with crown water interception, during periods of soil water stress, has the potential to influence many other aspects of plant form and function. For example, a unidirectional SPAC model would predict a strong influence of $\Psi_{Soil} + \rho Gh$ on the upper limit of tree height *via* constraints on Ψ_L, turgor pressure, and cell expansion (Koch et al. 2004; Woodruff, Bond & Meinzer 2004; Ishii et al. 2008). If $\Psi_{Soil} + \rho Gh$ does limit maximum tree height *via* constraints on maximum Ψ_L, it is likely that increased Ψ_L associated with crown water interception and direct foliar uptake could provide for greater potential cell growth and expansion through increased turgor pressure or $C_L(d\Psi_L/dt)$ for a given $\Psi_{Soil} + pGh$. Overall, our data, when combined with previous research, suggests that a unidirectional SPAC model is unable to describe whole-plant water relations and growth in response to variation in soil water availability.

Fog Interception and Leaf Gas Exchange

Our results suggest that fog interception by tree crowns provides a significant water subsidy that can have a positive influence on leaf level gas exchange when plants are exposed to an otherwise desiccating environment. Leaf level gas exchange is influenced by several environmental factors that either directly or indirectly influence variation in Ψ_L (Dewar 2002; Buckley, Mott & Farquhar 2003; Tuzet, Perrier & Leuning 2003). Because maximum Ψ_L is thought to approach but not exceed Ψ_{Soil}, both A_{area} and g_s are considered strongly coupled to variation in soil water availability as measured by Ψ_{Soil} (Sperry et al. 1998, Tuzet et al. 2003). In our study, A_{area} and g_s were positively correlated to modeled pre-dawn Ψ_{Soil} for *S. sempervirens* trees in the dry-down treatment but less so for trees in the fog treatment. Saplings in the dry down treatment showed an immediate decrease in leaf level gas exchange when exposed to minor soil water stress, whereas saplings exposed to fog interception showed a more gradual decrease in leaf level gas exchange, and maintained greater A_{area} and g_s for a given Ψ_{Soil} (Figure 5C and 5D). The observed increase in mid-day A_{area} and g_s suggests that fog water subsidies, *via* crown interception and subsequent foliar uptake, are not short-lived but are in fact great enough to impact leaf gas exchange throughout a majority of the day. Thus, our results show that foliar uptake of intercepted water can increase Ψ_L and improve photosynthetic carbon assimilation during periods of mild soil water stress. In this way fog, rain, or dew, intercepted by plant crowns can be viewed as an alternative water source for leaves that has a positive influence on leaf carbon gain.

Although crown wetting events *via* fog, rain or dew have previously been shown to increase leaf water content for many distantly related plant species, the potential benefits of crown interception on photosynthesis and growth is generally ignored or even dismissed. Instead, previous research has focused on the negative effects of leaf wetting events on plant

performance (Ishibashi & Terashima 1995) and the adaptive response of plants with an emphasis on morphological features that reduce leaf wettability and water retention (e.g. leaf drip tips, stomatal plugs; Lightbody 1985, Field et al. 1998; Ivey & DeSilva 2001).   The increased rates of leaf level gas exchange observed in our study suggest a cost/benefit analysis is necessary to fully understand the potential adaptive outcomes of crown wetting events on leaf form and function. Although leaf wetting can reduce the rate of CO_2 uptake by leaves, this temporary reduction can lead to increased gas exchange after leaf surfaces have dried due to the positive effects of leaf wetting on Ψ_L. To the best of our knowledge only three previous studies have shown a significant positive effect of crown water interception on leaf-level gas exchange (Grammatikopoulos & Manetas 1994; Muné-Bosch & Alegre 1999; Martin & Willert 2000). Unlike these previous studies, however, our study was conducted on whole plants, not individual leaves, and occurred over an extended period of time spanning a large range of soil water availability. Our research and the previous work cited above suggests that leaf wettability and the frequency of leaf wetting events are likely to be a strong selective pressure on leaf form and function during periods of mild soil water stress. For redwoods, the finely divided and closely arranged needles lead to a great deal of water retention in tree crowns particularly when compared with other tree species with long needle (e.g., *Pinus*) or elongate (e.g., *Eucalyptus*) leaf shapes (T. Dawson, pers. obs.). Thus, variation in leaf shape, wettability and water retention may be particularly important for not only crown water uptake but other aspects of a species' ecophysiology within the same community where some species may shed water from leaf surfaces much more quickly and therefore may not accrue the same benefits as redwood does.

CONCLUSION

Researchers and naturalists alike have long noted that the distribution of *S. sempervirens* along the California coast strongly coincides with the frequent occurrence of summer fog. However, most descriptions have generally focused on the enhanced soil, and therefore root, water availability resulting from summer fog inputs as an explanation for this unique range distribution. Our results illustrate how crown water interception and uptake of fog can provide another readily available water source that contributes to greater Ψ_L, resulting in greater midday gas exchange for a given soil water availability. The increased carbon gain associated with foliar water uptake may ultimately translate into increased fitness in these foggy coastal sites. Further, we demonstrate that foliar uptake of intercepted fog water can decouple plant water status from soil water availability. In doing so, foliar uptake can result in Ψ_L that is greater than the Ψ_{Soil} + ρGh potential gradient such that maximum Ψ_L may actually exceed Ψ_{Soil}. The importance of leaf wetting for the carbon gain of other tree species is unknown, yet given the frequency of rain, fog, and dew events in most forested ecosystems, it seems likely that similar effects are more widespread than generally acknowledged. This work and the research cited herein suggest that crown wetting events often represent an over looked aspect of plant water relations that can have a dramatic and positive effect on whole-plant and ecosystem water and carbon balance (e.g. Diaz & Granadillo 2005; Gabriel & Jauze 2008; Johnson and Smith 2008; Wiliams et al. 2008 Ewing et al. 2009).

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TABLES

Table 1. Repeated measures analyses for the effect of water regime (between-subject variation) through time (within-subject variation) on pre-dawn and midday leaf water potential (Ψ_P and Ψ_M, MPa), photosynthesis (A_{area}, µmol m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}) and chlorophyll fluorescence (fv/fm). P values in bold are significant at $\alpha = 0.05$.

Source	Tree response variable	F	Numerator *df*	Denominator *df*	P > F
Water regime	Ψ_{Pd} pre-treatment	1.35	2	9	0.305
	Ψ_{Pd} post-treatment	54.79	2	9	**<0.0001**
	Ψ_{Md}	6.00	2	9	**0.022**
	A_{area}	6.84	2	9	**0.015**
	g_S	3.62	2	9	0.070
	fv/fm	0.21	2	9	0.810
Water regime*time	Ψ_{Pd} pre-treatment	0.215	2	9	0.810
	Ψ_{Pd} post-treatment	9.99	6	14	**0.0002**
	Ψ_{Md}	3.94	6	14	**0.016**
	A_{area}	4.84	6	14	**0.007**
	g_S	2.48	6	14	0.075
	fv/fm	0.218	4	16	0.924

FIGURE LEGENDS

Figure 1. Diurnal variation in branchlet water potential (Ψ_L) measured at lower, mid, and upper crown positions of (A) an interior tree experiencing fog but no leaf wetting, (heights: 40 m = dotted line, 53.8 m = dashed line and 60.3 m = solid line), and (B) an exposed tree experiencing fog and leaf wetting at ~ 0700 h in the lower crown and ~0800 h in the mid and upper crown (heights: 32.9 m = dotted line, 55.5 m = dashed line and 67.5 m = solid line). Ψ_L was measured every 2 hours beginning at pre-dawn ~ 5:20 AM (Means ± 1 SD).

Figure 2. Branchlet water potential (Ψ_L) as a function of canopy height (m). Here we show a comparison between Ψ_L and canopy height for four time intervals during a single diurnal cycle: (A) 520 and 720 h, (B) 520 and 1120 h, (C) 520 and 1720 h. The natural range of Ψ_L, when soil water is the only readily available water source, is expected to occur below predawn water potential when Ψ_L is equal to $\Psi_{Soil} + \rho Gh$. Note that in Figure 3B, the lower crown foliage sat at or above $\Psi_{Soil} + \rho Gh$ for the entire day. At 11:20 AM foliage that was wetted by fog interception maintained water potentials that sat above the $\Psi_{Soil} + \rho Gh$ gradient (Means ± 1 SD). Trees: interior tree experiencing fog but no leaf wetting (open symbols), exposed tree experiencing fog and leaf wetting (closed symbols). Measurement times: 0520 h (circles); 0720 h (triangles) 1120 h (squares) 1720 h (diamonds).

Figure 3. Branchlet water potential measured at pre-dawn (Ψ_{Pd}; A) and midday (Ψ_{Md}; B), along with photosynthesis (A_{area}; C) and stomatal conductance (g_S; D) per unit leaf area, as a function of days since water withheld (Means ± 1 SD). The arrow denotes the point in time when water began to be withheld from the treatment plants.

Figure 4. Pre-dawn water potential (Ψ_{Pd}) as a function of volumetric soil water content for trees exposed to, and withheld from, overnight fog events (A; Means ± 1 SD). Figure 4B is a log-log plot using the absolute value of Ψ_{Pd}. The dotted lines represent the 95% confidence intervals. Note the distinct separation between the trees exposed to, and withheld from, overnight fog events.

Figure 5. Midday maximum photosynthesis (A_{area}) and stomatal conductance (g_s) as a function of pre-dawn (A and B) and modeled soil water potential (Ψ_{Soil}; C and D) for trees in the dry-down and fog treatments. Ψ_{Soil} was predicted using the following equation $y = -77.9x^{-1.8}$, see Figure 4.

Figure 6. Correlation between midday maximum photosynthesis (A_{area}) and stomatal conductance (g_s) for trees in the control, fog, and dry-down treatment groups.
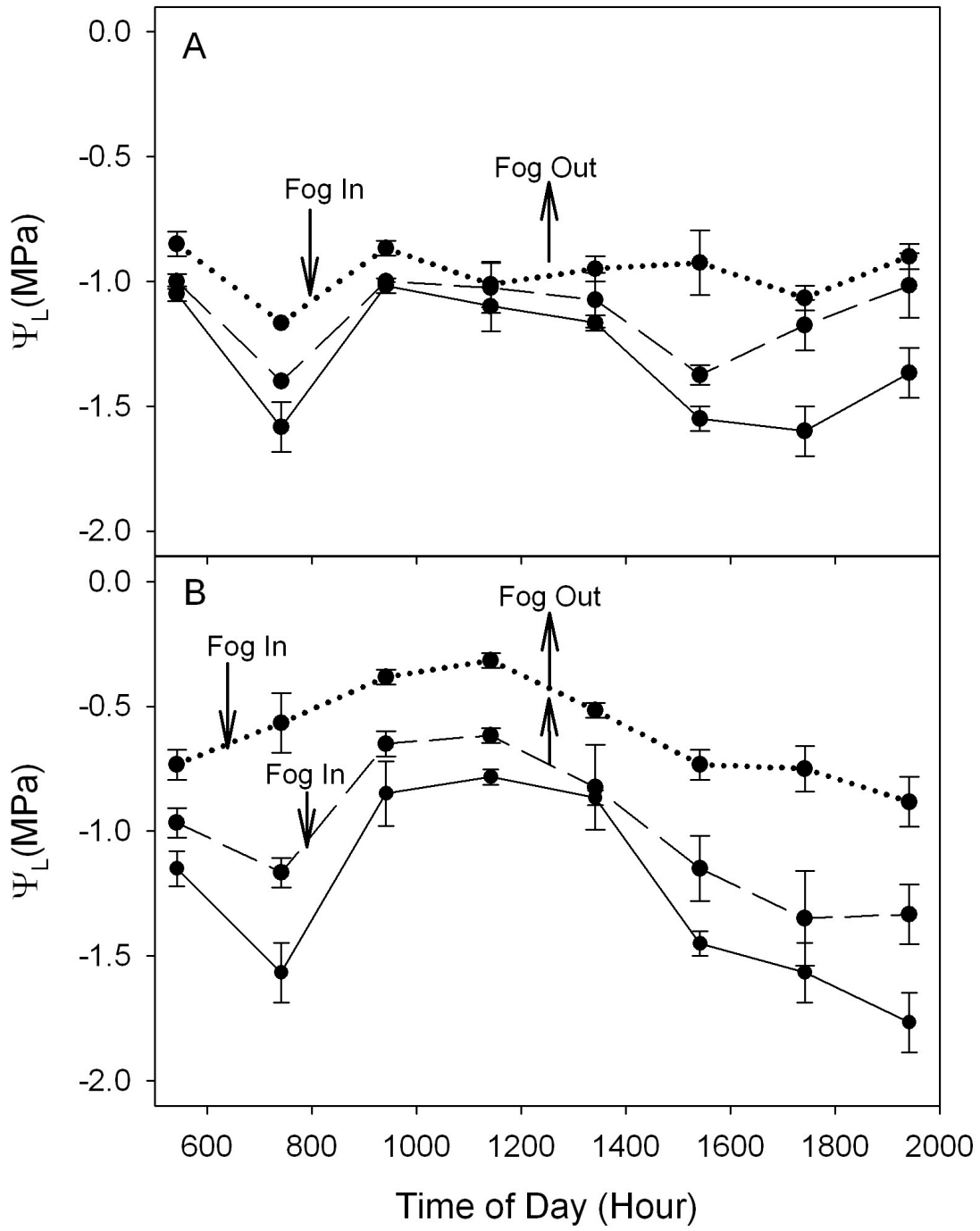
Figure 1.

