

Magnitude of nighttime transpiration does not affect plant growth or nutrition in well-watered *Arabidopsis*

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Received 1 December 2008; revised 24
January 2009

doi: 10.1111/j.1399-3054.2009.01216.x

Significant water loss occurs throughout the night via partially open stomata in many C₃ and C₄ plant species. Although apparently wasteful in terms of water use, nighttime transpiration (E_{night}) is hypothesized to benefit plants by enhancing nutrient supply. We tested the hypothesis that plants with greater E_{night} would have improved plant nutrient status and greater fitness, estimated as pre-bolting biomass, for *Arabidopsis thaliana*. Two very different levels of E_{night} were generated in plants by exposing them to high vs low nighttime leaf-to-air vapor pressure deficits (VPD_{leaf}) in controlled environment chambers. An assessment of responses of nighttime leaf conductance (g_{night}) to VPD_{leaf} indicated that E_{night} differed by at least 80% between the treatments. This large difference in E_{night} , imposed over the entire vegetative growth phase of *Arabidopsis*, had no effect on leaf nutrient content (N, Ca, K) or pre-bolting rosette biomass. The lack of response to differences in E_{night} held true for both a high and a low nitrogen (N) treatment, even though the low N treatment decreased leaf N and biomass by 40–60%. The N treatment had no effect on g_{night} . Thus, higher E_{night} did not provide a nutrient or growth benefit to *Arabidopsis*, even when the plants were N-limited.

Introduction

Nighttime leaf conductance (g_{night}) is observed in many C₃ and C₄ plants and can result in significant water loss without carbon gain (reviewed in Caird et al. 2007a). While the widespread occurrence of significant g_{night} has been shown in many recent studies (Bucci et al. 2004, 2005, Caird et al. 2007b, Cavender-Bares et al. 2007, Daley and Phillips 2006, Fisher et al. 2007, Howard and Donovan 2007, Hubbart et al. 2007, Kavanagh et al. 2007, Ludwig et al. 2006, Scholz et al. 2007, Seibt et al. 2007, Snyder et al. 2003, 2008), little is known

about the actual costs or benefits of incomplete stomatal closure at night. The implications of g_{night} and resulting nighttime transpiration (E_{night}) include consequences for plant water relations and mineral nutrition, which can ultimately influence plant productivity or fitness (reviewed in Caird et al. 2007a, Dawson et al. 2007, Marks and Lechowicz 2007, Scholz et al. 2007, Snyder et al. 2008). For example, high E_{night} results in reduced predawn plant water status (Bucci et al. 2004, Cavender-Bares et al. 2007, Donovan et al. 2003, Fisher et al. 2007, Hubbart et al. 2007, Kavanagh et al. 2007, Moore et al. 2008) and represents water loss without

Abbreviations – $\delta^{13}\text{C}$, leaf carbon isotope composition; A, daytime assimilation; E_{day} , daytime transpiration; E_{night} , nighttime transpiration; g_{day} , daytime leaf conductance; g_{night} , nighttime leaf conductance; PPF, photosynthetic photon flux density; RH, relative humidity; VPD_{leaf} , leaf-to-air vapor pressure deficit.

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simultaneous carbon gain, which could represent a cost to plants in water-limiting environments.

Alternatively, some of the consequences of high g_{night} and E_{night} may be beneficial to plants. For example, supply of soil-mobile nutrients (e.g., nitrate, NO_3^-) to roots is known to be affected by transpiration-driven mass flow of water toward roots (Barber 1995, Nye and Tinker 1977). E_{night} -driven water flux could thus provide a benefit to plants by enhancing nutrient supply to roots (Caird et al. 2007a, Scholz et al. 2007, Snyder et al. 2008). In addition, E_{night} may additionally affect distribution or supply of nutrients throughout the plant. Theoretical support for these hypotheses has come from circumstantial evidence of the general effects of transpiration on mineral nutrient supply and uptake (Masle et al. 1992, Nye and Tinker 1977, Polley et al. 1999), and modeling work using the Barber–Cushman model (Barber and Cushman 1981, M. Christman, unpublished). Empirical support has come from evidence of reduced nutrient accumulation when stomatal conductance and transpiration are reduced due to elevated CO_2 (McDonald et al. 2002) and previous studies where increased post-harvest fruit quality was linked to increased Ca supply when nighttime humidity is low in greenhouses (Armitage and Tsujita 1979), conditions which allow E_{night} to occur. More recently, Snyder et al. (2008) found decreased ^{15}N uptake when E_{night} was suppressed in plants under manipulated field conditions. However, they were unable to determine if mass flow supply driven by E_{night} affected the ^{15}N uptake or if the result was caused by a secondary effect of the E_{night} suppression treatment (e.g., effects on daytime gas exchange).

