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Atmospheric and soil drought reduce nocturnal conductance in live oaks

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Summary Nocturnal and daytime whole-canopy transpiration rate (E) and conductance ($g = E$/VPD, where VPD is leaf to air vapor pressure difference) were assessed gravimetrically in drought-treated and well-watered 3-year-old saplings of live oak species (*Quercus* series *Virentes* Nixon) from the southeastern USA (*Quercus virginiana* Mill.) and Central America (*Q. oleoides* Cham. & Schlecter). Our objectives were to: (1) quantify nocturnal and daytime E and g in a controlled environment; (2) determine the impact of severe drought on nocturnal E and g; and (3) examine whether unavoidable water loss through the epidermis could account for nocturnal water loss. We calculated daytime E during peak daylight hours (between 0930 and 1330 h) and nocturnal E during complete darkness (between 2200 and 0500 h). In addition to reducing E and g during the daytime, drought-treated plants reduced nocturnal E and g on a whole-canopy basis by 62–64% and 59–61%, respectively, and on a leaf-level basis by 27–28% and 19–26%, respectively. In well-watered plants, nocturnal g declined with increasing VPD, providing evidence for stomatal regulation of nocturnal transpiration. In drought-treated plants, g was low and there was no relationship between nocturnal g and VPD, indicating that water loss could not be reduced further through stomatal regulation. Both daytime and nocturnal g declined curvilinearly with predawn water potential for all plants, but nocturnal g was unrelated to predawn water potentials below –1 MPa. The reductions in daytime and nocturnal E and g during drought were associated with decreases in whole-plant and leaf hydraulic conductances. Observed nocturnal g was within the same range as epidermal conductance for oak species determined in previous studies under a range of conditions. Nocturnal E rose from 6–8% of daytime E for well watered plants to 19–20% of daytime E for drought-treated plants. These results indicate that, during drought, saplings of live oak species reduce g to a minimum through stomatal closure, and experience unavoidable water loss through the epidermis.

Keywords: epidermal conductance, hydraulic conductance, leaf hydraulic properties, nocturnal conductance, Quercus oleoides, Quercus virginiana, stomatal pore index.

Introduction

Despite the general assumption that stomata close at night in C_3 plants, there is growing evidence for nocturnal transpiration (Benyon 1999, Donovan et al. 1999, Feild and Holbrook 2000, Donovan et al. 2001, Matzner et al. 2001, Snyder et al. 2003, Bucci et al. 2005, Daley and Phillips 2006, Domec et al. 2006, Dawson et al. 2007, Fisher et al. 2007, Hubbart et al. 2007, Kavanagh et al. 2007, Scholz et al. 2007). This finding has challenged the widely held assumption that predawn plant water potential is in equilibrium with, and hence a good proxy for, soil water potential (Donovan et al. 2001, Donovan et al. 2003). It also prompts the question of whether nocturnal transpiration confers a functional benefit on the plant.

Because nocturnal transpiration rates are typically low, accurate quantification by instantaneous gas exchange measurements on small foliage samples is difficult (cf. Barbour et al. 2005). Estimation of nocturnal transpiration from sap flow measurements is prone to error due to flows of water into and out of tissue storage compartments. Daley and Phillips (2006) isolated the non-transpirational components of sap flow by means of a series of measuring probes distributed vertically up the tree, allowing them to quantify whole-tree nocturnal transpiration for three broad-leaved deciduous tree species in a mixed hardwood forest under mesic conditions. When recharge was accounted for, nighttime transpiration was 10% of total daily flux in paper birch, but was negligible in red oak and sugar maple.

In this study, we quantified nocturnal transpiration gravimetrically by measuring mass loss from large potted plants (Gaumann and Jaag 1936) . By measuring water loss by the entire plant canopy, short-term estimates of transpiration could be made accurately and independently of the movement of water into or out of tissue storage compartments.

If plants do not close their stomata fully at night, photosynthesis can begin without delay at sunrise when the leaf-to-air vapor pressure deficit (VPD) is low (Bucci et al. 2005, Dawson et al. 2007). However, it is unclear whether the positive effect that this will have on overall water-use efficiency outweighs the negative effect of nighttime water loss. Another possible reason for nocturnal transpiration is that sapflow is

necessary to supply oxygen to xylem parenchyma during the hours of darkness (Gansert 2003). A third possible explanation is that nocturnal transpiration promotes nutrient uptake by mass flow (e.g., Daley and Phillips 2006).

Nighttime water loss is not necessarily adaptive and may result from incomplete stomatal control. For example, Feild and Holbrook (2000) hypothesized that in *Drimys granadensis* L. nocturnal water loss is due to waxy plugs inside the stomata, which serve to reduce leaf wetting in wet forests, but which prevent complete stomatal closure. However, water loss through incompletely closed stomata has also been shown in detached *Sorghum bicolor* (L.) Moench. leaves placed in the dark in dry air (Muchow and Sinclair 1989). Significant nocturnal transpiration may also occur through the cuticle (Kerstiens 1996).

This study aimed to quantify nocturnal conductance in two closely related live oak species, whose native ranges encompass contrasting climatic regimes, and to determine the effects of low water availability on nocturnal water loss. We predicted that chronic drought would decrease nocturnal conductance and that, during drought treatment, nocturnal water loss would depend in part on VPD and stomatal aperture, and in part on cuticular permeability. We also examined the degree to which nocturnal transpiration is correlated with other water relations parameters, including hydraulic conductances and stomatal characteristics.

This study is part of a larger project examining the adaptive divergence of live oaks across their range from the temperate maritime forests in the southeastern USA to the dry tropics of Central America. Live oaks (*Quercus* series *Virentes* Nixon) form a small monophyletic lineage of interfertile evergreen oaks that occur in lowland and coastal areas (Muller 1942, Nixon et al. 1997). *Quercus virginiana* Mill. and *Q. oleoides* Cham. and Schlect. are the most broadly distributed species within the complex. *Quercus virginiana* extends from the outer banks of southern Virginia and North Carolina to northern Mexico, and *Q. oleoides* is a sister species that extends from northern Mexico to northwestern Costa Rica.