Figure 2.

A

$y = -0.011x + -0.336$
$r^2 = 0.99$
$F = 821.11$
$p < 0.0001$

$y = -0.029x + 0.271$
$r^2 = 0.86$
$F = 24.27$
$p = 0.0079$

B

$y = -0.013x + 0.127$
$r^2 = 0.99$
$F = 440.83$
$p = 0.0096$

$y = -0.0046x + -0.779$
$r^2 = 0.53$
$F = 1.12$
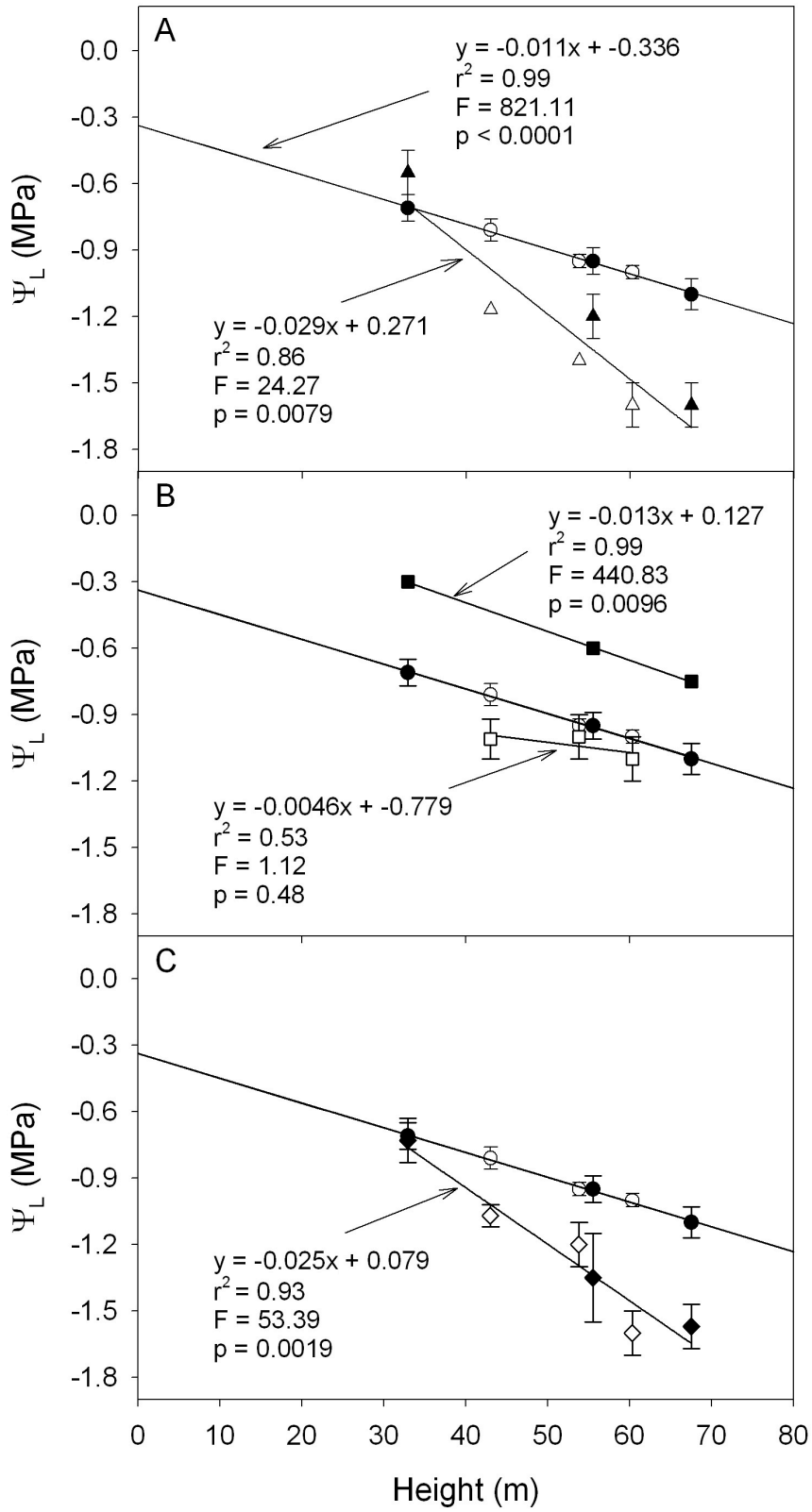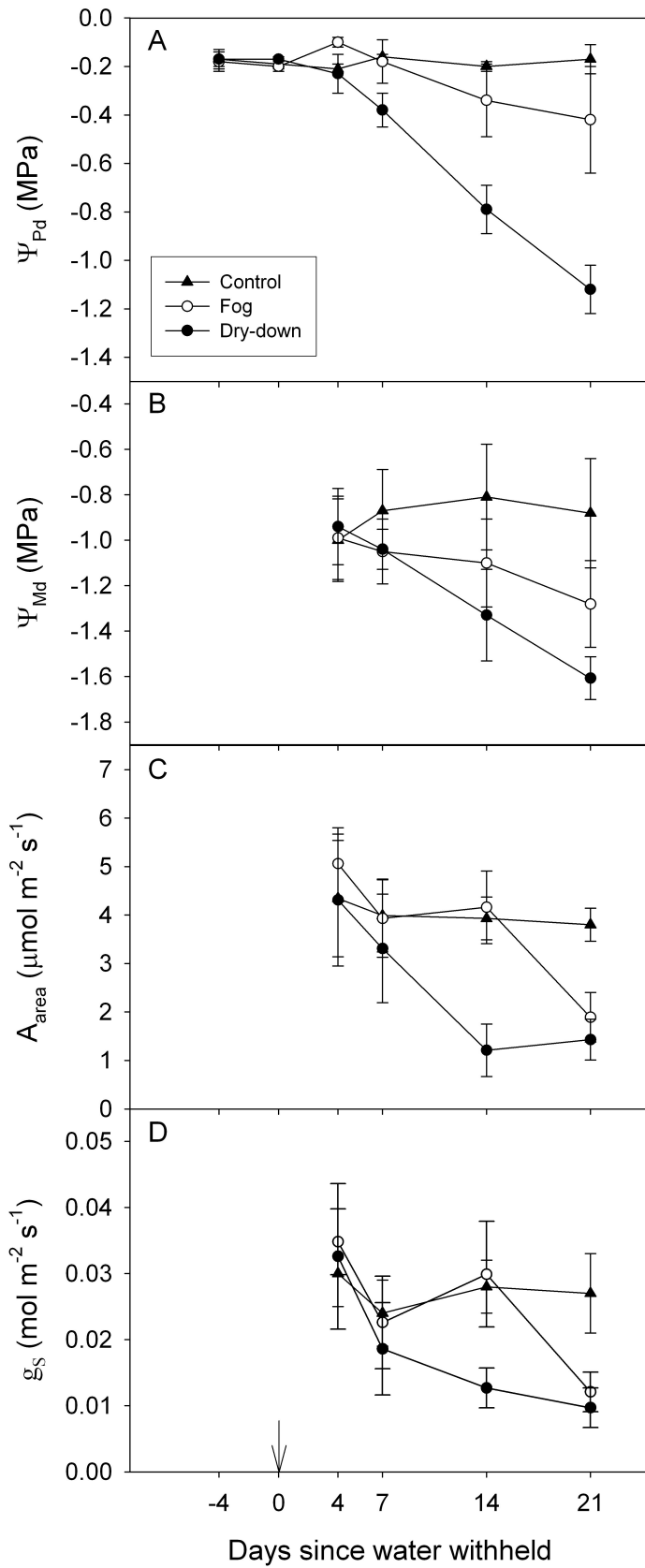$p = 0.48$

C

$y = -0.025x + 0.079$
$r^2 = 0.93$
$F = 53.39$
$p = 0.0019$

Ψ_L (MPa)

Height (m)

Figure 3.

Figure 4.

Figure 5.

Figure 6.

$r^2 = 0.86$

Y-axis: A_{area} (μmol m^{-2} s^{-1})

