Modeling Within-Plant Water Distribution in Current-Year

Shoots of the Climbing Vine Kudzu (*Pueraria lobata)*

by

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Environment
Duke University

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Thesis submitted in partial fulfillment of
the requirements for the degree of
Master of Science in Environment
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ABSTRACT

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Abstract

The purpose of this study was to investigate hydraulic limitations associated with long, tall stems. Longer stems may lead to decreased water delivery to leaves due to increased friction against the walls of the conduit, and taller stems lead to decreased water delivery due to increased hydrostatic pressure at greater heights. Leaves that receive less water must close stomata to avoid cavitation, and thus limit uptake and growth rates. Although both of these limitations have been demonstrated to lead to reduced growth in trees, there is evidence that some vines can compensate. I hypothesize that despite growing long stems, kudzu is able to maintain high rates of gas exchange and photosynthesis regardless of leaf position. This is accomplished through hydraulic compensations that manifest in structural changes of the conducting tissue along the pathway.

I observed only a slight (11%) decline in stomatal conductance as expected, but was unable to predict the small decline with height based on structural and hydraulic changes to the vine. Across treatment groups, leaf area increases with path length, so equal water delivery to each leaf would result in less water per unit leaf area to distal leaves, suggesting kudzu does not compensate by adjusting the sapwood to leaf area ratio. To compensate by increasing the driving force, leaf water potential would need to fall below the wilting point. To compensate by decreasing hydraulic resistance, kudzu

stems would need to be two orders of magnitude more conductive than measured. Since the total amount of water storage in kudzu based on volume is too small to maintain water supply for 20 minutes, leaves cannot rely on capacitance to compensate for slow delivery through the stem. I am unable to describe the mechanism that allows kudzu to maintain high gas exchange and growth rates in distal leaves.

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1. Introduction

Simple models of water flow from soil to leaf assume that water uptake equals water transpired (i.e. over a short period of time there is no change in the amount of water in plants). Further simplifications assume average hydraulic conductance between two points, one in the soil and another in the crown, defining the two end points for the water potential difference driving the flow from soil to leaves. Models based on these assumptions have been successfully used to describe variation in stomatal conductance at scales ranging from tree crowns to whole ecosystems (Mencuccini et al., 1997; Schäfer et al., 2000; McDowell et al., 2002a,b; Barnard & Ryan 2003; Meinzer et al., 2004; Novick et al., 2009; Ambrose et al., 2010; Gotsch et al., 2010).

However, the role of water stored in the plant is important for supporting transpiration (Meinzer et al., 2009; Phillips et al., 2009), and hydraulic conductance through each element of the soil-plant-atmosphere continuum (SPAC) is far from uniform. Both soil hydraulic conductance (Running 1980) and hydraulic conductance of the xylem pathway (Tyree & Sperry 1989; Sperry et al., 2003) decrease as water potential declines. Moreover, xylem hydraulic conductance varies by organ (Tyree et al., 1975; Ewers & Zimmermann 1984a,b; Andrade et al., 1998; Nardini & Pitt 1999; Sack et al., 2004), and conductance in each plant organ may respond differently to changes in water potential (Tsuda & Tyree 1997; Pivovaroff et al., 2014). Thus, coarse attributes (e.g., averages of path length from soil to leaves, root or sapwood area per unit of leaf area, and predawn to daytime leaf water potential gradients) may work reasonably well in capturing the variability of transport capacity to the average leaf among individuals, stands, and ecosystems, only if these attributes dominate over more nuanced sources of variation. One may therefore ask: what are the limits within which simple hydraulic approximations are useful for describing the variation of stomatal conductance within crowns? To answer this question one must account for the relative contribution to the total resistance to water flow in, and capacity for water supply of roots, stems, petioles and leaves, which relates to the size of each organ and its vulnerability to cavitation.

Climbing vines compete with trees both above (Kira & Ogawa 1971; Dillenberg et al., 1995) and belowground (Dillenberg et al., 1993), oftentimes to the detriment of the host tree (Clark & Clark 1990). In order to for a vine to maintain growth at great heights, it needs to be able to transport water efficiently through its stem to its leaves (Clearwater et al., 2004; Johnson et al., 2013). Indeed, maintaining high stomatal conductance in leaves along the entire stem length is essential for shade-intolerant, freeze-intolerant vines that die back each year, and yet must grow fast to overtop nearby vegetation. Maintaining high conductance near the tip of such vines would facilitate high photosynthesis and provide a local source of carbohydrates for extension growth. However, transporting large quantities of water to the tops of tall canopies is a challenge vines must overcome. It has yet to be shown how vines meet this challenge.

Compared to trees, vines have a much lower sapwood area per unit of leaf area, which in trees has been associated with reduced hydraulic supply per leaf (Whitehead et al., 1984; Phillips et al., 2003; Ewers et al., 2007). Furthermore, the length of flow along the stem in a vine is as long or longer than the path length through a tree stem, and much longer relative to the conducting cross-sectional area of the stem (Rosell & Olsen 2014; Filartiga et al., 2014). Despite restrictions to maximum water flow rates through long, narrow stems, woody vines can contribute a significant proportion of total evapotranspiration in ecosystems (Restom & Nepstad 2001). This implies that these vines must have some mechanism compensating for hydraulic limitations. Among proposed mechanisms for increasing hydraulic flow are deeper rooting systems (Chen et al., 2015), increased stem hydraulic conductivity (Schultz & Matthews 1988; Gartner 1991), increased xylem wall thickness (Masrahi 2014), increased vessel size and number of vessels (Rosell & Olsen 2014), alternate water routing to bypass breaks in the hydraulic line (Tateno & Taneda 2007; Kamakura & Furukawa 2009), and segmentation of hydraulic resistance (Taneda & Tateno 2011).

There are several models that can account in detail for the different and variable resistances along the pathway, based on both Ohm's law analogy, Darcy's flow, or porous media formulations (Edwards et al., 1986; Williams et al., 1996; Ogée et al., 2003; Bohrer et al., 2005; Ewers et al., 2007). I used one such model developed by Tateno & Taneda (2007, 2011), which partitions the above-ground portion of plant hydraulic conductance using an Ohm's law analogy. Taneda & Tateno (2011) found the model to overestimate by ~80% the decrease of stomatal conductance with distance from the base in horizontally growing stems of *Pueraria lobata* (kudzu) . However, the model may match the behavior of stems growing vertically because tissues developing under more negative water potential trade-off safety for conductivity – these tissues may develop such that their hydraulic conductivity is lower but so is their vulnerability to cavitation and loss of hydraulic function (Schubert et al., 1999; Sperry et al., 2002; Schultz 2003; Tombesi et al., 2014). If the greatest resistance to flow in vines such as kudzu is the lateral flow through the petiole and leaf (Taneda & Tateno 2011), a dynamic pattern of lateral resistance where top leaves that are less prone to loss of conductivity may be as well supplied with water as bottom leaves.

I investigated the ability of the Tateno & Taneda (2007) flow resistance formulation and their lateral flow formulation (Taneda & Tateno 2011) to distribute water along stems of vines growing at different angles from fully horizontal to fully vertical. I compared model-base estimates of stomatal conductance to those measured with gas-exchange system. I tested whether the generality of Taneda & Tateno's (2011) finding that a large lateral relative to axial resistance to flow, mostly located in leaves rather than petioles, is responsible for an even flow distribution to leaves along the stem. This should result in little or no decrease of stomatal conductance with distance from the stem base. I also assessed the degree to which stomatal conductance controlled photosynthesis, permitting fast growth rates at long distances from the water source.

1.1 Theory

The Hydraulic Limitation Hypothesis (HLH) predicts that taller individuals will have lower gas exchange rates, reflecting the effect of height on liquid water transport (Ryan and Yoder, 1997; Bond, 2000; Bond and Ryan, 2000; Ryan et al., 2006). The effects of increasing height include an increase of resistance to flow with greater path length (*L*), and a decreasing water potential gradient driving the flow from soil to leaf, as the counter force of hydrostatic pressure increases with the height of the water column. A simplified representation of hydraulic controls over stomatal conductance (g_s) based on a Darcy's law analogy is (Whitehead et al., 1984; Whitehead and Hinckley, 1991; McDowell et al., 2002b):

$$g_s = \frac{k_s \cdot \Delta\Psi \cdot A_s \cdot K_g(T_A)}{L \cdot A_l \cdot D} \qquad (1),$$

where k_s is the xylem specific hydraulic conductivity, $\Delta\Psi$ is the water potential difference driving the flow from the soil to the leaf, A_s is the sapwood area, A_l is the leaf area, and *D* is vapor pressure deficit (see Table 1 for units). K_g is a coefficient that fluctuates with air temperature (T_A) based on changes in the psychometric constant, latent heat of vaporization, specific heat of air at constant pressure, and the density of air

(Ewers et al., 2001). Hereafter I refer to Eqn. 1 as the Simplified Hydraulic properties Formulation (SHF).