Materials and methods

Study plants

Seeds of *Q. virginiana* were collected in North Carolina (NC) and north central Florida (FL). Seeds of *Q. oleoides* were collected in Belize (BZ) and northwestern Costa Rica (CR).

Planting, experimental design and watering regime

Seeds were germinated in 27-cm Deepots (Steuwe & Sons, Inc., Corvallis, OR) and transplanted after one year to 15-l Treepots (Steuwe & Sons, Inc., Corvallis, OR) containing a 1:1 (v/v) mixture of sand and loam topsoil. Plants were grown in three replicated temperature-controlled chambers in greenhouses at the Franklinville Experimental Station in Franklinville, NY (42°18′ N, 78°33′ W), with the study plants randomly allocated across the three chambers. Greenhouse chamber temperatures varied between minimum and maximum set points that tracked the mean monthly temperatures in northwestern Costa Rica (Figure 1A). Relative humidity and temperature were monitored and vapor pressure deficit calculated every 60 s (Pearcy et al. 1989). Volumetric soil water content was monitored daily in a subsample of plant pots in each chamber by time domain reflectometry as described by Cavender-Bares and Holbrook (2001). A drought was imposed for > 3 months in the second and third summer of growth by watering every 3 to 5 days so as to maintain volumetric soil water content at about 7% in the drought treatment and 15% in the well-watered treatment (Figure 1B). Predawn leaf water potential values corresponded to soil water potentials from –1.5 to –2.3 MPa in the drought treatment and –0.05 to –0.3 MPa in the well-watered control. All reported measurements were made in the third growing season when plants were about 1.5 m tall.

Measurement of whole-plant transpiration and conductance

Whole-plant water loss was determined by placing each potted plant on a top-loading balance and recording the pot mass ± 0.1 g every 5 minutes for 24 h. Data were logged every 5 minutes. To prevent water loss through soil evaporation, the entire pot was placed inside a black plastic bag, which was sealed around the stems with adhesive tape. Plants were al-

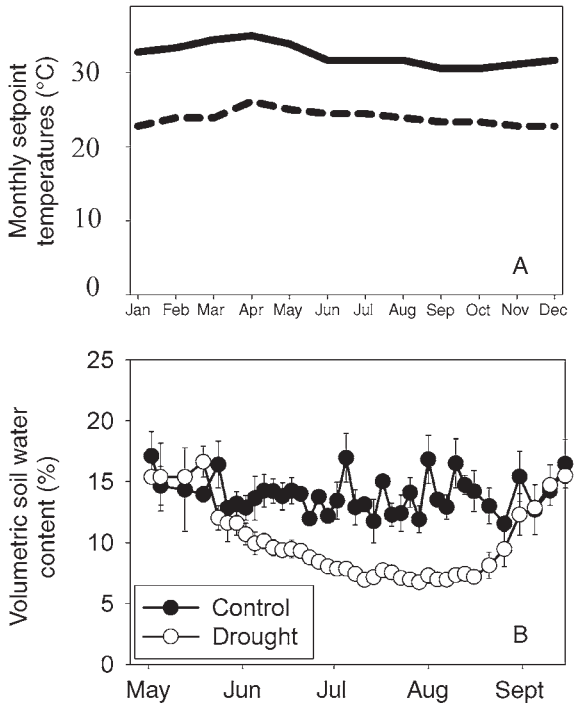

Figure 1. A: Monthly maximum and minimum temperature setpoints. B: A drought treatment was imposed from May–August, corresponding with the drought season in northwest Costa Rica. The volumetric soil water content of the control (●) and drought-treated (○) pots is shown for May–September 2005. Error bars are ± SE, *n*=24 per treatment.

ways measured two days after watering. A single plant was measured each day, and 30 plants were measured between early July and early August, alternating drought-treated and well-watered plants. Relative humidity varied over this period, but ANOVA showed that the vapor pressure deficit for nights when the drought-treated plants were measured were not significantly different from nights when the well-watered plants were measured.

Nocturnal transpiration rate (E) was measured as the mean total water loss per second between 2200 and 0500 h. Sunrise was between 0540 and 0600 h throughout the measurement period. Daytime transpiration was calculated as the mean water loss between 0930 and 1330 h. The whole-plant transpiration rates were divided by total leaf area (see below) to determine transpiration rate per leaf surface area. Total canopy conductance (g_t) was calculated as the molar loss of water per second (E), corrected for mass flow of water vapor through the stomata, divided by the mole fraction leaf-to-air vapor pressure difference ($w_l - w_a$) according to Pearcy et al. (1989):

$$g_t = \frac{E\left(1 - \frac{w_l + w_a}{2}\right)}{w_l - w_a} \quad (1)$$

where w_l is the molar concentration of water vapor within the leaf (mol H_2O mol air^{-1}) and w_a is the molar concentration of water vapor of the air. The w_l term was calculated assuming leaf temperature was equivalent to air temperature and that RH was 100% inside the leaves. The molar concentration of water vapor of the air (w_a) was calculated from relative humidity, temperature and total atmospheric pressure (97.8 kPa determined from elevation). The correction for mass flow represents a 2.7% decrease in the conductance value and allows direct comparison with measurements of instantaneous conductance (see below). Two circulation fans in the greenhouse compartment where the plants were measured maintained well-mixed air and a minimal boundary layer so that total canopy conductance should approximate canopy stomatal conductance.