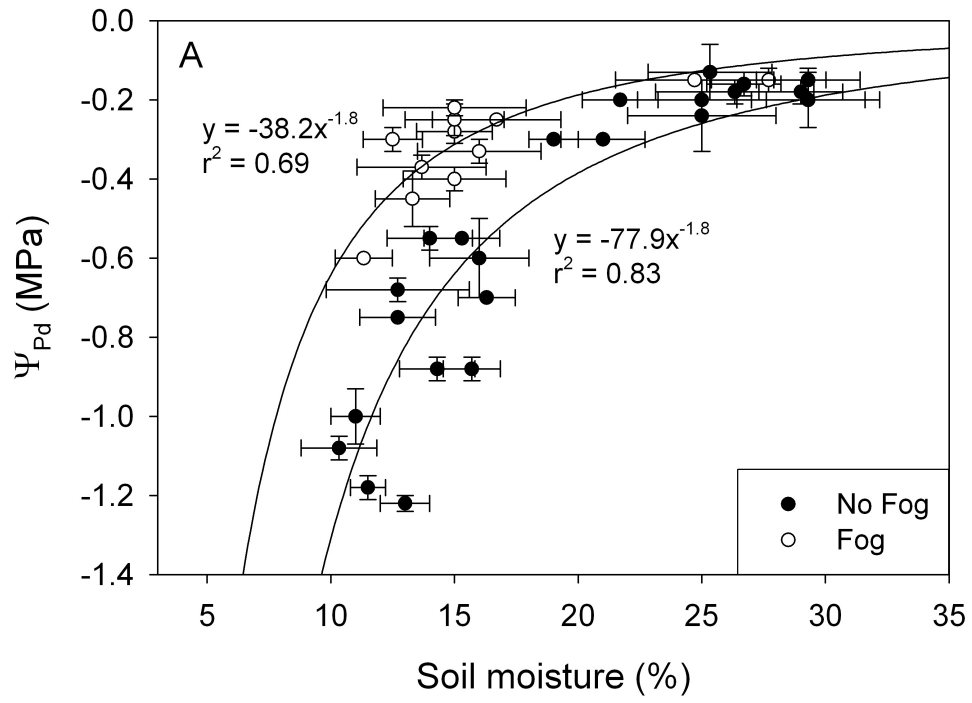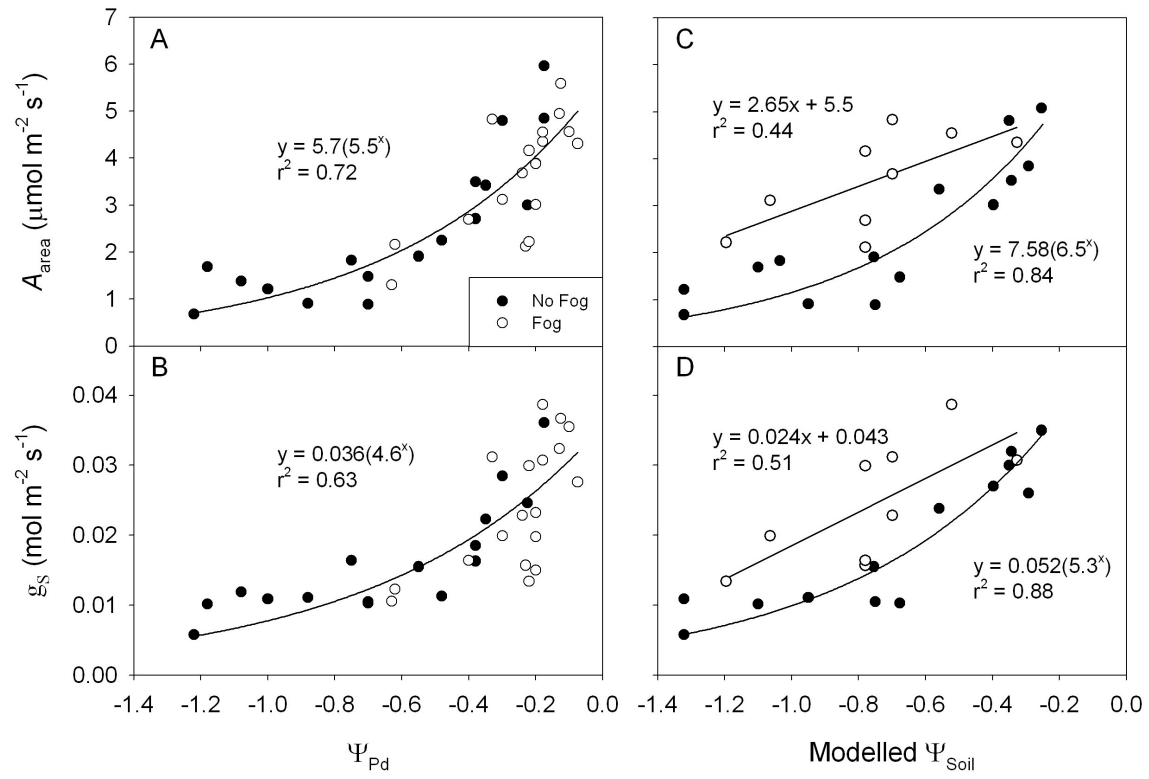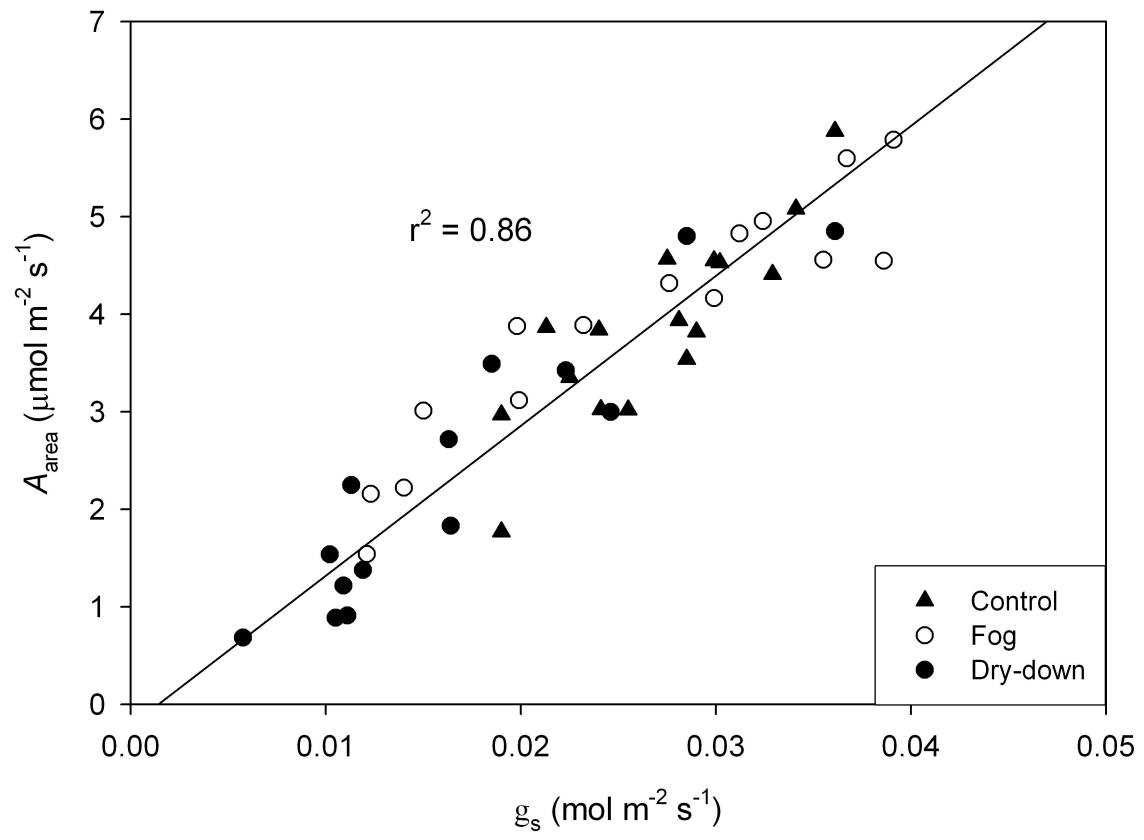X-axis: g_s (mol m^{-2} s^{-1})

Legend:
▲ Control
○ Fog
● Dry-down
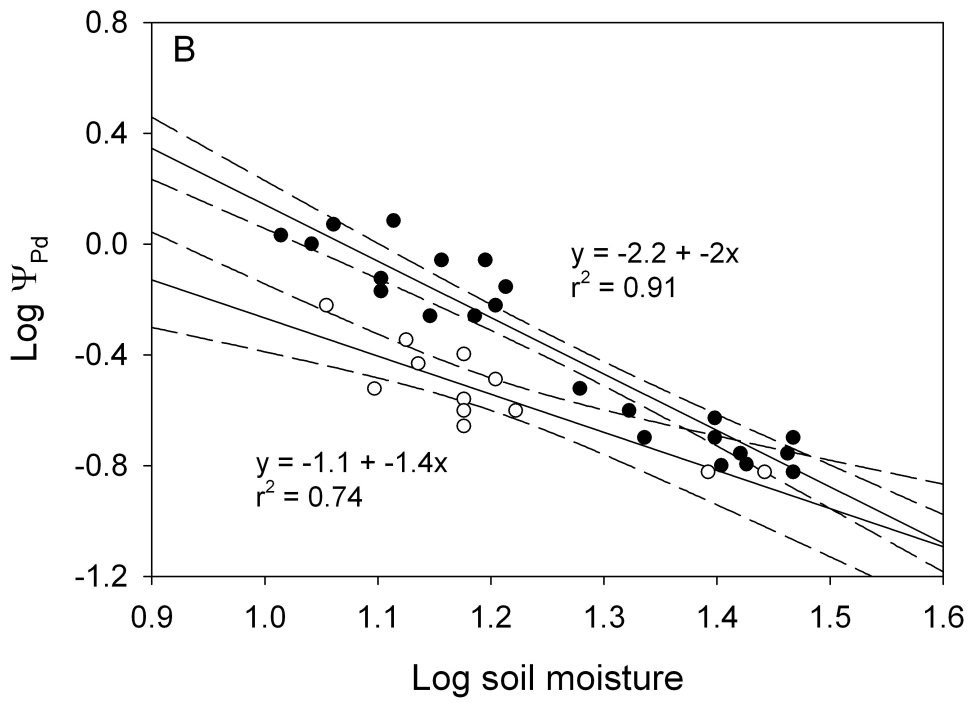
Chapter 2. When size matters: the influence of leaf size on plant function.

ABSTRACT

Most carbon, water, and energy exchange between plants and the atmosphere occurs at the leaf surface. Consequently, variation in leaf size has critical impacts on plant water use and primary productivity. Recent research describing relationships between the size, chemistry and function of leaves suggests increasing leaf surface area (LA) has negative consequences that result in a diminishing return on dry mass (M_D) investment towards light interception. However, these previous interpretations relied on indirect assessments of leaf gas exchange and photosynthetic metabolism. To re-explore these issues, we used a coupled photosynthesis-leaf water balance model and direct measurements of maximum leaf hydraulic conductance (K_{Leaf}) and leaf carbon isotope composition ($\delta^{13}C$) to test for the instantaneous rate of return on dry mass investment (i.e. carbon gain) in LA. Analysis of the leaves of C3 angiosperm species reveals that K_{Leaf} scales isometrically with LA but that there is no relationship between $\delta^{13}C$ and LA. The observed relationships between K_{Leaf} and LA suggests that, all else being equal (e.g. soil water and nutrient availability), the instantaneous return on resource investment in LA is size invariant.

INTRODUCTION

Plants vary greater than five orders of magnitude in leaf size, and this variation influences whole-plant biomechanics, light interception, and energy balance (Horn 1971; Niklas 1992; Wright et al. 2006). This is because plant traits that contribute to variation in crown architecture are directly coupled to changes in leaf surface area (*LA*). For example, large leaves often occur on long petioles and thick twigs with long internodes, whereas small leaves generally occur on relatively small petioles and thin twigs with short internodes (Corner 1949; Ackerly and Donoghue 1998; Poorter and Rozendaal 2008). These correlations between leaf size and crown architecture result in greater spacing between leaves, reduced within canopy shading and improved whole-plant light interception efficiency (Falster and Westoby 2003; Poorter and Renzendall 2008). Since plant primary productivity is dependent upon light interception by leaves, recent attention has been devoted to describing general scaling relationships between the size, chemistry, and physiology of leaves and the impact of these scaling relationships on dry mass investment in *LA* (Niklas *et al.* 2007; Milla and Reich 2007; Niinemets *et al.* 2007b).

Evaluations of size-dependent variation in organismal form and function are often described using allometric scaling relationships in the form of $Y = \beta X^{\alpha}$ (Thompson 1966; Givnish 1986; Kerkhoff and Enquist 2007). For plants, Y represents a particular function like photosynthesis, X is a metric of size such as *LA*, and β and α represent the elevation and slope of the log-transformed function vs. size regression. Using this framework the return on resource investment in *LA* would be constant and therefore independent of size if α = 1. If, however, α > 1, an increase in *LA* results in an increasing return on resource investment in size. Conversely, if α < 1, an increase in *LA* results in a diminishing return on resource investment in size.

Two basic biophysical constraints on the growth and maintenance of leaves are thought to have functionally negative consequences as *LA* increases resulting in a diminishing return on dry mass (M_D) investment in *LA* (Niklas et al 2007; Niinemets et al. 2007b; Milla and Reich 2007). As light interception increases, large leaves must rely on greater evaporative cooling in order to maintain leaf temperature within a viable range (Parkhurst and Loucks 1971; Givnish 1978; Osborne et al. 2004). This is a consequence of increasing boundary resistance to convective heat loss as *LA* increases (Gates 1980). Therefore, in warm environments, increasing *LA* is likely to impose a substantial cost to plants through greater M_D allocation to non-photosynthetic vascular tissue in order to maintain high rates of evaporative cooling (Givnish 1978). In addition to hydraulic considerations, increases in *LA* are linked to greater M_D allocation to biomechanical structure (Givnish 1978; Niklas 1999). For leaves, biomechanical structure is coupled to leaf hydraulic architecture and water use strategy. This is because leaf vascular architecture contributes directly to leaf rigidity via turgor maintenance (i.e. hydrostatic skeleton) as well as enhanced structural stiffness due to the higher lignin content of xylem cells (Givnish 1978; Niklas 1986, 1989). Recent research has shown that as *LA* increases a greater fraction of M_D is allocated to non-photosynthetic tissues that maintain both high rates of evaporative cooling and provide biomechanical support (Niinemets et al. 2006, 2007a). Thus, greater dry mass allocation to the non-metabolic vascular network may eventually limit the ability of leaves to most effectively use light energy to drive photosynthetic metabolism as *LA* increases.

Although recent research has shown strong support for a diminishing return on M_D, these interpretations relied on indirect assessments of gas exchange and carbon balance. Here we test

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for the return on M_D investment in *LA* using a coupled photosynthesis-leaf water balance model and 100 angiosperm species spanning three orders of magnitude in *LA*. The model framework used here is based on the hypothesis that natural selection acts to maximize the return on resource investment in surface area for energy exchange by maximizing the efficiency of resource transport to the sites of energy exchange. However, unlike previous models of vascular allometry, the analysis we present here makes no assumptions about the architectural design of the vascular transport network (e.g. Price et al 2007; West, Brown & Enquist 1999). Instead, we use plant-based parameters in a photosynthesis model that relies solely on first principles of mass conservation and fluid flow through porous media. If natural selection acts to maximize the return on resource investment in *LA* (i.e. photosynthesis) then vascular network efficiency should be size invariant (i.e. $\alpha = 1$) despite the potential for significant variation in vascular network architecture (Becker et al. 2003; Zwieniecki et al. 2004b). We therefore hypothesize that leaf hydraulic efficiency scales isometrically with *LA* in order to maximize photosynthetic carbon return and M_D investment in light interception regardless of variation in leaf vascular architecture.