According to SHF, and consistent with HLH, L increases with height, limiting g_s of upper leaves and thus the average g_s of the crown (Pennisi, 2005). Not only does increasing height, as a proxy of increasing L, increase resistance to water flow, it also reduces $\Delta\Psi$ since (Zimmermann 1983; Schäfer et al., 2000; Koch et al., 2004; Domec et al., 2008):

$$\Delta\Psi = \Psi_s - \Psi_l - \rho_w g h \cong \Psi_s - \Psi_l - 0.01h \qquad (2),$$

where Ψ_s is the soil water potential, Ψ_l is the leaf water potential, ρ_w is the density of water, g is the acceleration of gravity, and h is the mid-crown height above the ground, vaguely the height of the leaf of which Ψ_l represents the crown or canopy. The SHF can explain the variation of g_s among trees of different height at a given stand (Schäfer et al., 2000; Ambrose et al., 2010), between undisturbed crowns and those of reduced height following fire-induced epicormic branching (Nolan et al., 2014), between shorter, young and taller, old stands (Ryan et al., 2000; McDowell et al., 2002b; Barnard & Ryan 2003; Phillips et al., 2003), and among ecosystems (Novick et al., 2009).

Nevertheless, given the simplifications inherent in the SHF, it is not likely to perform well when average hydrological properties are used to describe wide distributions of these properties, especially for properties that are non-linearly related to g_s. In such cases, the behavior of the system computed based on the average of a property will not equal the average of the behavior computed from the distribution of the property. Furthermore, SHF cannot be readily implemented to produce a distribution of within-plant variation of g_s. Instead, building on Ohm's Law and an electrical circuit analogy, the flow of water through a stem with leaves distributed along its length can be approximated as an electrical circuit with resistors in parallel (Van den Honert, 1948; Tuzet et al., 2003; Tateno & Taneda 2007). As such, the resistance between two leaf nodes can be expressed as in Tateno & Taneda (2007):

$$R_{tot}(n) = R_I(n) + \left(\frac{1}{R_{pet}(n)} + \frac{1}{R_{tot}(n-1)}\right) \quad (3),$$

where $R_{tot}(n)$ is the total resistance in the internode below node n. The most distal node is node 1, and the most basal node is node N. $R_I(n)$ is the specific resistance in the stem between two nodes, defined as:

$$R_I(n) = \frac{I}{k_s * A_s} \quad (4),$$

where I is internode length. Similarly,

$$R_{pet}(n) = \frac{l_{pet}}{k_{pet} * A_{pet}} \quad (5),$$

where $R_{pet}(n)$ is the specific petiole resistance of the n^{th} petiole, l_{pet} is the petiole length, k_{pet} is the petiole conductivity, and A_{pet} is the petiole cross-sectional area.

Once resistance is partitioned between stem and petiole segments, $R_{tot}(n)$, the total resistance through any internode, is computed. In order to determine flow through each leaf, Taneda and Tateno (2011) employ Kirchhoff's Voltage Law:

$$E_k = \sum_{i=k}^{N}(I_{AX,i} * R_{AX.i}) + (I_{lt,i} * R_{lt,i}), \quad (6),$$

where E_k is the driving force for flow, $I_{AX,n}$ is the flow through the axial portion of the soil-to-leaf pathway at node n, $R_{AX,n}$ is the resistance through the axial portion of the pathway at node n, $I_{LT,n}$ is the flow through the lateral portion of the soil-to-leaf pathway at node n, and $R_{LT,n}$ is the resistance through the lateral pathway at node n. Furthermore, Taneda and Tateno (2011) define the axial flow rate at node n as the sum of the lateral flow rates distal to that node according to Kirchhoff's Current Law:

$$I_{AX,k} = \sum_{i=1}^{k} I_{LT,i}, \qquad (7).$$

To run this Distributed Hydraulic Formulation (DHF) model, I solve equation (6) for lateral flow rate at each node simultaneously. For axial resistance, I use $R_l(n)$ or $R_{tot}(n)$ and for lateral resistance, I sum the petiole and leaf resistances. For E_k, I use measured water potential gradient at each node. Axial flow is defined by equation (7), leaving only lateral flow rates as unknowns in the complete set of equations.

In this study, I examined hydraulic traits and gas exchange rates of a model system: a nitrogen-fixing kudzu grown in full sun at various angles, thus allowing some degree of separation between the distance traveled from the soil surface, affecting resistance and potentially causing hydraulic adjustments (Sperry et al., 1988; Magnani et al., 2000; Koch et al., 2004), and height which may affect the driving force as well. However, in field settings, g_s of individual leaves is often not hydraulically controlled, but limited mostly by light intensity on leaf surfaces (Niinemets, 2010), the distribution of which may be correlated to nutrient concentration and photosynthetic capacity

(Leuning et al., 1991; Niinemets, 1998; Bond et al., 1999; Kull & Kruijt 1999; Gonzalez-Real & Baille 2000). I utilized a data reduction approach to account for and remove the effects of changing light intensity and *D* on g_s during field measurements before assessing hydraulic controls of g_s.

1.2 Hypotheses

H1: Although the SHF would predict a sharp decline in stomatal conductance as the distance from the base of the stem increases, equal distribution of leaf water use and segmented resistance to flow according to DHF (Taneda & Tateno 2011), will result in no pattern in g_s along stems of kudzu. However, g_s will decline with vertical distance from the ground due to an increased gravitational discount to the water potential gradient driving flow (Bond 2000; Ryan et al 2006).

H2: Structural and hydraulic alterations within long kudzu stems will allow sufficient water delivery to leaves to support observed gas exchange rates, and should be sufficient to predict uniform water supply rates to all leaves.

H3: Since g_s per unit of leaf area will vary little over the length of the stem, I predict that net photosynthesis per unit of leaf area will similarly show little decline with respect to leaf position.

2. Methods

2.1 Setting

The experiment was established in a field located in the Duke Forest (Durham County, USA: 36.01 °N, 79.00 °W) where the long-term annual mean of temperature and precipitation are 15.5 °C and 1145±180 mm (all means expressed as mean ± standard deviation), respectively. The soil is mostly clay to a depth of 1-1.5 m. Kudzu seeds were grown in pots at the Duke University Phytotron in June 2010, and moved to outside conditions in early July 2010. The seedlings overwintered outside in pots, were trimmed before transplantation to increase the root-to-shoot ratio and planted at the study site in June 2011. Plants were well watered to avoid water limitations, averaging three times per week during the first growing season. During the 2012 growing season, plants were watered at least every other day except in case of rain.

To study leaves along a height and length gradient, a 15-m tall tower was erected at the center of the field, with twelve 15-m long wires attached at four different angles from the horizontal (0°, 30°, 60°, and 90°), between the tower and each of twelve anchor points on the ground. A seedling was planted at the anchor point of each wire (3 replicates x 4 treatment angles = 12 plants). Each plant was trained to grow multiple shoots along the ground at 0° and around a wire at one of the four treatment angles in order to compare horizontal leaves to angled leaves within the same plant in addition to between plants. Browsing by deer affected early season growth in 2012, and may have contributed to different growth patterns among individuals. Deer were prevented from browsing once this was recognized. In September 2012, the length of *P. lobata* stems along vertical wires was 12.1±1.8 m, and exceeded (ANOVA, $p=0.005$) that of all other treatments (7.5±1.5, 2.4±0.6, 2.7±1.9, and 4.1±1.0 m for the 90°, 60°, 30°, 0° on wire and 0° on ground, respectively.

In September 2012, leaves were sampled for gas exchange and leaf water potential measurements. Each day's measurements were blocked to account for variation in conditions. Following these paired water potential and gas exchange measurements, maximum RuBisCO carboxylation rates were measured in situ before stems and leaves were sampled for hydraulic conductivity and pressure-volume relations.

2.2 Gas Exchange

On September 7-9 gas exchange on 54 leaves (20 vertical, 9 at 60°, 4 at 30°, 21 horizontal) was measured using a LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE). For each leaf, the chamber was set to match prevailing environmental conditions assessed immediately prior to the measurement: ambient atmospheric CO_2 concentration (376-413 ppm), ambient relative humidity (26-61 %), ambient leaf-level photosynthetically active radiation (PAR; 300-2000 μmol m^{-2} s^{-1}), and ambient leaf temperature (27-39 °C). All gas exchange results were expressed on a leaf area basis.