Measurement of leaf and stem water potential

Leaf water potential (Ψ_{leaf}) was measured with a Scholander pressure chamber (Soil Moisture Equipment Co., Santa Barbara, CA), generally six times over the course of the 24-h period including at night (2100–2200 h), predawn (0400–0500 h), midmorning (0900–1000 h), midday (1200–1300 h), mid-afternoon (1500–1600 h) and evening (2000–2100 h). At midday, water potential was measured for a leaf that had been enclosed in parafilm and covered with reflective aluminum foil the previous evening. This measurement was taken as an estimate of the water potential in the stem subtending the leaf (Ψ_{stem}, Brodribb and Holbrook 2003). After measuring its water potential, the fresh mass of each excised leaf was determined, as well as the mass of the parafilm and aluminum foil, and these values were used to correct for mass loss.

Measurement of whole-plant hydraulic and leaf hydraulic conductance

Whole-plant leaf-specific hydraulic conductance (K_{plant}) was calculated as daytime transpiration per unit leaf area divided by the leaf-to-soil water potential difference: $E/(\Psi_{leaf} - \Psi_{soil})$, with Ψ_{soil} estimated from predawn Ψ_{leaf}. This calculation uses the Ohm's Law analogy, which assumes steady-state transpiration (Nardini and Salleo 2000), an assumption that is most valid during the midmorning and midday periods (0930–1330 h). It also assumes that, at predawn, the plant canopy is in water potential equilibrium with the soil, so that soil water potential can be estimated from predawn leaf water potential. Two factors that may contribute to predawn disequilibrium between the soil and leaves are nighttime transpiration and accumulation of apoplastic solutes in leaves (Donovan et al. 1999, Donovan et al. 2001). In drought-treated plants, breaks in the soil–root hydraulic contact may also contribute to predawn disequilibrium between leaves and soil (Nobel and Cui 1992), and would again lead to an underestimate of soil water potential. To determine whether there was a significant drop in water potential across the leaf at predawn (0400–0500 h) (Bucci et al. 2005), we measured predawn Ψ_{leaf} and Ψ_{stem} for all plants on one day at the end of the experiment (August 11).

Leaf hydraulic conductance (K_{leaf}) was calculated as transpiration per unit leaf area divided by the difference in water potential from the leaf to the stem: $E/(\Psi_{leaf} - \Psi_{stem})$. Transpiration rate per unit leaf area was determined from mass loss, averaged over 1-h before the water potential measurements.

Measurement of leaf area

Leaf area (LA) of individual leaves was estimated from leaf length (LL). Leaves of a range of sizes from about 30 plants of each species (about 90 leaves per species) were harvested and measured for length (nearest mm) and for leaf area with a LI-1000 portable leaf area meter (Li-Cor, Lincoln, NE). The tightest curve fit resulted from power laws, with different relationships for the two species: for *Q. oleoides* $LA = 0.4355(LL^{1.8386})$, $R^2 = 0.92$; and for *Q. virginiana* $LA = 0.2867(LL^{1.9201})$, $R^2 = 0.91$. Before putting a plant on the balance, the length of every leaf was measured and the area of each leaf and the plant estimated.

Measurement of instantaneous gas exchange

Instantaneous transpiration and stomatal conductance were measured between 0900 and 1130 h on individual leaves from a total of 30 plants (68 plants per species in each treatment) over a 2-week period with an open gas exchange system (LI-6400, Li-Cor). A cuvette fan minimized boundary layer resistance, which was estimated for hypostomatous leaves, and removed from the calculation of stomatal resistance by the instrument software. The clear cuvette top allowed measurement of gas exchange under ambient conditions of irradiance, temperature, CO_2 and RH for comparison with the mass loss method.

Measurements of stomatal traits

Stomatal densities and apertures were measured on impressions from abaxial nail varnish peels. These peels were taken between the midrib and the leaf margin. If present, trichomes were removed with a razor blade. Leaves were sampled from the top of the canopy of each plant. Digital images of five locations per peel were captured with SPOT Advanced software (Diagnostic Instruments, Sterling Heights, MI) with an Olympus BX50 microscope. Two stomatal pores were measured per image at a magnification of 400×, giving a total of 10 pore lengths per leaf. Stomatal density was measured at a magnification of 200× with an Image J cell counter. An index of total stomatal pore area per unit leaf area (SPI) was calculated as stomatal density × (pore length)^2 (Sack et al. 2003, Sack et al. 2006).

Statistical analysis

The effect of species and watering treatments on the measured variables were evaluated by analyses of variance (ANOVA) as main fixed effects using Data Desk v. 5.0 software (Velleman 1995; Data Description, Ithaca, NY). Correlates of nighttime and daytime transpiration and conductance were determined by Pearson product-moment correlations. Analysis of covariance (ANCOVA) was used to determine if relationships for well-watered and drought treatments differed significantly (Sokal and Rohlf 1995), with vapor pressure difference, canopy leaf area, daytime transpiration and daytime K_{leaf} treated as covariates, and daytime and nocturnal g and E as dependent variables. If slopes and intercepts did not differ, a single regression line was fit to the combined data.

Results

Species differences

During the morning, instantaneous gas exchange rates were significantly higher in *Q. virginiana* than in *Q. oleoides* in the control treatment but values were similar in drought-treated plants (Table 1). There was a greater drop in water potential across the leaf ($\Psi_{leaf} - \Psi_{stem}$) of well-watered plants of *Q. virginiana* than of *Q. oleoides*. Other measured traits did not differ significantly between species. Differences among populations within species were generally not detected, although, in *Q. oleoides*, the length of the stomatal aperture was significantly greater in the Costa Rica population (14.4 ± 4.3 μm control, 13.7 ± 2.9 μm drought-treated) than in the Belize population (12.5 ± 0.86 μm control, 12.4 ± 3.2 μm drought-treated).

Diel patterns

Diel time courses (Figure 2) indicated that well-watered (control) plants of both species had significantly higher daytime and nighttime transpiration rates, conductances and predawn and midday leaf water potentials than drought-treated plants (Table 1). Compared with drought-treated plants, well-watered plants showed a greater drop in water potential across the leaf ($\Psi_{leaf} - \Psi_{stem}$) at midday, corresponding to higher transpiration rates (Table 1).