Coupled photosynthesis-leaf water balance model

The transport of water vapor and CO_2 across leaf surfaces can be described from first principles using Fick's law of diffusion. The general formulation for the rate of water loss from leaf to atmosphere is:

$$E = g\left(e_i - e_a\right) \tag{1}$$

where E is the rate of leaf water loss, g is whole-leaf conductance to water vapor, e_i is the vapor pressure inside the leaf and e_a is the vapor pressure outside the leaf. The relationship between net photosynthesis (A_n) and g is described as:

$$A_n = g(c_a - c_i)/1.6 \tag{2}$$

where c_a is the molar concentration of CO_2 in the air immediately outside the leaf, c_i is the molar concentration of CO_2 inside the leaf and 1.6 is the diffusivity correction between water vapor and CO_2. Combining Eqn 1 and 2 we can begin to evaluate the relationship between A_n and E as (Katul et a. 2003):

$$A_n = \frac{E}{1.6\left(e_i - e_a\right)} \times \left(c_a - c_i\right) \tag{3}$$

Since C3 angiosperms generally work across a narrow range of c_i (Wong et al. 1979; Yoshie 1986) increased resource allocation to leaf tissues that maintain high rates of E are required to increase A_n, as *LA* increases (i.e. $E \propto A_n$).

Using a simple water balance model we can begin to evaluate biophysical constraints on E. For example, if water supply from the petiole (J) is unable to keep up with increases in E (i.e. $J < E$) then leaf water content (W) will decrease at the following rate $\Delta W = E - J$. As W decreases leaf water potential (Ψ_{Leaf}) decreases resulting in lower whole leaf conductance (g) in order to reduce E and maintain both W and Ψ_{Leaf} above the point of turgor loss (Brodribb and Holbrook 2003). Biophysical constraints to maintaining turgor and hydraulic mass balance between E and J, can be described using a whole-leaf water balance model based on Darcy's Law:

$$E = K_{Leaf}\left(\Delta\Psi_{St-Leaf}\right) + C_{Leaf}\left(\Delta\Psi_{Leaf}\right) \tag{4}$$

where $\Delta\Psi_{St-Leaf}$ is the driving force for water transport from stem to the evaporative sites within the leaf, K_{Leaf} is the rate of water transport per unit $\Delta\Psi_{St-Leaf}$ (i.e. J), and C_{Leaf} is leaf capacitance defined as the change in leaf water content (ΔW) per unit change in leaf water potential ($\Delta\Psi_{Leaf}$) prior to turgor loss. Leaves often work at a relatively narrow range of $\Delta\Psi_{Leaf}$, as K_{Leaf} is sensitive to large $\Delta\Psi_{Leaf}$ (Sack and Holbrook 2006). Therefore in order to maintain high rates of E as LA increases, with minimal metabolic cost, plants can either optimize K_{Leaf} or C_{Leaf}. Research on a broad range of distantly related angiosperm species with the C3 photosynthetic pathway has shown a strong coupling between E and K_{Leaf} per unit leaf surface area (Sack and Holbrook 200). This is because, unlike gymnosperms or angiosperm plants with Crassulacean acid metabolism (CAM), C_{Leaf} provides a relatively minor contribution to E prior to turgor loss (Brodribb et al. 2005). This allows Eqn 4 to be simplified to:

$$E \approx K_{Leaf}\left(\Delta\Psi_{St-Leaf}\right) \tag{5}$$

Since hydraulic mass balance is required over the life of a leaf we can combine Eqn 5 and Eqn 3 to test for the coordination between maximum K_{Leaf} and maximum net photosynthesis:

$$A_n = \frac{\left[K_{Leaf}\left(\Delta\Psi_{St-Leaf}\right)\right]}{1.6\left(e_i - e_a\right)} \times \left(c_a - c_i\right) \tag{6}$$

Thus, for C3 angiosperms, the maximum return on resource investment in LA is coupled to the size-dependent scaling of maximum K_{Leaf} since hydraulic mass balance between E and J is required to maintain cell turgor and a positive carbon balance (Franks 2006). Using the above framework we evaluated the scaling of maximum A_n and LA and discuss how the scaling of K_{Leaf}, LA and M_D constrains the coordination between leaf and stem traits and variation in whole-crown architecture.

MATERIALS and METHODS

We evaluated the size-dependent scaling of LA and K_{Leaf} for 60 Angiosperm species, including both woody and herbaceous taxa, using the general scaling relationship $Log_{10}(K_{Leaf}) = \beta + Log_{10}(LA)\alpha$. The 60 species used in this study vary in LA by three orders of magnitude and represent a combination of previously published data and recently gathered data (Table 1). Maximum K_{Leaf} was evaluated using the evaporative flux method, described in detail by Sack et al. (2002). Although we were unable to constrain previously published data to the evaporative flux method, research has shown comparable results between the evaporative flux method and other currently accepted methods for measuring leaf hydraulic conductance (Sack et al. 2002). If multiple methods were used in a single study, we included only data obtained using the evaporative flux method when available. Additionally, total leaf nitrogen, and time integrated intercellular CO_2 concentration (c_i) was measured for 40 species spanning three orders of magnitude in LA by analyzing the stable carbon isotope composition ($\delta^{13}C$) of bulk leaf material (Table 2). The 40 species used were grown in green houses and outdoors at the UC Berkeley Botanical Garden (Table 2). The ratio of ^{13}C to ^{12}C for bulk leaf material was calculated using delta notation (δ):

$$\delta = \left(R_{sample} / R_{standard} \right) \times 1000$$

(7)

Time integrated c_i was calculated as (Farquhar *et al.* 1982):

$$c_i = c_a \left(\delta_a - \delta_p - a \right) / (b - a)$$

(8)

where c_a was measured as 400 ppm, δ_a is the $\delta^{13}C$ of source CO_2 (-11‰ for the green house plants and -9‰ for plants grown outdoors), δ_p is the $\delta^{13}C$ of bulk leaf material, a is the fractionation due to diffusion in air (4.4‰), and b is the net fractionation due to carboxylation (RuBP carboxylase, 27‰). Calculation of slope and intercept for the scaling of K_{Leaf} vs LA was accomplished using SMATR version 2.0 (Falster et al. 2006; http://www.bio.mq.edu.au/ecology/SMATR/).

RESULTS

The maximum K_{Leaf} data obtained from the sixty C3 angiosperm species used in this study (see methods) supports isometric scaling between maximum K_{Leaf} and LA with $\alpha = 1$ (Figure 1A; $r^2 = 0.85$, $p < 0.0001$). This very strong relationship exists despite large variation in leaf shape. We found minor variation in time integrated c_i obtained from leaf carbon isotope analyses of forty species grown in a common garden (Table 2), and no relationship between time integrated c_i and LA (Figure 1B). The lack of a correlation between time integrated c_i and LA suggests small variations in c_i can occur for a given E and therefore g (Eqns 1-6), and this finding is consistent with previous research (Wong et al. 1979; Yoshie 1986). Further the observed variation in c_i was minor relative to the wide variation observed for K_{Leaf} (Figure 1).

DISCUSSION

We found maximum K_{Leaf} scales isometrically with LA (i.e. $K_{\text{Leaf}} \propto LA$, $\alpha = 1$) across woody and herbaceous C3 angiosperm species and no correlation between long-term integrated c_i and LA (Figure 1). Taken together these results suggests maximum A_n scales isometrically with LA i.e. $A_n \propto K_{\text{Leaf}} \propto LA$, $\alpha = 1$. Therefore, the maximum return on resource investment in LA (i.e. $A_n \propto M_D$) is dependent upon the scaling relationship between LA and M_D. For example, Niklas et al. (2007) observed isometric scaling between LA and M_D in tree species and near isometric scaling across forb and shrub species. Since $A_n \propto K_{\text{Leaf}} \propto LA$ ($\alpha = 1$) and $LA \propto M_D$ ($\alpha = 1$) the return on M_D investment in LA is independent of leaf size for these plant groups (i.e. $A_n \propto M_D$; $\alpha=1$). Further, since $A_n \propto K_{\text{Leaf}} \propto LA \propto M_D$ ($\alpha = 1$), Eqn 6 provides a leaf-level mechanism for why growth rate (G; dry matter plant^{-1} time^{-1} or ΣA_n) scales isometrically with total foliar biomass (ΣM_D) across different tree species (Niklas & Enquist 2001). In contrast, Niklas et al. (2007) found increasing foliar M_D results in progressively smaller increases in LA (i.e. $LA \propto M_D$; $\alpha < 1$) across fern, graminoid and vine species. Isometric scaling between K_{Leaf} and LA ($\alpha = 1$) and non-isometric scaling between LA and M_D ($\alpha < 1$) suggests a diminishing return on M_D investment in LA for these plant groups (Niklas et al. 2007).

If the instantaneous return on resource investment in LA is size invariant for many plant groups, then why does mean LA vary across broad climate gradients? The coupled photosynthesis-leaf water balance model described in Eqn 6, provides a framework for answering this question and evaluating the impact of environmental change on the scaling of A_N and K_{Leaf}. According to the model described in Eqn 6, under a constant c_a, the return on M_D investment in LA is coupled to $K_{\text{Leaf}}/(e_i - e_a)$, and other factors that drive variation in c_i independent of leaf size (e.g. soil nitrogen and water availability). Although max $A_N \propto K_{\text{Leaf}}/(e_i - e_a)$ and $K_{\text{Leaf}} \propto LA$, $\alpha = 1$, the scaling of e_i and LA is strongly size dependent such that all else being equal the slope of e_i *vs.* net radiation is greater for large *vs.* small leaves (Gates 1980; Jones 1992). Therefore, as LA increases we would predict an increasingly negative effect of radiation on maximum A_n via decreasing $K_{\text{Leaf}}/(e_i - e_a)$. For example, LA generally decreases within and between angiosperm trees as radiation interception increases (Zweniecki et al. 2004a). Thus, $K_{\text{Leaf}} \propto LA$, $\alpha = 1$, and the size-dependent slope of e_i *vs.* net radiation provides a mechanistic explanation for within and between species variation in LA, in response to gradients of radiation and/or e_a. These findings also highlight the importance of landscape structure for generating gradients in radiation and e_a and thus increasing diversity in LA at local and regional scales. For example lower radiation and/or increased e_a that occurs in the riparian zones of valley bottoms should support larger leaves then ridge tops with high radiation and/or lower e_a.

Additionally, previous research has shown a strong positive correlation between forest leaf area index (ΣLA per unit ground area; LAI), site water balance and primary productivity (Grier and Running 1977; Nemani and Running 1989; Eagleson 2002). Since forest water balance and ($e_i - e_a$) are coupled via leaf energy balance and transpiration (Grier and Running 1977; Eagleson 2002), isometric scaling between K_{Leaf}, LA, M_D and tree growth rate, suggests that forest LAI is optimized in response to among site variation in radiation, e_a and soil water availability. In turn, this establishes testable predictions for how maximum forest LAI should vary with latitudinal gradients in radiation, e_a and soil water balance. For example Eqn 6 and the

foliar allometries described above would predict forest *LAI* to be greater in low latitudes with high annual radiation and e_a compared to high latitudes with low annual radiation and low e_a (Dai 2006; Garrigues et al. 2008). Furthermore the lack of correlation between *LA* and c_i suggests other factors such as nutrient availability secondarily modifies the impact of (e_i - e_a) on the scaling of A_n, K_{Leaf} and *LA,* independent of leaf size. As such variation in soil nutrient availability is expected to secondarily modify the impact of soil water balance, radiation, and e_a on maximum *LA, LAI* and primary productivity (Fonseca et al. 2000, McDonald et al. 2003; Meier and Leuschner 2008).

Describing the environmental constraints on leaf metabolism as *LA* increases also provides information on the selective pressures influencing plant crown development and the covariation between stem and leaf traits. For example, across tree species $A_n \propto K_{Leaf} \propto LA \propto M_D$, $\alpha = 1$, and thus for an entire twig $\Sigma A_n \propto \Sigma LA \propto \Sigma M_D$, $\alpha = 1$. Since the spacing of leaves is positively correlated with leaf size (Corner 1949; Horn 1971; Poorter and Rozendaal 2008) and $\Sigma A_n \propto \Sigma LA \propto \Sigma M_D$, $\alpha = 1$, the increased carbon allocation to stem length, as *LA* increases, should come at the cost of lower wood density. Previous research has shown a strong negative correlation between leaf size and wood density (Pickup et al. 2005; Wright et al 2006). Thus, the design space for potential leaf-twig trait combinations is strongly constrained by stem and leaf hydraulics and therefore biomechanics (Olson et al. 2009). Further, the mass conservation framework used here suggests that variation in the photosynthetic metabolic pathway is coupled to variation in plant vascular architecture. This provides an explanation for why plant species which utilize the Crassulacean acid metabolism pathway rely on C_{Leaf} to maintain E and thus A_n before turgor loss whereas angiosperms with the C3 photosynthetic metabolism rely mostly on K_{Leaf} (Eqn 4; Franks 2006; Brodribb et al. 2007).