The maximum Rubisco carboxylation rate (V_{cmax}) was assessed on 24 leaves on September 10-13, 2012 using the Li-6400 to compare photosynthetic capacity between leaves grown at the top of the tower (N=15) and at the base of the tower (N=9). Net CO_2 assimilation rate versus internal CO_2 concentration (A/C_i-curves) were measured setting cuvette conditions to 30 °C leaf temperature, 50±10 % relative humidity and 1500 μmol m^{-2} s^{-1} PAR. CO_2 concentrations were set to ambient and sequentially lowered towards the CO_2 compensation point (50 ppm).

2.3 Water Potential

Along with the instantaneous measurement of gas exchange in field conditions, leaf water potential was measured using a pressure chamber (PMS Ins., Albany, OR) every 3 m along both the height and length gradients of one wire of each treatment angle, for n= 4 different plants each day for three days. Thus, three leaves at each of 54 locations (162 total) at ~3 m intervals were sampled for water potential. To assess maximum (least negative) leaf water potential, one leaf at each location was sampled at pre-dawn. After the pre-dawn sample was excised, a second leaf at each location was wrapped in aluminum foil-covered plastic bags to obtain leaf water potential in equilibrium with the water potential of the adjacent xylem (Richter et al, 1997). Between 8:30 and 11:00 each day, water potential was measured on the bagged leaf, and on a third, unbagged leaf at each location. The third leaf was excised immediately following measurement of its gas exchange rates.

From September 24 to October 4, 2012, 20 leaves were sampled to compare pressure-volume parameters between leaves at the tip and the base of the 90° and 0° treatment angles. Leaves were sampled before dawn and transported to the lab in plastic bags keeping the cut end of the petioles submerged. For at least one hour prior to measurement, leaves were allowed to absorb water and rehydrate in open air at 25 °C while petioles were kept in water. Following, leaf mass and leaf water potential were repeatedly measured as leaves dried on a lab bench and lost turgor pressure. At least five points were measured before turgor loss point, and at least six points after turgor loss to accurately approximate the linear portion of the relation between the inverse of leaf water potential and leaf relative water content. The parameters obtained were water potential at turgor loss point (Ψ_{TLP}; MPa), osmotic potential at full turgor (Ψ_{FT}; MPa), maximum bulk modulus of elasticity (ε_{max}; MPa), absolute capacitance at full turgor per unit leaf area (C_{FT}; g H_2O m^{-2} MPa^{-1}), and time constant of adjustment of water potential to a change of transpiration rate (*s*). Following pressure-volume curve measurement, leaves were dried in an 80 °C oven for at least 24 hours before measuring dry weight.

2.4 Hydraulic Conductivity

Liquid-phase resistances to water transport aboveground were estimated from stem, petiole, and leaf specific hydraulic conductivity along the height and length gradients. Stem samples roughly 1.8 m long from the base and tip of each plant were transported to the lab, keeping the cut ends submerged in water. Estimates of longest vessel length were made using the compressed-air method of Ewers and Fisher (1989), whereby air is forced into the proximal end of the segment at 75 kPa and the distal end is submerged in water. Stem segments of 5 cm are cut from the distal end until air bubbles are seen, and the final length of the segment is then taken as the longest vessel length. Before hydraulic measurements, stem segments that were at least 20% longer than the mean vessel length (0.74±0.3 m) were recut at both ends underwater, stripped of leaves and flushed with filtered (0.22 μm), deionized water to refill embolized vessels. At each end of the stem, the xylem was exposed by removing the tissue surrounding it and attached to a tubing system suffused with filtered, deionized water. At the proximal end of the stem, 5 kPa of pressure was applied, and efflux was measured at the distal end with a 1 mL graduated pipette. Water temperature was measured before and after each measurement to account for changes in the viscosity of water with temperature and normalized to conductivity at 20 °C. Specific conductivity (k_s) was calculated as the mass flow rate of the perfusion solution divided by the pressure gradient across the segment, normalized by the xylem cross-sectional area and length.

Vulnerability curves were constructed using the air-injection method (Sperry and Saliendra, 1994). Recent work has shown that reliable measurements of hydraulic vulnerability can be obtained by using this method, especially when using a small pressure sleeve (Ennajeh et al. 2011). After the maximum flow rate was measured (k_{smax}), stems were placed in a double-ended pressure sleeve (9 cm in length) and pressurized for 1 min. The stem was then removed from the pressure sleeve and variables necessary to estimate k_s measured again as described above, except that the hydraulic pressure head was adjusted to less than 4 kPa to avoid refilling of embolized vessels. Pressure was applied at increments of 0.5 MPa between consecutive k_s estimates until it dropped to 10% or less of k_{smax}.

Variation in petiole conductivity with stem length and height was measured on the 162 petioles of 54 locations (three leaves per location) on which water potential was determined in the field. After being excised for water potential measurements, leaves were transported to the lab in plastic bags and kept in the refrigerator for a maximum of seven days. Petioles were cut from each leaf and flushed with filtered, deionized water to remove embolisms prior to measuring petiole specific hydraulic conductivity, k_{pet}, using a high-pressure flow meter (HPFM, Dynamax Inc., Houston, TX).

2.5 Plant Structure

After the water potential measurements performed in 2011, fresh and fully hydrated leaf mass was measured for each leaf. Leaf area was measured using a scanner and ImageJ image analysis software. For each leaf water potential sample excised in 2012, leaf area was measured after petioles were removed using a leaf area meter (LI-3100, Li-Cor, Lincoln, Nebraska). Leaf area measurements (mean=166 cm^2) may be slight overestimates (<3%) because the petiolules (< 5 cm^2) connecting each leaflet to the petiole were not removed from leaves. Leaves were then dried in an 80 °C oven for two days to assess dry mass.

After performing vulnerability curve assessments for each stem sample, sapwood diameter was measured using a caliper and converted to sapwood area (A_s; m^2). The distance between each leaf (I; m) was measured using a tape measure to estimate the distance for water flow between leaves as well as the number of leaves per stem. Stems were tightly coiled around the training wires, resulting in a three-dimensional shape after sampling. Thus, despite stretching, measured I may be slightly underestimated. Petiole diameter was measured with a caliper prior to k_{pet} assessment, and converted to petiole cross-sectional area (A_{pet}; m^2). In June 2014, 78 leaves from shaded stems, and 33 leaves from unshaded stems in a nearby location were sampled for petiole length and petiolule length. Since the center kudzu leaflet (of three leaflets) has a petiolule increasing the path for flow beyond the petiole, one-third the petiolule length was added to the petiole length to estimate average petiole path length to each leaflet, l_{pet}.

3. Results

3.1 Gas Exchange

Decreasing stomatal conductance from both atmospheric and hydraulic effects was directly related to changes in C_i/C_a, but only over low g_s values, which reflect conditions of moderate-low PAR and high *D* (Fig. 1A). Thus, once these low g_s values were exceeded, increases in g_s led to a nearly linear and rapid increase of net photosynthetic rate (A_{net}; Fig. 1B). Although leaves at the top of the stem had somewhat lower V_{cmax} and CO_2 compensation point, these differences were not significant (Table 2).

Stomatal conductance (g_s) decreased with increasing *D*, and generally increased with light intensity (PAR) at each *D* (Fig. 2A; minimum r^2 was 0.59 for the lowest PAR interval, ranging from 0.70 – 0.71 for higher PAR intervals; maximum p=0.005). Each of the four PAR intervals was represented by stems of all four treatment angles. The sensitivity of g_s to ln(*D*) (the parameter b of the regression fit $g_s = g_{s\text{-}ref} - b * \ln(D)$) increased with the reference conductance, i.e. g_s at *D*=1kPa (Fig. 2B). The line representing an average for many species (Oren et al. 1999) is shown for reference. However, $g_{s\text{-}ref}$ increased significantly with PAR, showing little tendency to saturate at high irradiance (Fig. 2C). Thus, although variation in stomatal conductance due to soil moisture were controlled by having the plants well irrigated, the variation of g_s caused by atmospheric water demand and light conditions must be considered before attributing the remaining variation to hydraulics.