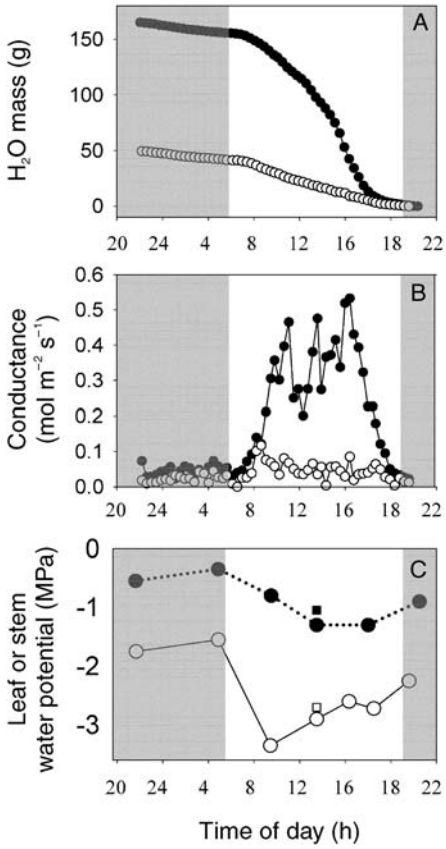

Figure 2. Representative examples of the diel course of transpirational water loss (A), total conductance to water vapor per unit leaf area (B) and leaf or stem water potential (C) for a drought-treated (open symbols) and well-watered (closed symbols) plant. Symbols: leaf (○,●); and stem (□,■).

Leaf area

Well-watered plants had a 74% greater total leaf surface area than drought-treated plants (Table 1), a result of higher absolute growth rates (as assessed by aboveground measures of stem diameter and stem height), not leaf loss, relative to drought-treated plants. There was no significant difference in the % leaf drop (arcsin transformed) between the treatments based on leaf counts (data not shown).

Nighttime transpiration and conductance of the whole-plant canopy were significantly correlated with total canopy leaf surface area (see Figure 5A). The correlation was equally strong for daytime transpiration (not shown).

Nighttime transpiration and conductance

Nighttime transpiration rates were significantly lower for drought-treated plants than for well-watered plants for both species (Table 1). As expected, nighttime transpiration rates were driven by evaporative demand, as indicated by the dependence of nocturnal E on nighttime VPD for all plants combined ($r = 0.56$, $P < 0.001$, data not shown). Slopes for the dependence of nocturnal transpiration on nocturnal VPD did not

Table 1. Comparison of morphology, leaf and stem water status, water loss, epidermal conductance and hydraulic properties for drought-treated and well-watered *Quercus virginia* and *Quercus oleoides*. Values are means ± SE. Significant effects of treatment (Tmt) and species (Spp) from ANOVA (total df = 29) are indicated by asterisks: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; and ms, $P < 0.09$. The Spp × Treatment interaction was not significant for any variable except morning conductance, for which $P = 0.03$. Morning transpiration and conductance were measured at the leaf level with a gas exchange system; all other transpiration and conductance measurements were assessed gravimetrically.

	Q. virginiana		*Q. oleoides*		Tmt	Spp
	Control	Drought	Control	Drought		
Plant morphology						
Canopy leaf area (m^2)	0.205 ± 0.016	0.120 ± 0.016	0.255 ± 0.018	0.135 ± 0.016	***	ms
Specific leaf area ($cm^2\ g^{-1}$)	83.8 ± 2.8	98.1 ± 10.8	89.0 ± 4.0	90.8 ± 3.2		
Stomatal pore index	0.165 ± 0.01	0.094 ± 0.006	0.15 ± 0.010	0.127 ± 0.013	***	
Stomatal density (mm^{-2})	934 ± 50	705 ± 71	822 ± 61	723 ± 60	**	
Stomatal aperture length (μm)	13.3 ± 0.43	11.6 ± 0.40	13.6 ± 0.51	13.2 ± 0.31	*	
Leaf and stem water status						
$Y_{PD\ leaf}$ (MPa)	−0.47 ± 0.10	−2.33 ± 0.43	−0.40 ± 0.04	−1.67 ± 0.19		
$Y_{MD\ leaf}$ (MPa)	−1.93 ± 0.15	−3.51 ± 0.31	−2.03 ± 0.20	−3.29 ± 0.19		
$Y_{MD\ stem}$ (MPa)	−1.38 ± 0.11	−3.14 ± 0.28	−1.57 ± 0.15	−3.04 ± 0.19		
$Y_{MD\ stem}$ - $Y_{MD\ leaf}$ (MPa)	0.62 ± 0.13	0.37 ± 0.09	0.46 ± 0.11	0.26 ± 0.07		
Water loss						
Daytime canopy transpiration ($mmol\ s^{-1}$)	0.384 ± 0.083	0.044 ± 0.004	0.446 ± 0.053	0.046 ± 0.005	***	
Daytime transpiration per leaf area ($mmol\ m^{-2}\ s^{-1}$)	2.00 ± 0.45	0.41 ± 0.08	1.73 ± 0.18	0.38 ± 0.06	***	ms
Morning transpiration ($mmol\ m^{-2}\ s^{-1}$)	3.641 ± 1.009	0.579 ± 0.143	2.184 ± 0.405	0.69 ± 0.196	***	*
Nocturnal canopy transpiration ($mmol\ s^{-1}$)	0.0214 ± 0.0012	0.0083 ± 0.0008	0.0248 ± 0.0025	0.0084 ± 0.0014	***	
Nocturnal transpiration per leaf area ($mmol\ m^{-2}\ s^{-1}$)	0.111 ± 0.013	0.074 ± 0.011	0.098 ± 0.009	0.070 ± 0.015	**	
Nocturnal transpiration as % of daytime transpiration	8.4 ± 1.6	19.9 ± 2.9	6.3 ± 1.1	18.5 ± 2.3	***	
Epidermal conductance						
Daytime conductance ($mol\ m^{-2}\ s^{-1}$)	0.1081 ± 0.0200	0.0181 ± 0.0034	0.0818 ± 0.0031	0.0228 ± 0.0033	***	ms
Morning conductance ($mol\ m^{-2}\ s^{-1}$)	0.1665 ± 0.0653	0.0135 ± 0.0033	0.0644 ± 0.0151	0.0199 ± 0.0065	***	*
Nocturnal conductance ($mol\ m^{-2}\ s^{-1}$)	0.0111 ± 0.0011	0.0080 ± 0.0010	0.0092 ± 0.0011	0.0075 ± 0.0012	*	
Nocturnal diffusion (permeance) ($m\ s^{-1} \times 10^5$)	13.8 ± 1.25	9.94 ± 1.13	11.46 ± 1.31	9.35 ± 1.41	*	
Nocturnal conductance as % of daytime conductance	12.2 ± 1.9	48.3 ± 6.6	10.8 ± 1.0	34.3 ± 4.3	***	ms
Hydraulic properties						
Whole plant hydraulic conductance ($mol\ s^{-1}\ MPa^{-1}$)	0.30 ± 0.059	0.073 ± 0.035	0.28 ± 0.035	0.035 ± 0.004	***	
Whole plant leaf specific hydraulic conductance ($mol\ m^{-2}\ s^{-1}\ MPa^{-1}$)	1.57 ± 0.26	0.74 ± 0.44	1.11 ± 0.13	0.31 ± 0.06	**	
Leaf hydraulic conductance ($mmol\ m^{-2}\ s^{-1}\ MPa^{-1}$)	4.43 ± 0.54	1.65 ± 0.52	3.71 ± 0.74	2.21 ± 0.62	**	