Using first principles of mass conservation and fluid flow through porous media we have shown strong support for isometric scaling between vascular efficiency and leaf size that optimizes the instantaneous return on foliar M_D. Further, the foliar allometries described here provide a mechanistic framework for interpreting previously observed co-variation between *LA, LAI* and climate. Additionally, isometric scaling between K_{Leaf}, *LA* and M_D in tree species provides insight into the covariation between leaf and stem traits.

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TABLES

Table 1. Mean leaf surface area (*LA*), leaf hydraulic conductance (K_{Leaf}) for species used in the analysis. Methods for measuring species mean K_{Leaf} are as follows: evaporative flux method (EFM), High-pressure method (HPM) and Vacuum pump method (VPM).

Species	*LA* (cm^2)	K_L (mmol s^{-1} MPa^{-1})	Method	Reference
Acer macrophyllum	306.0	0.200	EFM	This study
Acer rubrum	57.0	0.040	EFM	Sack et al. (2002)
Acer sachrum	57.0	0.027	EFM	Sack et al. (2002)
Aesculus hippocastanum	434.40	0.175	HPM	Nardini et al. (2005)
Alberta magna	39.10	0.053	EFM	Scoffoni et al. (2008)
Aleurites moluccana	277.7	0.413	VPM	LoGullo et al. (2005)
Alliara petiolata	217.5	0.191	HPM	Nardini et al. (2005)
Annona glabra	38.0	0.086	HPM	Sack and Frole (2006)
Anthurinum sp.	900.0	1.13	EFM	This study
Arbutus menziesii	52.4 ± 8.4	0.026 ± 0.0004	EFM	This study
Betula papurifera	33.0	0.048	EFM	Sack et al. (2002)
Callophylum longifolium	50.0	0.038	HPM	Sack and Frole (2006)
Calycanthus floridus	32.0	0.043	HPM	Nardini and Salleo (2000)
Castanea sativa	64.0	0.030	HPM	Nardini and Salleo (2000)
Cercis siliquastrum	93.4	0.056	HPM	Nardini et al. (2005)
Cercis siliquastrum	55.1	0.038	HPM	Nardini and Salleo (2000)
Cordia allidora	62.0	0.061	HPM	Sack and Frole (2006)
Corylus avellana	53.9	0.041	HPM	Nardini and Salleo (2000)
Corylus avellana	111.0	0.060	HPM	Nardini et al. (2005)
Dendropanax arboreus	28.0	0.021	HPM	Sack and Frole (2006)

Eucalyptus erythrocorys	25.5	0.017	EFM	Scoffoni et al. (2008)
Gunnera sp.	1000	0.865	EFM	This study
Hedera canariensis	40.0	0.026	EFM	Scoffoni et al. (2008)
Hedera helix	60.0	0.036	EFM	Sack et al. (2002)
Heteromeles arbutifolia	10.8	0.017	EFM	Scoffoni et al. (2008)
Helianthus annus	188.8	0.362	HPM	Nardini et al. (2005)
Helianthus annus	165.8 ± 14.13	0.178 ± 0.038	EFM	This study
Helianthus annus	261.0 ± 10.7	0.336 ± 0.043	EFM	This study
Hymensporum flavum	21.7	0.024	EFM	Scoffoni et al. (2008)
Juglans regia	174.0	0.150	HPM	Nardini et al. (2005)
Juglans regia	63.3	0.032	HPM	Nardini and Salleo (2000)
Laurus nobilis	11.8	0.003	HPM	Nardini and Salleo (2000)
Magnolia soulangeana	55.9	0.044	HPM	Nardini and Salleo (2000)
Malnus domestica	15.6	0.026	HPM	Nardini and Salleo (2000)
Miconia argenteum	224.0	0.350	HPM	Sack and Frole (2006)
Myrtus communis	3.40	0.003	HPM	Nardini et al. (2005)
Paulownia tomentosa	231.6	0.122	HPM	Nardini et al. (2005)
Persia americana	108.1	0.051	VPM	LoGullo et al. (2005)
Phaseolus vulgaris	35.9 ± 2.1	0.056 ± 0.015	EFM	This study
Platanus orientalis	285.40	0.181	VPM	LoGullo et al. (2005)
Posoqueria latifolia	94.0	0.041	HPM	Sack and Frole (2006)
Populus spp.	112.5 ± 17.3	0.102 ± 0.02	EFM	This study
Populus spp.	39.9 ± 1.5	0.041 ± 0.008	EFM	This study
Protium tenufolium	66.0	0.038	HPM	Sack and Frole (2006)

Prunus laurocerasus	78.9	0.055	HPM	Nardini and Salleo (2000)
Prunus laurocerasus	152.0	0.456	HPM	Nardini et al. (2005)
Quercus kellogii	52.4 ± 10.4	0.064 ± 0.017	EFM	This study
Quercus lobata	24.8 ± 9.3	0.015 ± 0.004	EFM	This study
Quercus rubra	81.0	0.169	EFM	Sack et al. (2002)
Quercus rubra	78.5	0.041	VPM	Lo Gullo et al. (2005)
Raphiolepsis indica	28.8	0.044	EFM	Scaffoni et al (2008)
Ricinis communis	262.1 ± 24.8	0.270 ± 0.058	EFM	This study
Ricinis communis	658.5 ± 59.7	0.511 ± 0.08	EFM	This study
Runnunculus spp.	419.41	0.293	EFM	This study
Sambucus nigra	236.6	0.268	HPM	Nardini et al. (2005)
Senecio thomasii	157.96 ± 10.1	0.088 ± 0.022	EFM	This study
Smilax aspera	118.0	0.078	HPM	Nardini et al. (2005)
Swartzia simplex	30.0	0.006	HPM	Sack and Frole (2006)
Terminalia amazonia	20.0	0.031	HPM	Sack and Frole (2006)
Viburnum tinus	14.9	0.010	HPM	Nardini and Salleo (2000)
Vitis labrusca	190.0	0.177	EFM	Sack et al. (2002)
Vitis vinifera	296.7	0.196	HPM	Nardini et al. (2005)

Table 2. Mean leaf surface area (*LA)* and the integrated internal CO_2 concentration (c_i) for species used in the analysis.

Species	*LA* (cm^2)	$\delta^{13}C$	c_i
Acer macrophyllum	33.10	-27.62	252
Anthurinum sp.	1230.0	-29.69	288
Aralia spinosa	33.10	-27.82	255
Arctostaphylos refugioensis	7.14	-29.14	278
Ceanothus coeruleus	13.94	-28.0	258
Cedrela salvadorensis	6.15	-27.23	245
Cercis canadensis	62.76	-25.49	214
Chiranthodendron pentadactylon	205.80	-27.24	245
Citharexylum hidalgense	10.50	-25.19	209
Cornus floccosa	14.57	-25.71	218
Cornus spp.	31.24	-28.58	269
Helianthus annus	174.16	-29.97	254
Liquidambar styracifluas	73.53	-29.51	285
Magnolia cambellii	249.06	-25.51	214
Magnolia dealbata	800.0	-27.81	255
Magnolia delavayi	545.0	-29.92	292
Phaseolus vulgaris	74.86	-30.46	266
Philadelphus lewisii	14.11	-30.12	292
Populus fremontii	105.06	-28.93	275

Quercus agrifolia	13.43	-27.28	245
Quercus berberdifolia	2.22	-26.60	234
Quercus castanea	38.32	-27.56	251
Quercus gravesii	24.61	-27.24	245
Quercus grisea	15.39	-26.20	227
Quercus kellogii	52.9	-27.41	248
Quercus lobata	20.21	-27.56	251
Quercus muehlenbergii	46.34	-28.29	263
Quercus pacifica	3.15	-29.69	288
Quercus palmeri	12.92	-27.13	243
Quercus risophylla	64.57	-27.50	250
Quercus saltillensis	3.01	-27.09	242
Rhododendron arboreum	22.30	-28.00	258
Rhododendron protistum	298.27	-27.96	258
Salix chilensis	484	-27.49	249
Salvia mexicana	24.34	-28.10	260
Senecio aeschenborianus	57.53	-25.51	214
Senecia thomasii	179.95	-30.74	271
Simmondsia chinensis	6.76	-30.05	295
Sorbus aria	124.52	-26.65	234
Urera caracasana	188.52	-26.64	234

FIGURE LEGENDS

Figure 1. Maximum leaf hydraulic conductance (K_{Leaf}) versus leaf surface area for sixty C3 angiosperm species (A) and time-integrated intercellular CO_2 concentration (c_i) versus leaf surface area (*LA*) for forty C3 angiosperm species grown in a common garden (B).

FIGURES

Figure 1.

The scatter plot (A) shows Log K_L versus Log LA with the regression equation:

Log K_L = 1.08*LogLA - 3.23
r^2 = 0.86
p = 0.0001
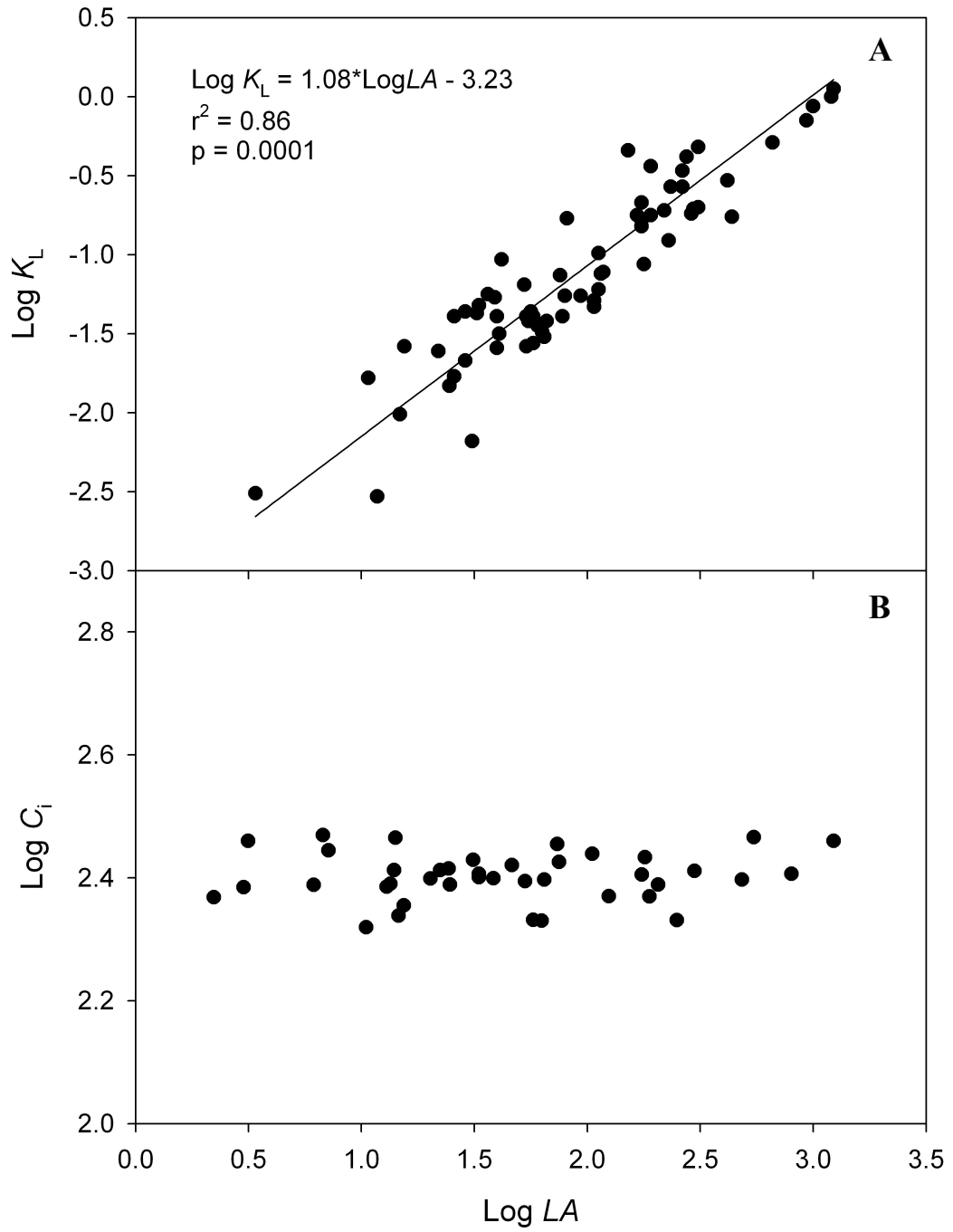
Chapter 3. Are leaf carbon balance and water use independent of leaf longevity?