To account for variation of g_s caused by D and PAR, I first calculated the residual of each value in Fig. 2A from its respective fit representing a PAR range, and added the value to the $g_{s\text{-}ref}$ obtained from the same fit (Fig. 2C), thus normalizing all g_s values to their expected values at 1 kPa, i.e., to expected $g_{s\text{-}ref}$, *or* $g_{s\text{-}ref'}$. Following, I related these $g_{s\text{-}ref'}$ values to the actual PAR in which each original g_s value was measured, obtaining a relationship that allowed us to estimate the average maximum $g_{s\text{-}ref'}$ (Fig. 3A). Taking the residuals of this fit, and adding them to the fitted maximum $g_{s\text{-}ref'}$ (1.1 mol m^{-2} s^{-1} at 2000 PAR; Fig. 3A) I obtained a maximum g_s normalized to 1 kPa D and maximum PAR ($g_{s\text{-}ref'}$m). This value, likely controlled by hydraulic architecture only, was then related to leaf height and distance from the soil, showing a similar relationship with both (Fig. 3B,C). Because I kept sample averages of hydraulic properties for each location rather than values of each leaf, I focus my hydraulic analysis on explaining the departure of the observed average decrease from the SHF-based expected effects of height and path length.

3.2 Water Potential

While no pattern emerged in daytime leaf water potential measurements, for either the transpiring (90°: p=0.52, 0°: p=0.94) or non-transpiring leaves (90°: p=0.37, 0°: p=0.41), the pre-dawn water potentials decreased significantly with height in the vertical treatment (Fig. 4A, p=0.0003), but not with length in the horizontal treatment (p=0.44), reflecting the expected effect of hydrostatic pressure. Thus, although daytime leaf water potential decreased with light (reflecting increasing stomatal conductance) in transpiring leaves, and showed similar though not significant trend in non-transpiring leaves (Fig. 4B), there was no clear difference in leaf water potential of either transpiring or non-transpiring leaves among the treatment angles (Fig. 4C). Thus, when modeling, I only accounted for the effect of the hydrostatic pressure depending on leaf height on the potential difference driving water flow from soil to leaf.

All pressure-volume parameters were similar regardless of height ($p>0.05$) except for the pressure at turgor loss point, which was more negative in leaves at the top of the tower ($p=0.047$, Table 3).

3.3 Plant Structure

The area of individual leaves increased with path length (A_l; Fig. 5A), as did the cross-sectional area of the stem xylem (A_s; Fig. 5B). The length between leaves, l, was conserved, but was greater (two-tailed t-test, $p=0.00110$) in vertical stems (21.7±0.8 cm) than horizontal stems (17.4±0.9 cm). Along with the increase of A_l along the stem, the cross sectional area of the petiole also increased with distance from the stem base (Fig. 5C). Although petioles sampled in 2014 in a partially shaded area (27.3±0.8 cm) were longer than those sampled in an exposed area (21.0±1.1 cm; two-tailed t-test, $p=0.009$), all leaves in this study were developed under full sky exposure, and the mean length of the shorter petioles were used in the hydraulic calculations

3.4 Hydraulic Conductivity

With increasing height, leaf conductivity decreased, although this relationship was only statistically significant in the 90 degree treatment group (Fig. 6A; p=0.0127). Along the same height gradient, petiole conductivity increased (Fig. 6B, p<0.0001) and stem conductivity did not significantly change (Fig. 6C, p=0.39).

Stems sampled from the top and base of each vine showed a similar pattern of reduction in conductivity as applied pressure increased. The applied pressure at which 50% of conductivity is lost was not different between the tops and bases of vines on wires or on the ground (ANOVA, p=0.921). The average P50 value for all stems was 2.1±0.1 MPa. The loss in conductivity between measured stem pressure pre-dawn and mid-day (mean=3.5%) was similar in the base and tops of vines on wires and on the ground (ANOVA, p=0.127).

Although significant, decreases of $g_{s\text{-}ref}$m with both height and distance to stem base (from Fig. 3B,C) were small relative to the theoretical decrease according to the SHF as well as the DHF (Fig. 7A), indicating either an appreciable compensation or that path length has little effect on the total resistance to flow to any leaf. In the following discussion, I will assess potential explanations for the differences between the pattern of estimated $g_{s\text{-}ref}'m$, with distance to stem base and the more abrupt reduction estimated based on DHF.

4. Discussion

4.1 Gas Exchange

The purpose of this study was to investigate the effect of hydraulic limitations along vine stems on leaf water supply, and how this relates to total leaf photosynthesis. Within a single plant, g_s has been shown to respond to variable light (Ewers et al., 2007) and nitrogen supply (Niinemets, 2007) along the length of the stem. In this ideal system, light and nutrient limitations were minimized since kuzdu, a nitrogen-fixer, was growing in the open. I hypothesized that once these potential sources of variation in leaf photosynthesis are removed, hydraulic supply along the stem will account for the remaining variation in g_s.

Despite observations of steep declines in gas exchange rates due to height increases in trees (Hubbard et al., 1999; Magnani et al., 2000; Schäfer et al., 2000; McDowell et al., 2002; Philips et al., 2003; Renninger et al., 2003; Fang et al., 2013), and vines (Oliver & Scene 1992), my results agree with previous findings in kudzu (Taneda & Tateno 2011) and other lianas (Gartner 1991; Molina-Freaner et al., 2004; Zhu & Cao 2009; Masrahi 2014; Chen et al., 2015), and supports the hypothesis that stomatal conductance will remain high regardless of leaf position along the stem of long vines. Once the effects of variation in incoming light and *D* during the measurement period were removed (Fig. 2), I found only a slight (11%) reduction in g_s between the most basal and the most distal leaves (Fig. 7A). Indeed, the decrease was even less than the 27% that could be attributed to the gravitational discount of the driving force (Fig.4A). This suggests that most of the variation in g_s observed in my study was associated with changes in environmental conditions rather than leaf hydraulic supply rates.

These high g_s values, $g_{s\text{-}ref}'m$, represent conductance unlimited by soil moisture and light, and at a reference D = 1 kPa. Therefore, $g_{s\text{-}ref}'m$ is higher than the stomatal conductance representing ambient field conditions (mean g_s=375 mmol m^{-2} s^{-1}) in which light and D are variable. Indeed, only few of the measured g_s values were obtained under such condictions (Fig. 1B), while g_s values representing more common field conditions were similar to previous field observations of kudzu (Forseth & Teramura 1987; Taneda & Tateno 2011) and *Vitis* (Williams et al., 2012), but greater than others reported for *Vitis* (Lovisolo & Schubert 1998) and other vine species (Santiago & Wright 2007; Doughty 2011; Leuzinger et al., 2011; van der Sande et al., 2013).

4.2 Hydraulic Compensation

I hypothesized that structural alterations would compensate for the path length limit to conductance as stem length increased in kudzu, but that there would still be a slight reduction in vertical stems due to the declining water potential gradient, as observed in trees (Ryan et al., 2006, Domec et al., 2008) and vines (Leuzinger et al., 2011). While the effect of the gravitational pull on the increasingly large water column is seen in the pre-dawn pattern of leaf water potential (Fig. 4A), it is no longer apparent in the daytime organization of leaf water potential. Daytime leaf water potential in this experiment (mean=-0.41 MPa) was roughly half of daytime leaf water potential reported in kudzu (Ponder & Al-Hamdani 2011; Taneda & Tateno 2011) and two thirds to one quarter of leaf water potential reported in other vines (Schultz & Matthews 1988; Williams et al., 2012; Johnson et al., 2013; Tombesi et al., 2014). Thus, the gravitational effect should have had a relatively large effect on the g_s, but this was not observed on $g_{s\text{-}ref}{'}m$ (Fig. 3B).

Structurally, the kudzu stems and leaves were similar to reports of other kudzu and climbing vines. Stem and petiole diameters were similar to reported values for vines (Lovisolo & Schubert 1998; Taneda & Tateno 2011; Filartiga et al., 2014). However, the distance between nodes was 50% greater in the kudzu measured by Taneda & Tateno (2011), suggesting more leaves per length of stem in my samples. Although no change in conducting area was found along vertically growing stems in other studies of kudzu (Sakai & Suzuki 1999; Tateno & Taneda 2007) and *Vitis* (Schubert et al., 1999), in this study, increasing sapwood area with height was observed (Fig. 5B). Furthermore, leaf area increased from the base to the most distal nodes (Fig. 5A), whereas previous observations suggest that the largest leaves are located near the middle of the stem (Sasek & Strain 1989).