differ significantly between treatments ($P > 0.05$; ANCOVA).

In well-watered plants, daytime conductance was independent of VPD but nocturnal conductance declined with increasing VPD (Figures 3A and 3B). In contrast, in drought-treated plants, daytime conductance decreased with increasing VPD but nocturnal conductance did not (Figures 3C and 3D)

Daytime conductance declined exponentially with predawn leaf water potential, the decline being steeper in well-watered plants than in drought-treated plants (Figure 4A). Nighttime conductance (and nighttime transpiration, data not shown) also declined with predawn leaf water potential. The slope of this relationship was much steeper for the well-watered plants than for the drought-treated plants (Figure 4B).

Nighttime transpiration rates were 1.5 times higher in well-watered plants than in drought-treated plants on a leaf-area basis. If whole-canopy nighttime transpiration rates are considered, well-watered plants had 2.5 times greater nighttime transpiration rates than drought-treated plants because of their greater total leaf area (see below). Daytime transpiration rates were 4.8 and 9.1 times higher in well-watered plants than in drought-treated plants when calculated on a leaf area and whole-canopy basis, respectively. Hence, the difference in transpiration rates between the drought-treated and well-watered plants was much higher during the day than at night.

Total canopy transpiration at night was a function of total leaf area (Figure 5A). Nighttime transpiration and conductance on a leaf area basis were correlated with daytime transpiration and conductance (Figure 5B). For *Q. virginiana* and *Q. oleoides*, nighttime (2200–0500 h) transpiration rates were 18.5 and 20% of the daytime rates, respectively, for the drought-treated plants and 6 and 8% of the daytime rates, respectively, for the well-watered plants (Table 1). The higher proportion of nighttime to daytime transpiration for the drought-treated plants was a reflection not of higher absolute nocturnal transpiration rates, but of greater relative decline in transpiration rate from day to night in well-watered plants.

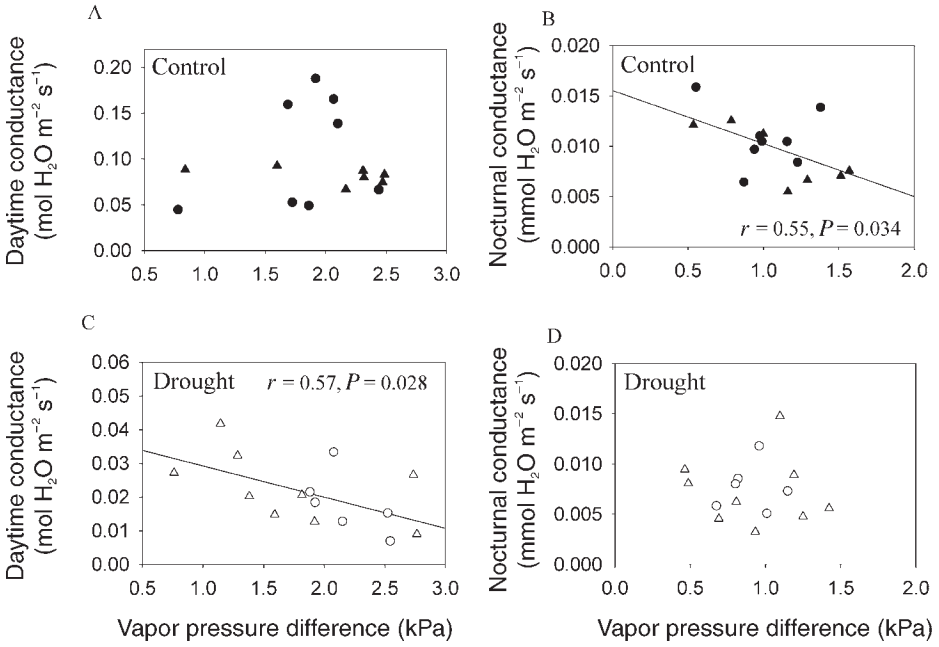

Figure 3. Nocturnal conductance decreases with increasing leaf-to-air vapor pressure difference in well-watered (control) plants (B), but there is no relationship in drought-treated plants (D). Daytime conductance does not vary with vapor pressure difference in well-watered (control) plants (A), but in drought-treated plants daytime conductance decreases with increasing leaf-to-air vapor pressure difference (C). Open symbols correspond to plants in the drought treatment and closed symbols to plants in the well-watered treatment for the live oak species *Q. virginiana* (○,●) and *Q. oleoides* (△,▲).