ABSTRACT

Previous research suggests the maximum lifetime carbon gain of a leaf is constrained by a biophysical trade-off between metabolism and structural rigidity as seen by the strong negative relationship between maximum photosynthetic capacity and leaf lifespan. However, the biophysical processes that contribute to this trade-off are not well defined. Because plant persistence is dependent upon the process of carbon fixation which in turn is limited by the ability of a plant to obtain and transport resources to the sites of photosynthetic metabolism, plants are ultimately flux-limited biological systems. If the aforementioned is true then natural selection should favor the functional coordination between photosynthetic metabolism and vascular transport efficiency. Here we present the results of an investigation that explored the nature of covariation between leaf lifespan, vascular network efficiency, and photosynthetic capacity using a coupled photosynthesis-leaf water balance model. We make no assumptions of vascular network design and instead test the emergent property, leaf hydraulic conductance, and its variation across leaves of varying longevity. We do so by relying on first principles of energy and mass conservation by way of Fick's law for the diffusion of gases across a porous surface, and Darcy's law for fluid flow through a porous media. We observed negative scaling between leaf hydraulic conductance per unit leaf mass and leaf lifespan. Further, the negative scaling slope describing the covariation between leaf hydraulic conductance per unit mass and leaf lifespan is not significantly different from one providing strong support for a constant net carbon gain per leaf despite significant variation in both leaf lifespan and environment. In turn, variation in gross primary productivity is a function of the number of leaves a plant maintains over a given unit of time.

INTRODUCTION

A large body of research has demonstrated a strong negative relationship between maximum photosynthetic capacity and leaf lifespan (Larcher 1975; Chabot and Hicks 1982; Reich et al. 1997; Wright et al. 2004). This relationship is generally considered the outcome of a trade-off between resource investment in long-lived photosynthetic tissues (i.e. leaves) and resource investment in rapid leaf turnover and increased growth rates (Reich et al. 1991, 1997; Westoby et al. 2000). The argument for this trade-off is as follows: high photosynthetic capacity requires significant nitrogen investment in photosynthetic machinery at the expense of nitrogen investment in defense or the ability to persist. Resource allocation to photosynthetic metabolism as opposed to defense usually results in highly desirable leaves for both herbivores and pathogens and therefore shorter leaf lifespan (Chabot and Hicks 1982; Coley 1983; Reich 2001). In contrast, longer-lived leaves require greater carbon allocation to rigid cell structures as a defense against biotic and abiotic stresses that would otherwise damage a leaf (Coley 1983, 1988). The fact that there are no leaves with a long leaf lifespan that also possess high photosynthetic rates suggests a fundamental trade-off exists between cells specialized for photosynthetic metabolism and those specialized for structural rigidity (Reich 2001). Further, a plant species possessing short lived leaves with low photosynthetic capacity would suffer from a low lifetime productivity, which in turn places it at greater risk of running at a carbon deficit, i.e. these plants might never make up the carbon cost of leaf construction and maintenance (Kikuzawa 1991). Therefore, the observed correlation between photosynthesis and lifespan suggests that the maximum lifetime carbon gain of a leaf is limited by a biophysical trade-off between persistence (i.e. cellular rigidity) and metabolism, while the minimum lifetime carbon gain is limited by the need to maintain a positive carbon balance (Figure 1A). Although a strong theoretical framework exists to explain a trade-off between photosynthetic metabolism and leaf longevity, the biophysical processes that contribute to this trade-off are not well defined and rarely quantified (Shipley et al. 2006; Mediavilla et al. 2008).

We know from first principles of energy and mass conservation that photosynthetic metabolism (e.g. C_3, C_4 and CAM photosynthesis) is ultimately limited by light energy and the transport of CO_2 and water to sites of photosynthetic metabolism inside the leaf (Wong et al. 1979; Farquhar et al. 1980; Schulze 1991; Farquhar et al. 2001; Brodribb, Feild and Jordan 2007). Put another way, the conversion of atmospheric CO_2 to simple sugars inside the leaf cannot exceed the rate of both CO_2 and water vapor exchange between the leaf and atmosphere. This is because for C3 plants it is physically impossible to transport CO_2 from the atmosphere to the sites of photosynthetic metabolism inside the leaf without, at the same time, loosing at least 1.6 times as much water to the surrounding atmosphere (depending upon the ambient humidity; see Gates 1980; Monteith 1995; Oren et al. 1999). Since plant persistence is limited by maintaining a favorable leaf carbon balance and therefore by leaf gas exchange (i.e. CO_2 and water vapor exchange between leaf and atmosphere), leaf traits that influence both photosynthetic metabolism and leaf water use are considered to be under strong selection (Cowan 1977; Givnish 1979; Mäkelä et al. 1996). Thus, the evolutionary pressure to increase photosynthetic metabolism is contingent upon similar increases in leaf hydraulic conductance or a decrease in the resistance to water transport between leaf and atmosphere (Beerling et al. 2001;

Osborne et al. 2004; Brodribb et al. 2005; Brodribb et al. 2007). This suggests that not only is there a tradeoff between leaf persistence and photosynthetic capacity but there must also be a tradeoff between leaf persistence and leaf hydraulic conductance since photosynthesis and water loss are physically coupled. If the aforementioned is true then a biophysical trade-off exists between resource allocation to live cells built to maximize both metabolism and resource transport (i.e. CO_2 and water) and those built for maximal structural rigidity and persistence.

Models describing functional coordination, or trade-offs, between traits often take the form of $Log(Y) = \beta + \alpha\ Log(X)$; where Y and X represent two continuous traits, such as leaf hydraulic efficiency and leaf surface area, β is a constant, and α describes the slope of the log-transformed relationship between, in this case, leaf hydraulic efficiency and leaf surface area. Using this framework, the coordination between changes in leaf hydraulic conductance and leaf surface area would be constant if $\alpha = -1$ or +1. If α is < or > $|1|$, then a change in leaf hydraulic conductance is coupled to a disproportionate change in leaf surface area. For example, if water transport is maximized in relation to surfaces for gas exchange (i.e. CO_2 and water vapor) between atmosphere and leaf, then leaf hydraulic conductance is expected to scale positively and isometrically ($\alpha = 1$) with leaf surface area, as previously shown by Simonin and Dawson (*Chapter 2*). Similarly, if leaf carbon balance is maximized for a given dry-mass invested in surfaces for gas exchange (i.e. whole-leaf mass), then maximum photosynthetic capacity and leaf hydraulic efficiency per unit leaf dry mass should scale isometrically with leaf lifespan ($\alpha = -1$). This in turn would suggest that lifetime leaf carbon gain is limited by a biophysical trade-off between resource allocation to leaf cells and tissues specialized for efficient water transport and photosynthetic metabolism and those specialized for structural rigidity and longevity (Figure 1B).

Here we extend the aforementioned arguments by asking the question, are previously observed negative correlations between leaf lifespan and photosynthesis the result of a fundamental trade-off between leaf hydraulic efficiency and structural rigidity as measured by the covariation between leaf hydraulic conductance and leaf lifespan? The plants used in this study were grown in a common garden to investigate the coordination between leaf hydraulic conductance and lifespan in the absence of climate variation. Our data were then combined with previously published values of leaf lifespan and leaf hydraulic conductance for species inhabiting a broad range of vegetation types and climate to explore the more general relationships between these traits. We hypothesized that if the return on dry mass investment in surfaces for gas exchange is maximized for a given environment, then leaf hydraulic conductance per unit leaf dry mass should scale isometrically and negatively with leaf lifespan resulting in a constant lifetime net carbon gain per leaf (Figure 1B).

METHODS
Common Garden
Study species and experimental design

We sampled four deciduous and four evergreen temperate forest tree species spanning a wide range of leaf lifespan for the common garden experiment: five angiosperms, *Acer macrophylum* (Aceraceae), *Arbutus menziesii* (Ericaceae), *Populus fremontii* (Salicaceae), *Quercus kelloggii* (Fagaceae), and *Umbellularia californica* (Lauraceae), and three

gymnosperms, *Metasequoia glyptosrtoboides* (Cupressaceae), *Pinus ponderosa* (Pinaceae) and *Sequoia sempervirens* (Cupressaceae). Four to five saplings of each species were grown from seed in 20L pots and transferred to a single site (common garden), in full sun, on a ridge top at the University of California Botanical Garden (32°52´N 122°14´W, ~256 m elevation) between 1-7 of March 2007. Individual saplings from each species were randomized spatially throughout the common garden. Saplings ranged from ~1.5 to 2.5 m in height. Plants were watered every other day using drip irrigation. Data were collected when leaves were fully expanded, ~ 1 month after the start of leaf emergence, which occurred in late May / early June of 2008 and 2009 for *A. macrophylum*, *M. glyptostroboides*, *P. fremontii*, *Q. kelloggii* and mid to late July for *A. menziesii*, *P. ponderosa*, *U. californica*, *S. sempervirens*. Six temperature and relative humidity sensors were placed ~ 2m off the ground at mid-crown height near the potted plants with mean values recorded every 30 minutes (EL-USB-2, Lascar Electronics INC; Oakton 35710, Oakton Instruments). Additionally, air temperature and relative humidity was measured with a Li-1600 steady-state porometer (Licor Inc., Lincoln NE, USA) in close proximity to the leaves we measured for gas exchange and water potential. Photosynthetically active radiation intercepted by the adaxial surface of the leaf was measured with a quantum sensor (Model Li-190SB, Licor Inc., Lincoln NE, USA).

Leaf life span (*LL*)

　　　Leaf lifespan (LL) was calculated by monitoring the time between first bud break and leaf senescence on marked branches over a two year period (2007-2008). Estimates of LL for species with LL > 2 years (i.e. *P. ponderosa*, *S. sempervirens* and *U. californica*) were based on the number of successive cohorts maintained on a branch during the time of study. Our estimate of LL for *P. ponderosa*, *A. menziesii*, *P. fremontii*, and *S. sempervirens* fall in the range of previous evaluations (Ackerly 2004; Reich et al. 1998; Espinosa-Garcia & Langenheim 1990).

Leaf hydraulic conductance (K_{Leaf})

　　　Diurnal variation in leaf hydraulic conductance (K_{Leaf}; mmol s^{-1} m^{-2} MPa^{-1}) for sun exposed leaves was measured *in situ* on four to five individuals of each species as:

$$K_{Leaf} = E/(\Psi_{St} - \Psi_{L})$$

(Eqn. 1)

where E is the evaporative flux, Ψ_{St} is stem xylem water potential and Ψ_L is leaf water potential. This *in situ* technique requires sampling two adjacent leaves, one leaf is used to measure Ψ_{St} while the adjacent leaf is sampled for E and Ψ_L. Leaves used as an assay for Ψ_{St} were covered in plastic film and aluminum foil the evening prior to the measurement period to allow for equilibration between stem xylem water potential and the covered leaf water potential. Evaporative flux (E) was measured with a Li-1600 porometer (as above). Due to the open canopy structure of the saplings, the wide spacing between trees, and the ridge top exposure of the common garden, we assumed leaf boundary layer conductances were high. Furthermore, during each measurement of E, leaf orientation, ambient humidity and radiation interception was conserved. Therefore we assumed that E measured by the Li-1600 was similar to the actual E

immediately before the measurement. Covered leaves were sampled as an assay for Ψ_{St} at the start of the E measurement. Immediately following determination of E, the uncovered leaf was excised, wrapped in plastic and placed in a Scholander-type pressure chamber for determination of Ψ_L (SAPS II, Soil Moisture Equipment Corp., Santa Barbara CA, USA). Balancing pressure was recorded when xylem exudates reached the cut stem surface as verified by a dissecting scope at 25X magnification. We measured E, Ψ_{St} and Ψ_L every ~2.5 hours over the course of a 14-18 hour period, beginning at pre-dawn (0400-0500 h).