I combined regressions of tissue area and length with regressions of hydraulic conductivity to predict hydraulic resistance in each tissue at each node. The proportion of tissue specific resistance and total plant resistance was 48% for leaves, 39% for petioles, and 3% for the stem (Fig. 6), suggesting the remaining 10% of hydraulic resistance is located below ground. This is similar to the findings of Taneda & Tateno (2011), who also found the most resistance through the leaf lamina, and the least through the stem. However, Taneda & Tateno (2011) report lamina resistance 2.4 times greater than mine (3.7 MPa s $mmol^{-1}$) and stem resistance 6.1 times less than mine (0.13 MPa s $mmol^{-1}$). Although I did not observe any effect of position or treatment angle on stem conductivity, Schubert et al., (1999) report that conductivity is significantly higher through stems that were grown vertically compared to stems angled downwards. In this study, petiole hydraulic conductivity increased with leaf distance from the stem base (Fig. 6B), while no pattern was observed in previous studies of vines (Taneda & Tateno 2011; Zuffrey et al., 2011; Tombesi et al., 2014). In this study, petioles had on average ~2 times less conductivity than other kudzu petioles (Taneda & Tateno 2011) and three orders of magnitude more petiole conductivity than *Vitis* (Zuffrey et al., 2011; Tombesi et al., 2014).

I hypothesized that structural changes along the length of the stem will hydraulically compensate for the path length and gravitational limitations, and hence provide distal leaves with sufficient water, supporting a nearly uniform transpiration rate along the stem. However, combining the hydraulic information into the two models, both models for water delivery failed to explain the nearly complete hydraulic compensation. For both SHF and DHF, the model suggests reduction of g_s by >98% over the length of 15 m (Fig.7A). Thus my hypothesis that structural changes along the stem would produce the only slight decline in g_s was not supported by either formulation of the hydraulic model. Although neither model agrees with the observations, the DHF may be more appropriate than the SHF in this case since it predicts a shallower decline in conductance with increasing leaf distance, and is thus closer to the estimated $g_{s\text{-}ref}'m$.

Contrary to the second hypothesis, the decline in the water potential gradient with respect to height (27%) did not parallel the decline in leaf gas exchange rates (11%) as predicted in SHF. Even though no direct compensation was observed, i.e. no reduction in leaf water potential as height increased to maintain a constant driving force to each leaf, it is reasonable in theory for kudzu to compensate in such a way given that the turgor loss point of the highest leaves is 24% more negative than TLP of leaves on the ground (Table 3). Indeed, the average transpiring leaf water potential was only ~32% of the average turgor loss point (-1.2 MPa) observed in this study and as little as 20% of the turgor loss point from other studies on lianas (Zhu & Cao 2009; Johnson et al., 2013), suggesting that leaf water potential could decline further without risking leaf function. Could higher leaves allow their water potential to decline further than lower leaves, theoretically compensating for the increased hydrostatic pressure at higher positions? Next I assess several potential explanations for the discrepancies between measured and modeled g_s.

4.3 Possible Explanations for Equal Water Delivery

To increase predicted water supply rates to match the maximum observed rate, the plant could either decrease the resistance along the pathway or increase the pulling force. Assessing the plausibility of these explanations, I solved the DHF for force or resistance, setting flow equal to $g_{s\text{-}ref}'m$. To reach maximum flow using the measured resistances, leaves at 15 m height would have to drop their water potential to lower than -20 MPa (Fig. 7B). This is nearly 19 MPa below the leaf turgor loss point at any position (Table 3). Based on this analysis, I conclude that it is unlikely that kudzu leaves can fully compensate for a low rate of water delivery by decreasing leaf water potential to increase the driving force.

Though plants have previously been shown to compensate for hydraulic limitations by increasing A_s:A_l (Schäfer et al., 2000), increasing leaf area with distance to stem base (Fig. 5A) leads to a decrease in A_s:A_l, suggesting that adjusting this ratio is not a mechanism that kudzu depends on to compensate for hydraulic limitation.

For the vine to reach the maximum transpiration along the entire stem (Fig. 3B,C) while maintaining a constant leaf water potential set to the average (Ψ_L=-0.41 MPa), it must reduce its total resistance to the most distant leaves. However, reducing the resistance in the stem will not have the same impact as reducing the resistance through the leaves or petioles. The relative water delivery rate at the base versus the tip of a stem is determined by the ratio of the axial to lateral resistance, such that a large lateral resistance relative to the axial resistance is required to equalize flow along the length of long stems (Taneda & Tateno 2011). This implies that either increasing lateral resistance or decreasing stem resistance is necessary to predict the observed uniform flow rate. However, increasing lateral resistance will reduce predicted flow to all nodes, causing the model to underestimate flow to all nodes. Hence, the only remaining hydraulic adjustment is to reduce axial resistance. Solving for axial resistance given lateral resistance and constant driving force, a flow matching uniform $g_{s\text{-}ref}'m$ is obtained when axial resistance is four orders of magnitude less than lateral resistance (Fig. 7C). I observed only two orders of magnitude lower axial than lateral resistances, but previous observations found three order of magnitude lower axial than lateral resistance in kudzu (Taneda & Tateno 2011), and lianas (van der Sande et al., 2013), closer to the four orders needed to match modeled values to observations (Fig. 7C). Although decreasing stem resistance could generate the observed uniform conductance, if my measurements of stem conductivity were accurate, I must also decline this as a possible explanation for the differences between modeled and measured patterns of $g_{s\text{-}ref}'m$.

I tested the possibility that each node is independent (a proxy of large local capacitance) by approximating flow using a SHF model of only local resistance to water flow. Instead of calculating flow simultaneously to each node as in DHF, I now assume that each leaf is decoupled from other leaves on the stem. As such, flow to each leaf can be modeled as a simple tree electric circuit where flow equals constant driving force divided by the sum of resistances through the local stem, petiole and leaf lamina. I find both a pattern and absolute values close to the measured $g_{s\text{-}ref}'m$ (dotted line in Fig. 7A), which is reasonable considering the structural, hydraulic conductivity and driving force patterns in these measurements (Fig. 4-6). I assessed the likelihood of this mechanism for supporting high transpiration in kudzu leaves by estimating the maximum supply of water stored locally within the internode, petiole and leaf that could be available during the day to compensate for a lack of water supply from the soil. To do this, I approximated the volume of each organ with respect to its position, and assumed that water comprises 67% of the stem by volume (Poblete-Echeverria et al., 2012), and that 50% of an organ's water volume is available to the leaf as capacitance. Then I converted this stored water volume to water mass per unit of leaf area and compared to the rate of transpiration. At a given rate of transpiration, I calculated how long stored water in the stem, petiole, and leaf can supply a leaf with water. Based on this, the supply of water would suffice to support transpiration at $g_{s\text{-}ref}'m$ in upper leaves for about 10 min, or at the average of measured conductance (assuming D=1 kPa) for 20 min (Fig. 7D). Thus local capacitance does not seem to be the mechanism supporting the small decline in water delivery observed along the stem.

4.4 Conclusions

I was unable to identify the mechanism supporting a nearly uniform supply of water per unit leaf area along the stem. This nearly even flow is essential to keep stomatal conductance high at increasing height along the stem. Coupling only slightly decreasing conductance along the stem (Fig. 3B,C) with increasing leaf area (Fig. 5A) provides for increasing leaf-scale photosynthesis with distance from the ground (Fig. 7E). This is regardless of whether $g_{s\text{-}ref}'m$ or mean g_s (reflecting the typical diurnal courses of PAR and *D*) is used, thus providing a local source of carbohydrates to support the high growth rate observed at the stem tops of Kudzu.