Daytime instantaneous stomatal conductance

Daytime instantaneous stomatal conductance, as measured with an LI-6400 gas exchange system on individual leaves, was similar to values from mass loss (Table 1). Daytime conductance, as measured by mass loss, decreased by 83% (*Q. virginiana*) and 72% (*Q. oleoides*) in the drought treatment. Similarly, morning stomatal conductance measured with the gas exchange system decreased by 91 and 69%, respectively. In the instantaneous measurements, estimated boundary layer resistance is subtracted from total resistance to water vapor in the calculation of stomatal conductance (LI-6400 manual). The close correspondence of total conductance based on mass loss with stomatal conductance indicates that boundary layer resistance was minimal during the experiment.

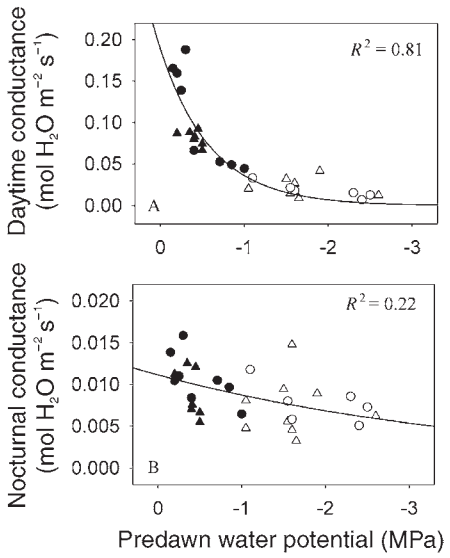

Figure 4. Daytime conductance (A) and nocturnal conductance (B) decrease exponentially with decreasing predawn water potential. Note the difference in scale on the *y*-axes of the two graphs. Open symbols correspond to plants in the drought treatment and closed symbols to the well-watered treatment for the two live oak species *Q. virginiana* (○,●) and *Q. oleoides* (△,▲).

Stomatal aperture length, density and SPI

Stomatal aperture length decreased by 7% in the drought treatment relative to the well-watered treatment, but the difference was only marginally significant. Stomatal density, however, decreased by almost 20% in the drought treatment (Table 1). These changes in aperture length and density resulted in a mean decrease of 29% in stomatal pore index (Figure 6A). Nocturnal conductance declined by about the same proportion as stomatal pore index (Table 1, Figure 6B).

Whole-plant hydraulic conductance and leaf hydraulic conductance

Whole-plant hydraulic conductance was 5.5 times higher in well-watered plants than in drought-treated plants. For well-watered and drought-treated plants the pressure difference across the plant at midday ($\Psi_{MD} - \Psi_{PD}$) was similar (Table 1) and comparable with other oak species under field conditions with varying water availability (e.g., Gebre et al. 1998, Reich and Hinckley 1989, Cavender-Bares and Bazzaz 2000). Thus, the higher K_{plant} for well-watered plants corresponded to a higher transpiration rate, driven by the greater aboveground growth (not shown) and leaf surface area, and higher transpiration rates per unit leaf area. Whole-plant leaf-specific hydraulic conductance was also significantly higher (2.6 times) in

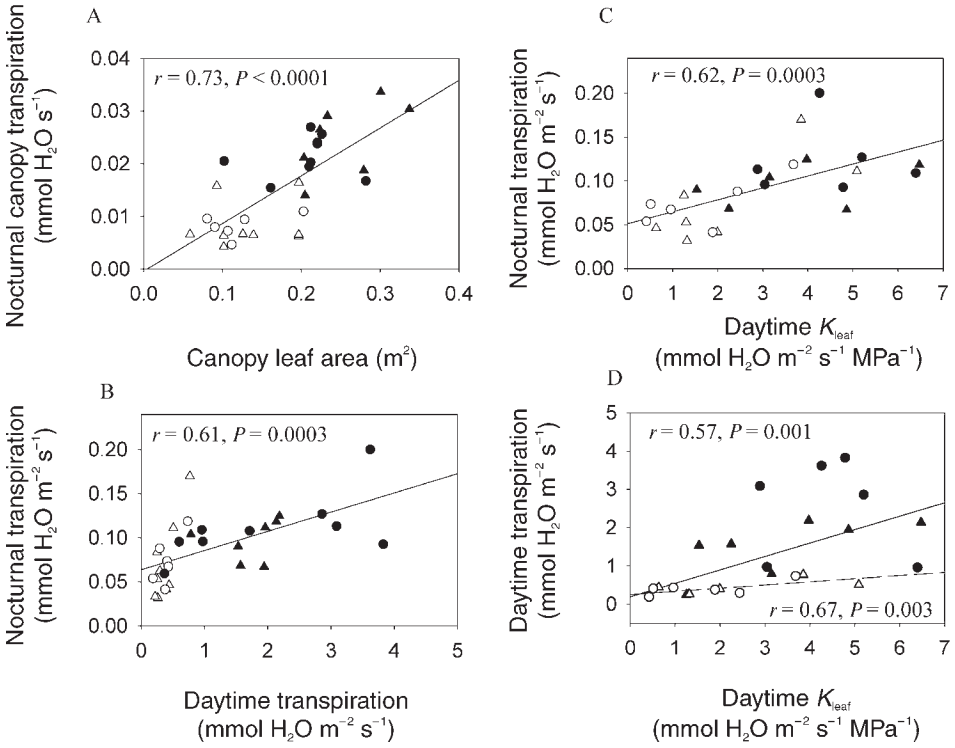

Figure 5. Correlates of nocturnal transpiration. Nocturnal whole canopy water loss is correlated with total canopy leaf area (A); nocturnal transpiration rate per leaf area is correlated with daytime transpiration rate (B), and with leaf hydraulic conductance (K_{leaf}) (C). For comparison, the relationship between daytime transpiration and K_{leaf} is shown (D). While there is a significant relationship, the daytime conductance values for the drought-treated plants show a tighter correspondence with K_{leaf} (dashed line) than the combined data (solid line). Daytime transpiration rates of the well-watered plants alone are not significantly correlated with K_{leaf}. Open symbols represent drought-treated plants and closed symbols represent well-watered plants of the live oak species *Q. virginiana* (○,●) and *Q. oleoides* (△,▲).