Literature survey

Database compilation

A literature survey was conducted to find studies where leaf lifespan, leaf hydraulic conductance, and leaf mass per unit area (LMA) were measured simultaneously. If leaf hydraulic conductance was measured and expressed per unit leaf area (i.e. $K_{Leaf, area}$) then leaf hydraulic conductance per unit leaf mass was calculated from $K_{Leaf, area}$ and LMA. Leaf trait data were collected from data tables, when available, and from figures if data were not presented in tabulated form (Graph Click version 3.0, Arizona Software). Studies that only reported K_{Leaf} and LMA were used if LL data for individuals grown in a similar climate were available from the GLOPNET database (Wright et al. 2004). Only one study reported all three leaf traits (Brodribb et al. 2005). The data set used to test for the covariation between K_{Leaf} and LMA spanned a wide geographic range and consisted of 50 angiosperm species and 8 gymnosperm species. The total number of species available for the K_{Leaf} vs. LL comparison was 24. Leaf lifespan data from the GLOPNET database were used for 13 of the 24 K_{Leaf} - LL pairs.

Statistical Analysis

Calculation of slope and intercept for the linear models describing the covariation between leaf traits was accomplished using SMATR version 2.0 (Falster et al. 2006; http://www.bio.mq.edu.au/ecology/SMATR/).

RESULTS and DISCUSSION

Similar to previous research (Tyree et al. 1999; Salleo & Nardini 2000; Sack et al. 2003), variation in LL and LMA were not significantly correlated with variation in $K_{Leaf, area}$ for the eight species grown in the common garden (Figure 2A and Figure 2B). When the common garden species were combined with previously published data, a significant, albeit weak, correlation was observed between LL and $K_{Leaf, area}$ (Figure 2B). In contrast to the area-based measures, a strong negative correlation was observed between both LMA and $K_{Leaf, mass}$, and between LL and $K_{Leaf, mass}$ for the temperate forest species (Figure 2C and Figure 2D) and also for the combined data set (Figure 4C and Figure 4D).

The lack of a consistent correlation between LL, LMA and $K_{Leaf, area}$, has led previous investigators to conclude that K_{Leaf}, a pivotal plant water relations trait, is orthogonal (i.e. statistically independent) to traits influencing leaf carbon economy (Sack et al. 2003; Sack & Frole 2006). However, expressing leaf trait information on an area basis obscures potential variation in the carbon cost of light interception, gas exchange and water transport that are in fact

present if one expresses them on a mass basis. For example, *A. menziesii, P. ponderosa,* and *U. californica* which all possess dense, long-lived leaves, showed higher maximum $K_{Leaf, area}$ and $g_{s,area}$ then *M. glyptostroboides* a species with short-lived leaves (Figure 2A; Witkowski & Lamont 1991; Hickman 1993; Williams 2005). Yet, when K_{Leaf} was normalized by leaf dry mass, *M. glyptostroboides* had greater $K_{Leaf, mass}$ than both *P. ponderosa* and *U. californica,* and was equivalent to *A. menziesii* due to a lower LMA. Similarly, Reich et al. (1997) and subsequent analysis by Wright et al. (2004) found that variation in LL and LMA were not strongly correlated with variation in photosynthetic capacity when expressed on an area basis (A_{area}). We therefore advocate that leaf-level water fluxes should be reported as a function of leaf dry-mass when evaluating the carbon costs of water loss and its impact on lifetime carbon gain.

Our analyses reveal a negative correlation between leaf hydraulic conductance per unit leaf mass ($K_{Leaf,mass}$) and leaf lifespan (LL) that we believe is the consequence of a biophysical trade-off between resource allocation to live cells specialized for resource transport (i.e. high leaf hydraulic conductance) and photosynthetic metabolism, and those specialized for structural rigidity. Further, the negative slope describing the covariation between $K_{Leaf, mass}$ and LL is not significantly different from one (i.e. $K_{Leaf, mass} \propto LL$, $\alpha = 1$). Therefore, lifetime leaf water use, the product of $K_{Leaf, mass}$ and LL, is a function of leaf mass. As such, if the lifetime water use of a leaf is optimized for carbon gain (i.e. ratio of water loss to carbon gain; see Parkhurst and Loucks 1972; Cowan 1977, 1982) then the lifetime carbon gain of all leaves is the same. This in turn would suggest that the lifetime carbon gain of a plant is a function of the number of leaves produced throughout its lifetime.

So how are these strong and potentially universal relationships best explained?   Our approach, outlined below, is to cast the explanation in terms of the leaf bio-mechanical processes that are likely to have contributed to the observed trade-off between hydraulic efficiency and leaf longevity. We also discuss whether or not previously observed patterns of variation in maximum photosynthesis, leaf lifespan, and water use efficiency (i.e. photosynthesis per unit water lost) support the notion of a realized constant net carbon gain per leaf in natural environments subject to variation in climate and resource availability.

Structural rigidity and leaf water use strategy

From a bio-mechanical perspective we first need to explore what processes constrain the ability of leaves to transport CO_2 and water vapor, and at the same time maintain a long lifespan via structural rigidity. Theory and empirical research suggests that variation in leaf structural rigidity and the liquid phase transport capacity of CO_2 and water are tightly coupled to variation in cell wall structure (i.e. carbon investment in being rigid; see Niklas 1989; Syvertsen et al. 1995; Sack et al. 2003; Aassama et al. 2005; Evans et al. 2009). This is because leaf structural rigidity is a function of both the apoplast (cell walls composing the organic skeleton of a leaf) and the positive internal pressure of the symplast (the cell cytosol pushing up against the plasma membrane and subsequently the cell wall). Therefore, the structural rigidity of a leaf can increase either through: (1) an increase in the ratio of cell wall volume (apoplast) to symplast volume, by way of an increase in cell wall thickness, or (2) an increase in the relative water content of cells and the hydrostatic pressure of the symplast, i.e. turgor pressure (Niklas 1989). Further, as the ratio of apoplast to symplast volume of a leaf cell increases, cell rigidity becomes

less sensitive to variation in cell water content and turgor pressure (Niklas 1989). Therefore, leaves constructed of mesophyll tissue with a high apoplast to symplast volume, by way of thick cell walls and low relative water content, are structurally more tolerant of abiotic and biotic stresses when exposed to decreases in water availability than leaves with a low apoplast to symplast volume (Balsamo et al. 2005).

Cell walls not only contribute to structural rigidity, and therefore longevity, but they also directly influence leaf water use and the liquid phase transport of CO_2 between the intercellular airspaces and the sites of photosynthetic metabolism. This is because mesophyll cell walls exposed to intercellular air spaces represent the site of evaporation and CO_2 uptake by leaf cells (Jarvis and Slatyer 1970; Meidner 1975; Farquhar and Raschke 1978; Jones and Higgs 1980). The water potential gradient utilized by plants for the uptake and transport of water and mineral nutrients from soil to leaf is first established at cell walls exposed to the internal air spaces of the leaf (Tyree and Zimmermann 2002). Further, the photosynthetic mesophyll cells represent the area of greatest resistance to water transport across the entire plant hydraulic pathway, from root to leaf (Boyer 1974; Cochard et al. 2004; Sack and Holbrook 2006; Mott 2007). Since the mesophyll is a major determinate of leaf hydraulic resistance and rigid mesophyll cell walls are resistant to water transport (Aasamma et al. 2005), short-lived leaves maintain high rates of water loss and CO_2 uptake by relying on a hydrostatic skeleton rather than on rigid cell walls with high hydraulic resistance to maintain leaf rigidity (Moore et al. 2008). In contrast, long-lived leaves are often sclerophyllous and rely on mesophyll tissue composed of small cells with thick, rigid cell walls to maintain leaf structure, thus decreasing instantaneous rates of water loss and CO_2 uptake (Balsamo et al. 2003; Moore et al. 2008). Additionally, the resistance to gaseous diffusion of CO_2 in leaves is considered relatively minor compared to the resistance to liquid phase transport of CO_2 (Evans et al. 2009). After CO_2 is transported from atmosphere to leaf, it enters the liquid phase in the cell wall and is subsequently transported through the cell wall and plasma membrane into the symplast before diffusing into the chloroplast where carboxylation occurs (Parkhurst and Mott 1990; Parkhurst 1994; Evans et al. 2009). Because the resistance to liquid phase transport of CO_2 is directly proportional to cell wall thickness, the cell wall is considered a major resistor along the CO_2 transport pathway (Evans et al. 2009). Therefore, our data when combined with previous evaluations suggests that the negative correlation between leaf lifespan and photosynthesis, is caused by a biophysical trade-off between cell walls designed for structural rigidity and those specialized for water transport and the liquid phase conductance of CO_2 (Figure 1B).

Is realized net carbon-gain independent of leaf lifespan despite variation in climate and resource availability?

Despite the strong support for isometric scaling between leaf hydraulic conductance per unit leaf mass ($K_{Leaf, mass}$) and leaf lifespan (LL) the scaling slope describing the correlation between leaf photosynthetic capacity per unit leaf mass (A_{mass}) and leaf lifespan, across different climate regimes (e.g. seasonal versus non-seasonal; xeric, mesic and tropical environments), is consistently > -1 (Reich et al. 1997; Wright et al. 2004). At first glance the difference in scaling slopes describing the covariation between LL, $K_{Leaf, mass}$ and A_{mass} would suggests that plants

have maximized the capacity to loose water for a given dry mass investment in leaves without an equivalent increase in photosynthetic metabolism. However, the covariation between photosynthesis and leaf hydraulic conductance is extremely sensitive to changes in climate, specifically the vapor pressure gradient from leaf to atmosphere. Therefore evaluations of the covariation between A_{mass} and LL should occur at the vapor pressure deficit (D) leaves experience when most active; i.e. flux-weighted D.

The influence of vapor pressure deficit on the covariation between A_n and K_{Leaf} can be explained using a coupled photosynthesis-leaf water balance model based on Fick's law for the diffusion of gases across a porous surface and Darcy's law for fluid flow through a porous medium. According to Fick's law, leaf water loss (E) can be modeled as:

$$E = gD$$

(Eqn. 2)

where g is leaf conductance to water vapor and D is the vapor pressure deficit from leaf to air. Similarly, net photosynthesis can be modeled as:

$$A_n = \frac{g}{1.6}\left(c_a\left(1 - \frac{c_i}{c_a}\right)\right)$$

(Eqn. 3)

where c_a is the ambient CO_2 concentration, c_i is the CO_2 concentration inside the leaf, and 1.6 is the diffusivity correction between water vapor and CO_2. Combining the two equations we can see the relationship between net photosynthesis and leaf water loss as:

$$A_n = \frac{E}{1.6D}\left(c_a\left(1 - \frac{c_i}{c_a}\right)\right)$$

(Eqn. 4)

Therefore, as vapor pressure deficit, D, increases the ability to maintain high rates of photosynthetic metabolism is directly coupled to a concurrent increase in leaf water loss.

For leaves, the ability to maintain high rates of water loss (i.e. E) is dependent upon an efficient vascular system (i.e. high K_{Leaf}). For example if water supply to the sites of evaporation in the leaf (J) is unable to keep pace with increases in leaf water loss then leaf water content (W) will decrease at the following rate, $\Delta W = E - J$. As leaf water content decreases leaf water potential and leaf conductance to water vapor decreases in order to maintain leaf water content above the point of turgor loss (Brodribb and Holbrook 2003). That being the case, in order to maintain high rates of E and A leaves must be hydraulically efficient. According to Darcy's law leaf hydraulic conductance can be modeled as:

$$K_{Leaf} = \frac{E}{\Delta\Psi}$$

(Equation 1 in methods)

Previous research has shown that the vast majority of cross-species variation in maximum E is due to variation in K_{Leaf} suggesting there is little variation in $\Delta\Psi$ between species (Brodribb and Holbrook 2004; Brodribb et al. 2005; Sack and Holbrook 2006). Indeed, a common slope describes the variation in stem and leaf water potential for species in the common garden as well as field grown individuals exposed to heat exposed to heat and water stress (Figure 3). Convergence in $\Delta\Psi$ suggests that maintaining high rates of leaf water loss (E) and photosynthetic metabolism (A_n) requires the ability to efficiently transport water from stems to the sites of evaporation in the leaf mesophyll. Thus leaf transport efficiency, K_{Leaf}, can be combined with Eqn. 1 and 4:

$$A_n \approx K_{Leaf} \frac{\left(c_a \left(1 - \frac{c_i}{c_a} \right) \right)}{1.6D}$$

(Eqn. 5)

Therefore, according to Fick's law and Darcy's law, the covariation between K_{Leaf} and A_{mass} is a function of the covariation between c_i/c_a and D:

$$\frac{A_n}{K_{Leaf}} = \frac{\left(c_a \left(1 - \frac{c_i}{c_a} \right) \right)}{1.6D}$$

(Eqn. 6)

Since the coordination between A_n and K_{Leaf} is a function of the covariation between c_i/c_a and D this begs the question, does flux-weighted c_i/c_a vary predictably with variation in vapor pressure deficit?