Table 1. Symbols, Units and Description of Variables

Symbol	Description	Units
A_l	Leaf Area	m^2
A_{net}	net rate of carbon assimilation	μmol CO_2 m^{-2} s^{-1}
A_{pet}	Petiole cross sectional area	m^2
A_s	Sapwood Cross Sectional Area	m^2
C_a	Atmospheric CO_2 concentration	ppm
C_i	Leaf internal CO_2 concentration	ppm
D	Vapor Pressure Deficit	kPa
E_k	Driving force for flow in Ohm's Law analogy	MPa
g	Acceleration due to gravity	m s^{-2}
g_s	Stomatal Conductance to water vapor	mol H_2O m^{-2} s^{-1}
$g_{s\text{-}ref}$	Reference stomatal conductance, at D=1 kPa	mol H_2O m^{-2} s^{-1}
$g_{s\text{-}ref}'m$	Maximum reference stomatal conductance, at D=1 kPa and 2000 PAR	mol H_2O m^{-2} s^{-1}
h	Height above the ground	m
I	Internode length, distance between adjacent leaves	m
$I_{AX,n}$	Water flow rate through axial pathway at node n	mmol H_2O s^{-1}
$I_{LT,n}$	Water flow rate through lateral pathway at node n	mmol H_2O s^{-1}
K_g	Conductance coefficient	kPa m^3 kg^{-1}
k_{pet}	Petiole hydraulic conductivity	mol H_2O m^{-1} s^{-1} MPa^{-1}
k_{plant}	Conductivity of the entire soil-to-leaf pathway	kg H_2O m^{-2} s^{-1} MPa^{-1}
k_s	xylem specific hydraulic conductivity	kg H_2O m^{-1} s^{-1} MPa^{-1}
k_{stem}	Stem specific hydraulic conductivity	kg H_2O m^{-1} s^{-1} MPa^{-1}
L	Path length	m
l_{pet}	Petiole length	m
n	Node number (distal node is number 1)	
PAR	Photosynthetically active radiation	μmol photons m^{-2} s^{-1}
$R_{AX,n}$	Resistance through axial pathway at node n	MPa s mmol H_2O^{-1}
R_I	Internode Resistance	MPa s mmol H_2O^{-1}
$R_{LT,n}$	Resistance through lateral pathway at node n	MPa s mmol H_2O^{-1}
R_{pet}	Petiole specific resistance	MPa s mmol H_2O^{-1}
R_{tot}	Resistance of the entire soil-to-leaf pathway calculated as a parallel circuit	MPa s mmol H_2O^{-1}

Table 1. Symbols, Units and Description of Variables cont'd

$\boldsymbol{T_A}$	Air Temperature	°C
$\boldsymbol{\Delta\Psi}$	Water potential difference between endpoints of the soil-to-leaf pathway	MPa
$\boldsymbol{\rho_w}$	Density of water	kg m^{-3}
$\boldsymbol{\Psi_l}$	Leaf water potential	MPa
$\boldsymbol{\Psi_s}$	Soil water potential	MPa

Table 2. A-C_i Results

Average Leaf Height (m)	CO_2 Compensation point (ppm)	Vcmax (µmol CO_2 m^{-2} s^{-1})
13.1±0.4	69.7±3.6	63.8±9.4
1.2±0.1	63.7±4.7	75.1±12.1

Table 2. Results from A-C_i analysis presented as mean±SE indicate no statistical difference between the upper leaves and the lower leaves on the vertical *P. lobata* vines in terms of CO_2 compensation point at 30 °C (t-test: p=0.34) and maximum rate of RuBisCO carboxylation (V_{cmax}; t-test, p=0.49). (N=24).

Table 3. Pressure-Volume Curve Analysis

Leaf Location	N	Ψ_{TLP} (MPa)	Ψ_{FT} (MPa)	ε_{max} (MPa)	Absolute Capacitance (g H_2O m^{-2} MPa^{-1})	Time Constant (s)
Top	6	-1.28±0.07[a]	-1.05±0.10	30.1±7.7	14.3±2.9	104.8±62.9
Base	6	-1.23±0.09[ab]	-1.01±0.11	19.7±2.9	24.4±5.8	53.3±23.2
Ground	8	-1.03±0.06[b]	-0.86±0.05	18.7±2.4	14.8±2.1	25.1±7.2

Table 3. Pressure-volume parameters shown as mean±SE for osmotic pressure at turgor loss point (Ψ_{TLP}), osmotic pressure at full turgor (Ψ_{FT}), maximum modulus of elasticity (ε_{max}), absolute capacitance per leaf area at full turgor, and time constant. No significant differences exist between the top, base, or ground vines for any parameters (p>0.1) except turgor loss point, which is more negative at the top of vertical vines than in leaves on horizontal stems (ANOVA, p=0.047).

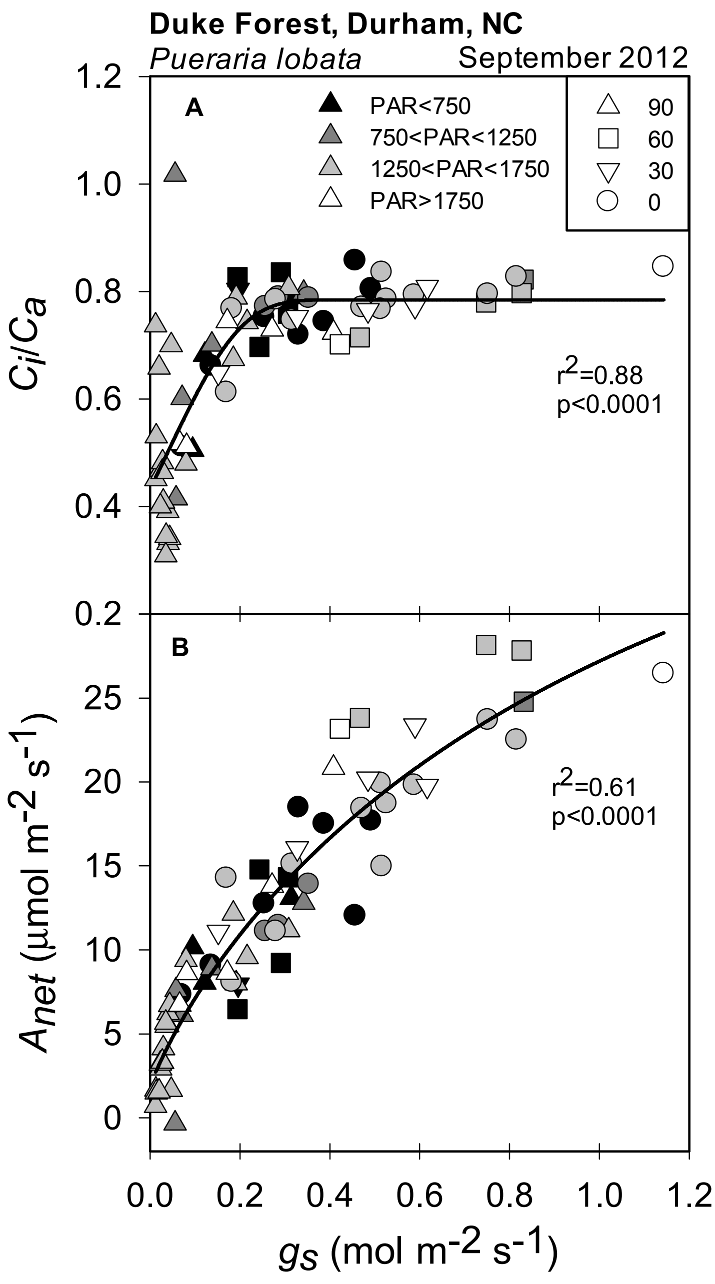

Fig. 1. Net rate of carbon assimilation (A_{net}) increases as stomatal conductance (g_s) increases. A_{net} increases at the fastest rate when C_i/C_a is also increasing. After C_i/C_a reaches its equilibrium (g_s >0.1), the increase in A_{net} is proportional to the increase in g_s.