well-watered plants than in drought-treated plants (Table 1). Leaf hydraulic conductance was 2.1 times higher in well-watered plants than in drought-treated plants. Nocturnal transpiration and conductance were significantly correlated with daytime leaf hydraulic conductance (Figure 5C) and total canopy conductance. Nocturnal and daytime conductances were correlated with whole-plant hydraulic conductance (not shown).

Predawn disequilibrium between leaves and stems

Drought-treated and well-watered plants showed significant differences in predawn leaf water potential, measured throughout July and early August. At the end of the experiment, all plants were measured for both leaf and stem water potential. The differences between the treatments remained significant, although the overall values were higher owing to recent watering on the day of measurement (August 11; Table 2). Mean values of predawn leaf and stem xylem water potential for drought-treated plants averaged across the two species were –1.59 MPa and –1.49 MPa, respectively, indicating a 0.11 MPa drop across the leaf at predawn. For well-watered plants, there was a difference of only 0.02 MPa. The mean predawn water potential was 3–5% higher for the covered leaves relative to the uncovered leaves in all plants (Table 2), which

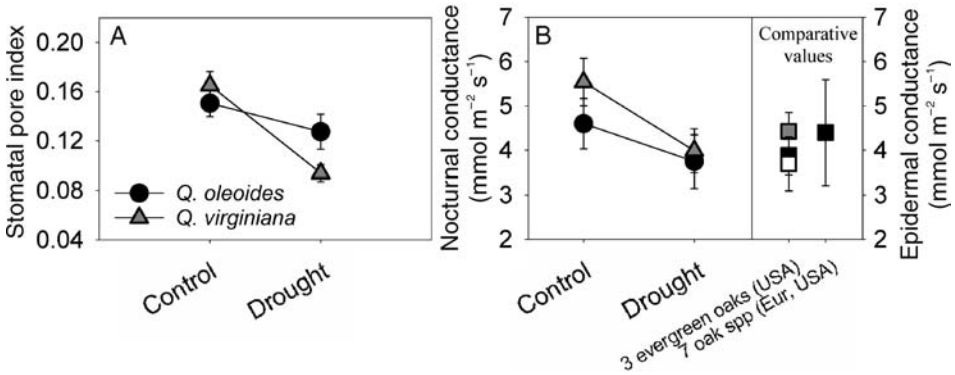

Figure 6. A: Stomatal pore index for the well-watered control and drought-treated *Q. oleoides* and *Q. virginiana* sapling leaves. B: Nocturnal conductance, calculated for a two sided leaf, compared to epidermal conductance for three evergreen oak species measured on detached leaves in a separate study (Cavender-Bares 2000) and to the mean value of epidermal conductance for 7 European and American oaks (Kerstiens et al. 1996). Symbols: *Q. oleoides* (●); *Q. virginiana* (▲); *Q. myrtifolia* (□); *Q. virginiana* (■) and *Q. geminata* (■). Error bars are ± SE.

Table 2. Mean predawn leaf and stem water potentials (MPa) and the absolute and percent difference between them. Values are means for *Quercus virginiana* and *Quercus oleoides* considered together. Significant treatment effects are indicated by asterisks: ***, $P < 0.001$; and *, $P < 0.05$. Abbreviation: WW, well-watered.

August 11	Drought	SE	WW	SE	Significance
Ψ_{PDleaf}	−1.59	0.18	−0.34	0.03	***
$\Psi_{PDxylem}$	−1.49	0.16	−0.32	0.02	***
$\Psi_{PDxylem} - \Psi_{PDleaf}$	0.11	0.03	0.02	0.02	*
% Difference	5.5	1.6	3.1	4.7	

was not statistically different from 0. The water potential drop across the leaf was relatively small compared with the range of values reported by Donovan et al. (2001) for 21 species that were well-watered, and with a range of species reported in the literature where nighttime transpiration was documented (e.g., 0.4 MPa in woody tropical savanna species (Bucci et al. 2004)), suggesting that, in our study, predawn leaf water potential provided a good estimate of soil water potential.

Discussion

Quantification of nighttime rates of water loss demonstrated that nighttime transpiration occurs in *Q. virginiana* and *Q. oleoides,* but at rates of only 8 and 6% of daytime rates in well-watered plants, respectively (Table 1). Nighttime transpiration of drought-treated plants declined in absolute terms, but not as much as daytime transpiration, and thus nocturnal transpiration of drought-treated plants as a percentage of daytime transpiration rose to nearly 20% for both species. The increased proportion of nocturnal transpiration relative to daytime transpiration in drought-treated plants suggests that nocturnal transpiration represents water loss that is not under direct control of the plants.

Our results indicate that nocturnal transpiration is regulated by the plant within a narrow range by variation in stomatal and cuticular resistance. However, some nocturnal water loss is unavoidable and, during drought, nocturnal transpiration was strictly a function of VPD, reflecting a close correspondence between nighttime conductance and epidermal conductance previously reported in seven oak species (Kerstiens 1996, Cavender-Bares 2000) (Figure 6B). Our values for nighttime conductance (recalculated as permeability rates) fall within published cuticular and epidermal permeability rates across a wide range of taxa (Kerstiens 1996).