If plant leaves are the products of selection to their local environment such that they maximize the return on dry mass investment in leaf tissue, then we would expect flux-weighted c_i/c_a to scale negatively with D. Put another way if the lifetime water use efficiency of a leaf (i.e. A_n/E) is relatively constant then isometric scaling between $K_{Leaf, mass}$ and LL provides strong support for a constant lifetime, net carbon gain per leaf. Although previous research has suggested optimal matching between leaf water use efficiency and the environment leaves inhabit (Parkhurst and Loucks 1972; Cowan 1977, 1982) relatively few, if any, studies have accurately characterized the flux-weighted c_i/c_a in response to variation in one of the key environmental variables that drives water loss (E) from leaves, namely the vapor pressure deficit during the periods of highest activity. Instead previous research has focused on short-term point measurements of instantaneous variation in c_i/c_a with variation in D. This has partly been due to methodological limitations in our ability to accurately characterize the lifetime flux weighted vapor pressure deficit from leaf to air (i.e. D). An early attempt to characterize the covariation between integrated c_i/c_a and D was made by Comstock and Ehleringer (1992) by weighting monthly average D by monthly average water availability in order to estimate D during the periods when leaves were most active (i.e. flux-weighted D). In a common garden study of different ecotypes, they found a negative linear relationship between integrated c_i/c_a and the average flux-weighted D an ecotype experienced in its home range. The fact that c_i/c_a of individuals grown in a common environment were negatively correlated with the vapor pressure

deficit of the site of origin suggests strong genetic differentiation in c_i/c_a in response to variation in the average D a leaf experiences when most active. A negative relationship between c_i/c_a and D suggests minor variation between A/E. At the ecosystem scale, A/E has also been found to be relatively constant across vegetation types and climate (Beer et al. 2009). Therefore, if c_i/c_a does scale negatively with increasing D, such that the ratio of A/E is relatively constant, then isometric scaling between $K_{\text{Leaf, mass}}$ and LL suggests a constant net carbon gain per leaf.

CONCLUSIONS

We observed a strong negative correlation between leaf lifespan and leaf hydraulic conductance. This negative relationship suggests that the ability of leaves to payback the carbon cost of construction and lifetime maintenance respiration is ultimately limited by a trade-off between resource investment in live cells specialized for photosynthetic metabolism and vascular transport versus resource investment in structural rigidity needed to display leaves for light capture. Additionally the trade-off between leaf hydraulic conductance and leaf lifespan observed here suggests that the lifetime water use of a leaf is directly proportional to leaf mass. Therefore if the amount of leaf water loss per unit carbon gain is constant then the net carbon gain of a leaf is independent of leaf lifespan.

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FIGURE LEGENDS

Figure 1. (A) Negative scaling between photosynthetic capacity per unit leaf mass (A_{mass}) and leaf lifespan is considered the outcome of evolution working within boundaries set by biophysical constraints and the need to maintain a positive carbon balance. Theory suggests that maximum lifetime carbon gain is limited by a trade-off between leaf tissues designed for metabolism versus structural rigidity (Roderick et al. 1999; Shipley et al. 2006) that prevents long-lived leaves from working at high photosynthetic rates per unit leaf mass. (B) Here we suggest that the trade-off between metabolism and structural rigidity is the result of a fundamental biophysical trade-off between the ability of living cells to transport water and resources (i.e. hydraulic conductance, K_{Leaf}) and maintain structural rigidity over long periods of time. Since photosynthetic metabolism is directly proportional to the ability of leaves to transport water to surfaces for energy and gas exchange on a mass basis (i.e. A_{mass} scales positively with $K_{Leaf, mass}$) a negative relationship between A_{mass} and leaf lifespan suggests a fundamental tradeoff between tissues specialized for liquid phase processes (e.g. resource transport and metabolism) versus tissues specialized to maintain structural rigidity.

Figure 2. (A) The independence of leaf hydraulic conductance per unit lamina area ($K_{Leaf, area}$) and lamina mass per area (LMA); (B) The covariation between leaf hydraulic conductance per unit lamina mass ($K_{Leaf, mass}$) and LMA; (C) The covariation between $K_{Leaf, area}$ and leaf lifespan (LL); (D) The covariation between $K_{Leaf, mass}$ and LL. Symbols: *Ac, Acer macrophyllum*; *Me, Metasequoia glyptostroboides*; *Po, Populus fremontii*; *Qu, Quercus kelloggii*. The evergreen species are: *Ar, Arbutus menziesii*; *Pi, Pinus ponderosa*; *Se, Sequoia sempervirens*; *Um, Umbellularia californica*, open circles represent species from the common garden, shaded squares represent previously published values for deciduous and evergreen woody species and herbaceous species (Wright et al. 2004; Brodribb and Holbrook 2005; Brodribb et al. 2005; Nardini et al. 2005; Sack et al. 2005; Scoffoni et al. 2008; Simonin and Dawson, *chapter 2*). Only one study reported both $K_{Leaf, area}$, LMA, and estimates of LL (Brodribb et al. 2005). The remainder of the studies reported both $K_{Leaf, area}$ and LMA with estimates of LL obtained from species mean values reported in the GLOPNET database (Wright et al. 2004). The solid regression lines were fit to the species used in this study, the bold dotted line was fit to the entire dataset.

Figure 3. Covariation between branch and leaf water potential during transpiration events (i.e. $E > 0$). All measurements of branch and leaf water potential were made over a diurnal time course in a common garden (see methods), except for *Heteromeles arbutifolia*. Branch and leaf water potential for *H. arbutifolia* was measured in the field during a heat wave and low soil water availability. The model describing variation in branch and leaf water potential does not change when the data from the common garden and the field (i.e. *Heteromeles arbutifolia*) are analyzed separately.
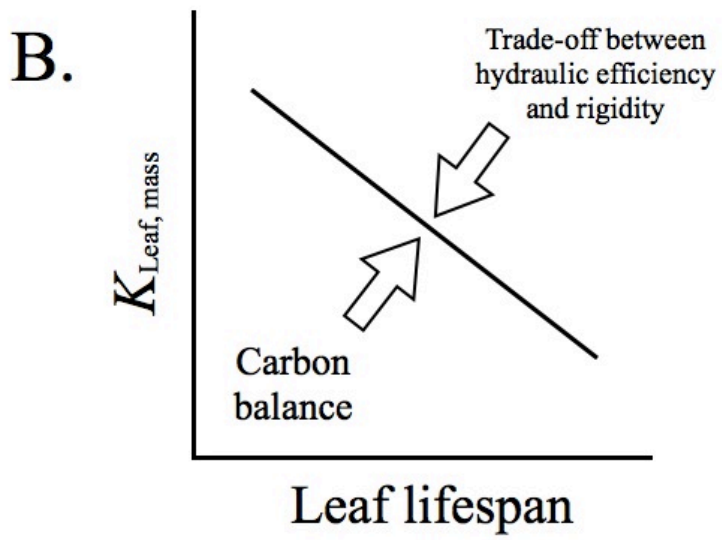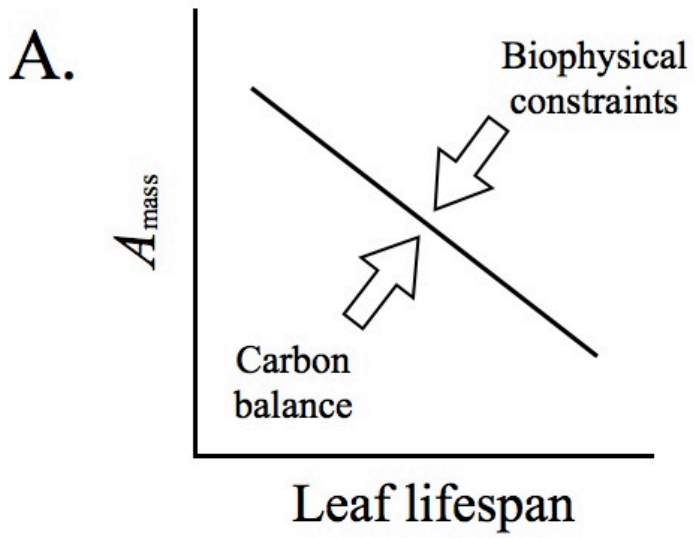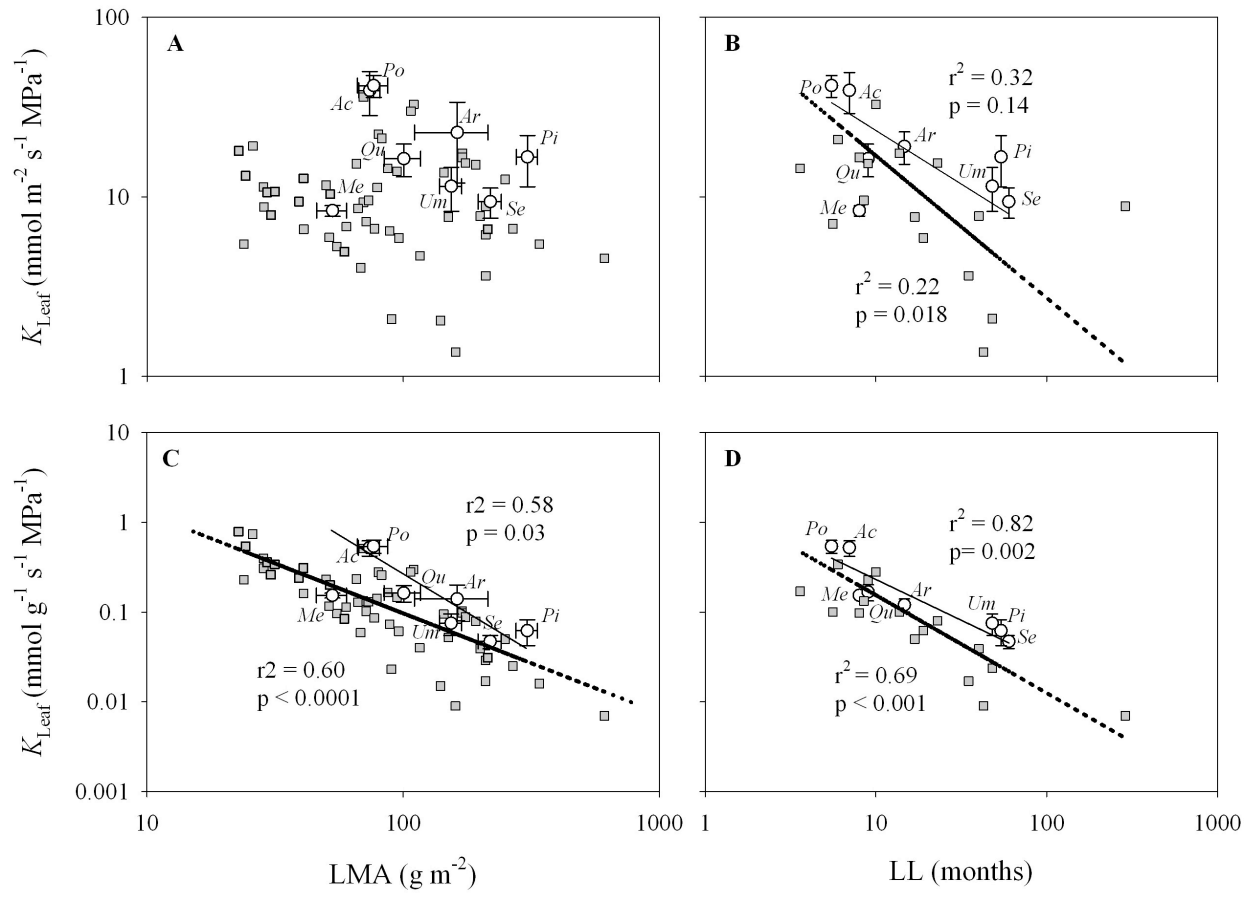
Figure 1.

Figure 2.

Figure 3.