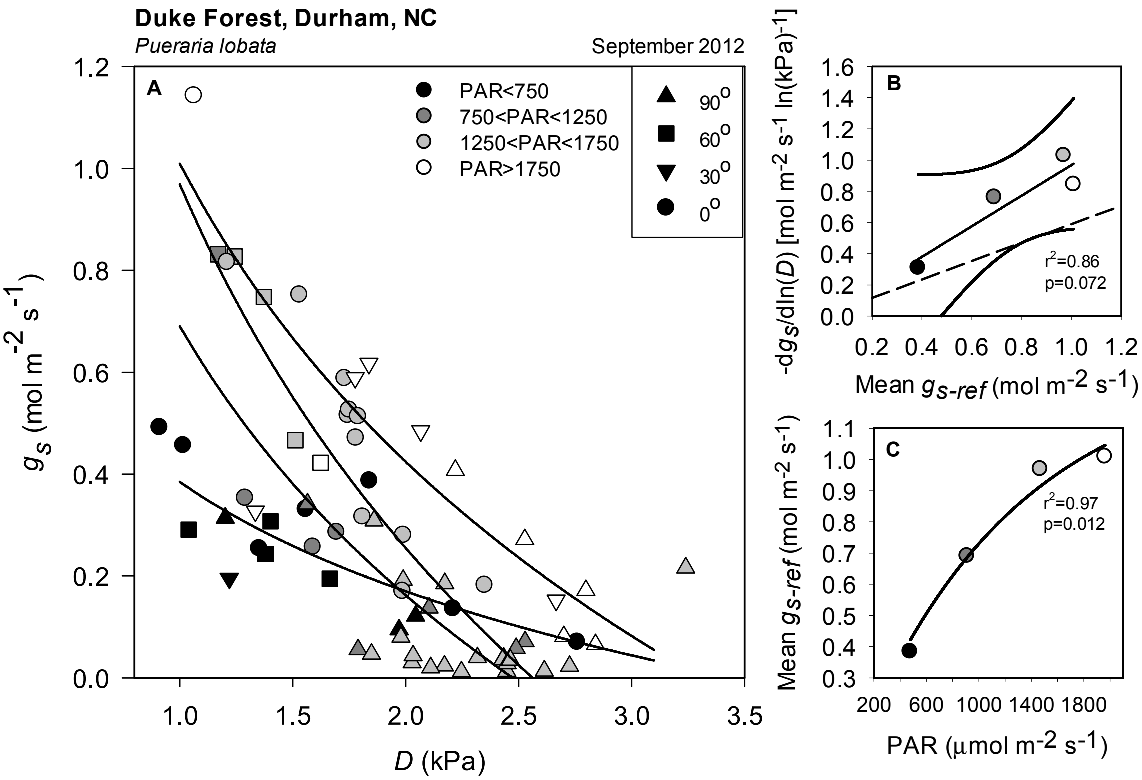

Fig. 2. Observed stomatal conductance (g_s) decreases as vapor pressure deficit (D) increases (Panel A). At low D, leaves exposed to high levels of radiation conduct water through the stomata faster than leaves exposed to low levels of radiation. Regressions are logarithmic best fits for each light class. Sensitivity of stomatal conductance to changes in D is illustrated in Panel B, such that as reference stomatal conductance increases, the sensitivity to D increases. Dashed line shows predicted sensitivity to D, with a slope of 0.59 (Oren et al., 1999). As radiation availability increases, the average reference g_s increases exponentially (Panel C), but reaches a maximum conductance at very high radiation.

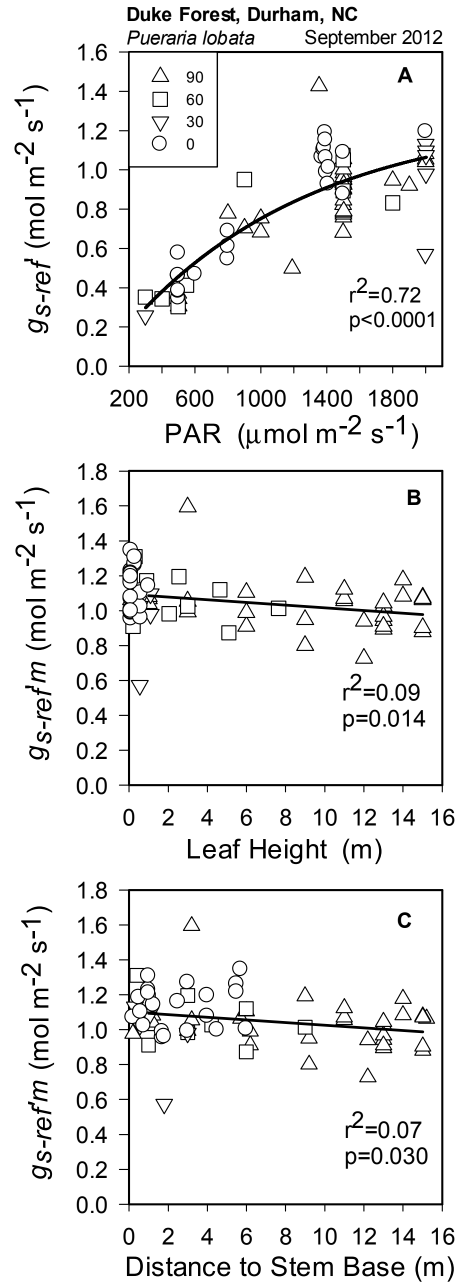

Fig. 3. Increase in reference stomatal conductance ($g_{s\text{-}ref}$) as light availability increases (Panel A). Maximum reference stomatal conductance ($g_{s\text{-}ref}$'m, assessed at PAR=2000 μmol m^{-2} s^{-1}) decreases slightly as leaf height (Panel B) and hydraulic path length increase (Panel C).

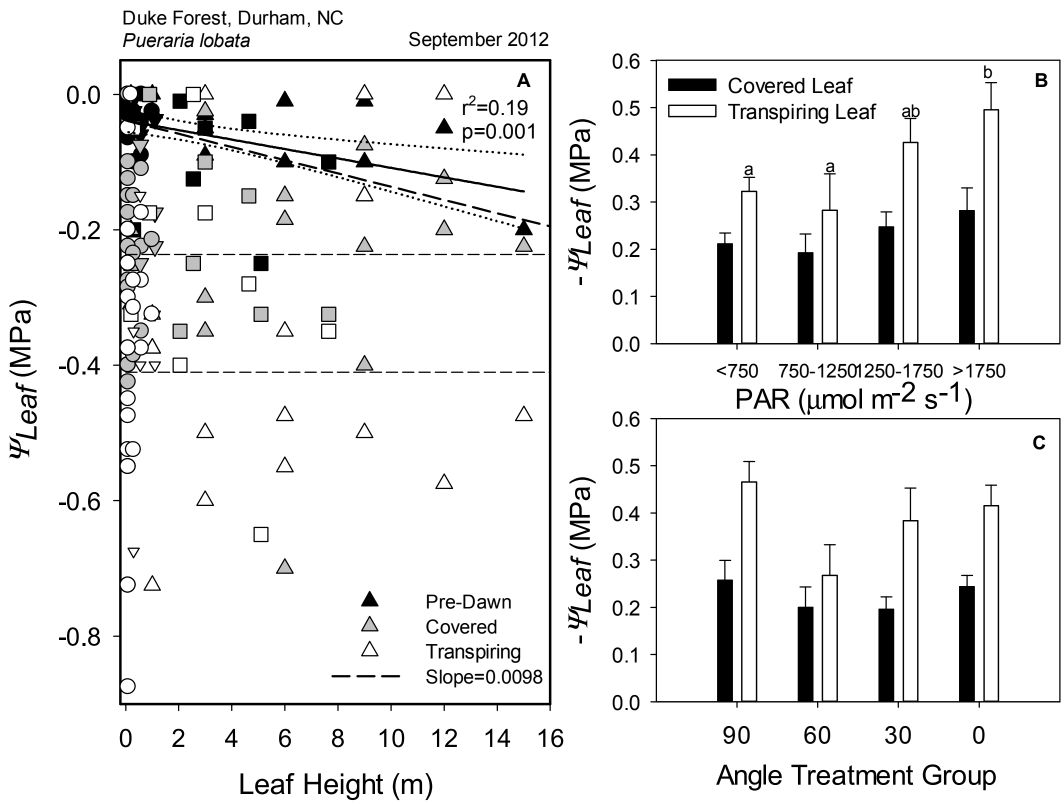

Fig. 4. Leaf water potential decreases further below zero as leaf height increases in the pre-dawn samples. The predicted slope for the pre-dawn group falls within the 95% confidence interval of linear regression (slope=0.007). No significant change in water potential for covered and open leaves at various leaf heights. Error bars +/- 1 SE.

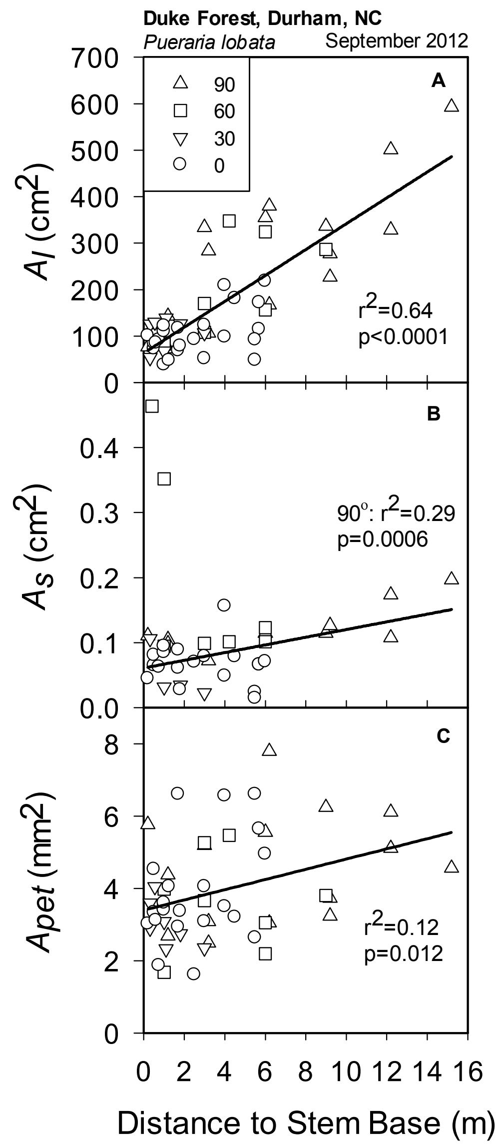

Fig. 5. Leaf area (A_l), stem xylem area (A_s), and petiole xylem area (A_{pet}) increase with respect to the distance from the base of the stem. In all treatment groups, A_l increases with leaf distance (Panel A), as does A_{pet} (Panel C). For A_s, positive relationship is significant in the 90 degree treatment group, but there is no trend in the horizontal group (Panel B).