Decreased nocturnal conductance in drought-treated plants

There are several plausible explanations for the decreased nocturnal conductance in response to drought. The first is that drought induces a reduction in stomatal pore area (Figure 6A). Epidermal resistance (reciprocal of conductance) includes the contribution of cuticular resistance and of stomatal resistance in parallel as well as boundary layer resistance in series (Figure 7). Boundary layer resistance is likely to be minimal, based on similar absolute rates of total conductance, measured gravimetrically, and instantaneous stomatal conductance (Table 1). Both diffusion through the cuticle and mass flow through incompletely closed stomata result in water loss (Kerstiens 1996, Pearcy et al. 1989). Drought-treated plants showed evidence of acclimation to lower water availability by producing leaves with a lower SPI, which had lower maximum stomatal conductance when stomata were open, and may have been less leaky when closed (Jordan et al. 2004, Muchow and Sinclair 1989).

A second explanation for reduced nocturnal conductance in drought-treated plants is that they have less permeable leaf cuticles than well-watered plants. However, a new technique for distinguishing between gas phase diffusion through stomatal pores and solid phase diffusion through the cuticle found that water flux across the astomatous cuticle on the adaxial surface of *Hedera helix* leaves was an order of magnitude lower than that across the stomatous abaxial leaf surface (Santrucek et al. 2004). The findings of Santrucek et al. (2004) suggest that the

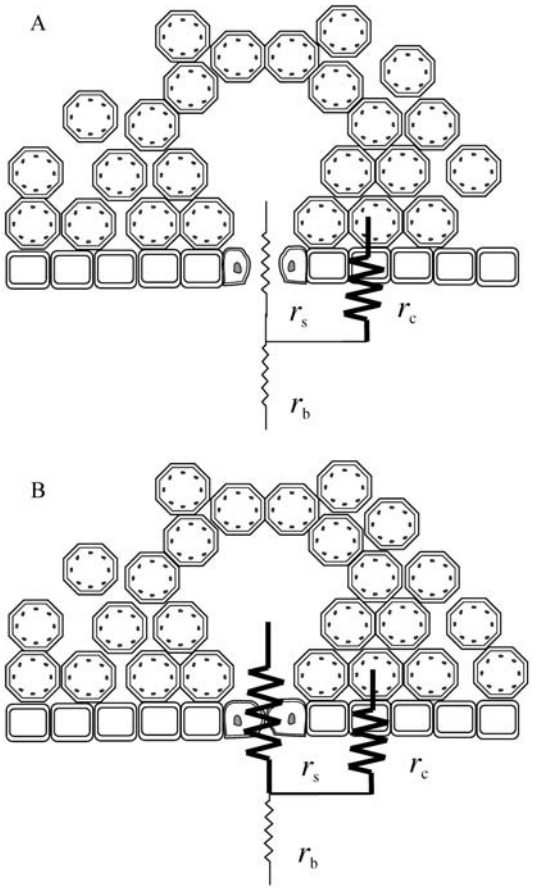

Figure 7. Schematic of resistances (reciprocal of conductance) to the diffusion of water vapor across the leaf surface when stomata are open (A) and closed (B) (adapted from Pearcy et al. 1989). Cuticular resistance (r_c) and stomatal resistance (r_s) are in parallel with each other and in series with boundary layer resistance (r_b). When stomata are open, stomatal and boundary layer resistances are much lower than cuticular resistance. When stomata are closed, both stomatal and cuticular resistances are high, making boundary layer resistance inconsequential.

most likely pathway for nocturnal water flux is through the stomata (Figure 7B). A lower surface area to volume ratio of leaves of drought-treated plants might also contribute to a lower nocturnal conductance if leaves of drought-treated plants have a reduced epidermal surface through which water could diffuse relative to leaf tissue volume (Hadley and Smith 1990). Our data do not support this explanation, however, because drought had no effect on specific leaf area in either species (Table 1). A fourth possible explanation for the lower nocturnal conductance observed in drought-treated plants is that the air surrounding the epidermal and mesophyll cell walls inside the leaves of drought-treated plants was drier than within leaves of well-watered plants. Although the assumption that air near the cell walls is saturated under a range of conditions is well-supported (e.g., Sharkey et al. 1982, Pearcy et al. 1989), lower vapor pressure inside the leaf would reduce the driving force for diffusion across the epidermis, resulting in an apparently lower nocturnal conductance (Canny and Huang 2006).

Correlates of nocturnal transpiration and conductance

Nighttime transpiration and conductance were correlated with daytime transpiration and conductance, as previously found for genotypes of a conifer species (Jordan et al. 2004). Additionally, nocturnal transpiration and conductance were correlated with daytime K_{leaf} (Figure 5C) for the study species in both the well-watered and the drought treatment. Nighttime transpiration and conductance were also dependent on leaf water status, although much less so than daytime transpiration and conductance (Figure 4). Thus, for the study plants, nocturnal and daytime conductances were related to stomatal pore area, which was correlated with K_{leaf}, as found previously for sets of temperate and tropical tree species (Sack et al. 2003, Sack et al. 2005). This linkage would have arisen because the drought-treated plants showed acclimation to lower water availability by reducing stomatal pore area with a concomitant reduction in K_{leaf}. The latter would presumably have been driven by xylem blockage as a result of embolism or tylose formation, or by acclimation of xylem anatomy, or both (Nardini and Salleo 2005).

Our findings indicate that nocturnal transpiration in live oak species is limited. Further, although additional investigations are needed to determine whether nighttime transpiration is adaptive in some plants, we show that unavoidable water loss may occur through the leaf epidermis. Distinguishing between unavoidable water loss and potentially adaptive water movement and loss at night via partially open stomata will be an important step in increasing our understanding of nocturnal transpiration.

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