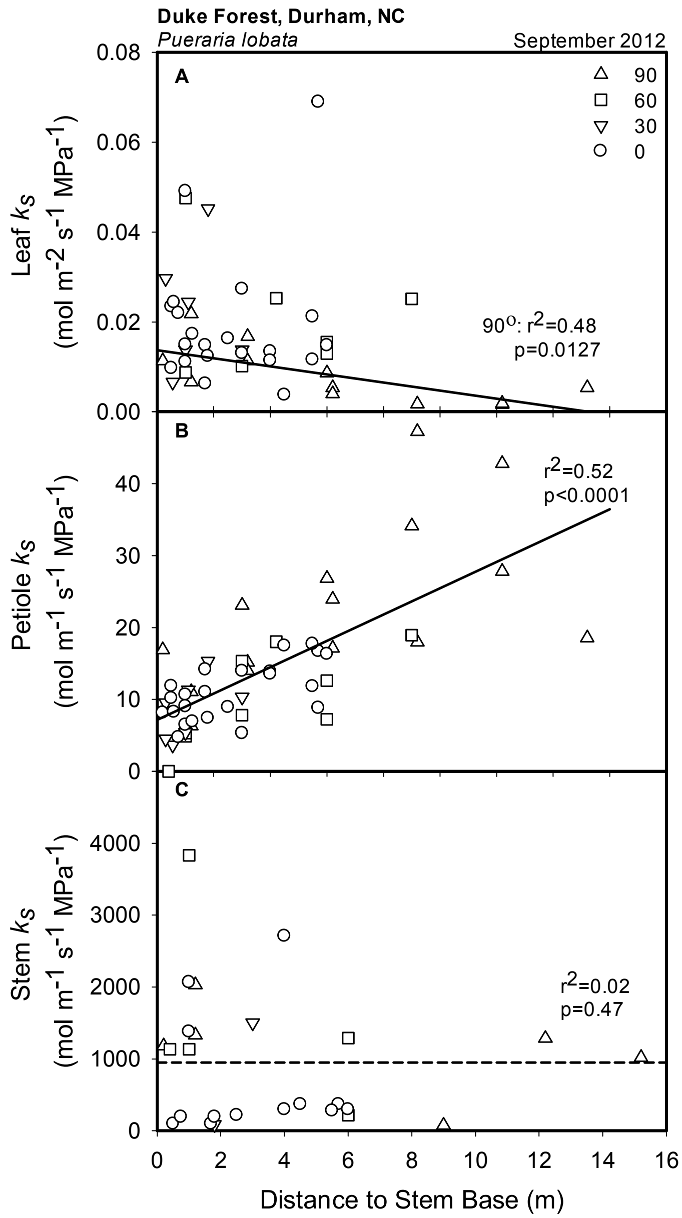

Fig. 6. Leaf conductance and petiole conductivity vary with respect to leaf position. In the 90 degree treatment group, leaf k_s decreases significantly as path length increases (Panel A). However, conductivity through the petiole increases with length (Panel B). Stem conductivity does not vary significantly with distance to stem base (Panel C).

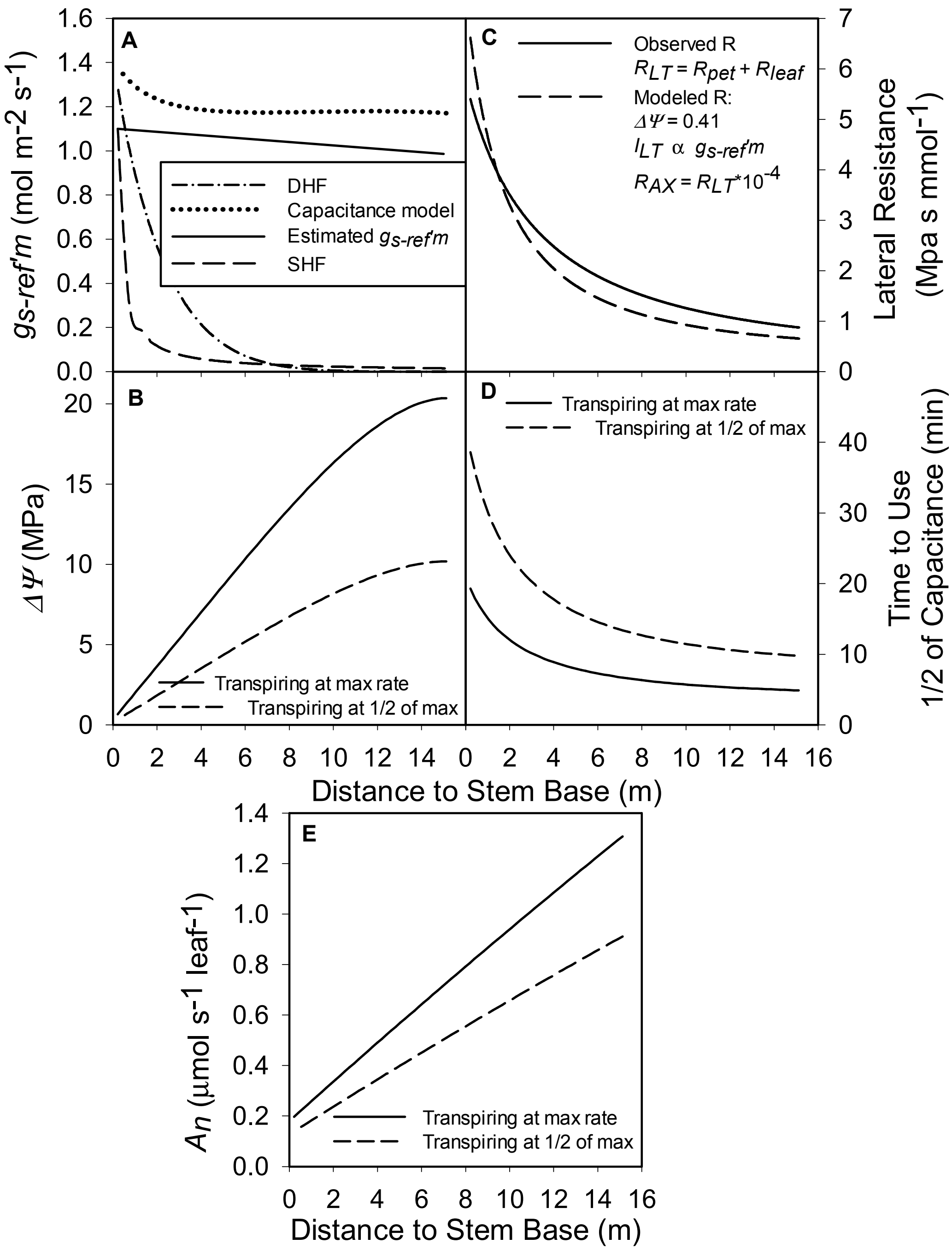

Fig. 7. Modeled and measured decline in max reference stomatal conductance ($g_{s\text{-}ref}'m$). Predicted decline in conductance according to SHF and DHF is not matched by observations (Panel A). The distribution of driving force required to maintain 90% of flow rate at most basal nodes in the most distal nodes (Panel B). When axial resistance is 4 orders of magnitude less than lateral resistance, lateral resistance required for flow at $g_{s\text{-}ref}'m$ (dashed line) is similar to measured resistance through

only local stem, petiole and leaf (solid line, Panel C). Net photosynthesis of each leaf when conducting at $g_{s\text{-}ref}'m$ (referenced to D=1 kPa and PAR=2000, Panel D). Amount of time that local capacitance can supply a leaf with sufficient water to transpire at two given rates representing $g_{s\text{-}ref}'m$ and half of this maximum conductance (Panel E).

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