The leaf-to-air vapor pressure deficit (VPD_{leaf}) is the gradient driving transpirational water loss. Thus, if nighttime VPD_{leaf} is small, very little water loss will occur despite high g_{night} . Under such conditions, incomplete stomatal closure may have little effect on the plant. Conversely, under conditions of high nighttime VPD_{leaf} , incomplete stomatal closure and thus high g_{night} results in increased water loss (E_{night}) relative to if stomata were more fully closed. Stomata respond to changes in VPD_{leaf} during the daytime, closing as VPD_{leaf} increases and thus reducing excess water loss. Several studies have observed negative correlations between nighttime VPD_{leaf} and sap flux (Benyon 1999, Bucci et al. 2004, Cavender-Bares et al. 2007, Daley and Phillips 2006, Dawson et al. 2007, Fisher et al. 2007, Kavanagh et al. 2007, Marks and Lechowicz 2007), suggesting that the nighttime response of stomata to VPD_{leaf} is similar to that during the day. This was subsequently confirmed with experimental manipulations of VPD_{leaf} for *Ricinus communis* (Barbour and Buckley 2007), although the

sensitivity of stomatal responses to VPD_{leaf} was lower at night than during the day. Despite such regulatory ability, high g_{night} is observed in plants when nighttime VPD_{leaf} is high enough to cause substantial water loss.

Under well-watered conditions, nighttime water loss may not present any substantial cost to the plant. Instead, plants with higher E_{night} may have an advantage by increased nutrient supply because water is not limiting. This may be particularly important for plants in nutrient-limiting, but water-sufficient conditions. However, whether plants regulate the magnitude of g_{night} to increase E_{night} and thus increase nutrient supply is unclear. Various studies have found conflicting responses of g_{night} to nutrient limitations. In two field studies, g_{night} was higher in N-limited than N-sufficient plants (*Helianthus anomalus*, Ludwig et al. 2006, savanna trees, Scholz et al. 2007). However, N nutrition had no effect on g_{night} in four *Helianthus* species in a greenhouse study (Howard and Donovan 2007). In studies where g_{night} was affected by nutrition, the direct effect of nighttime water loss on nutrient content of plant tissues was not quantified. Thus it is possible that even if there is a benefit of nutrient acquisition from E_{night} , it may only be a passive benefit and not one regulated by plants in response to nutrient availability.

This study investigated whether there is a nutrient benefit to losing water at night by manipulating nighttime VPD_{leaf} to generate two very different levels of E_{night} . *A. thaliana* was chosen because it is known to have high g_{night} and E_{night} (Christman et al. 2008, Lasceve et al. 1997), its small stature enabled the use of a large number of plants within growth chambers, and its short life cycle enabled manipulation of nighttime VPD_{leaf} over the entire vegetative phase of its life cycle. However, the propensity for *Arabidopsis* to bolt when subjected to drought conditions limited this study to investigating the effects of nighttime water loss on nutrient supply solely under well-watered conditions. Our primary objectives were to determine whether (1) higher E_{night} enhances plant nutrition under non-limiting water availability, and (2) the enhancement in plant nutrition by E_{night} affects plant fitness estimated as vegetative biomass. Two N treatments were included to investigate whether nutritional status affects the magnitude of g_{night} and to provide a more robust test of the effect of E_{night} on plant nutrient status and growth. We hypothesized that N-limited plants would benefit more from high E_{night} than N-sufficient plants because additional N uptake would be more important for N-limited than N-sufficient plants.

Materials and methods

Plant material

Seeds of *A. thaliana* (L.) Heynh. (Brassicaceae) accession Kas-1 were obtained from the Arabidopsis Biological Resource Center (www.arabidopsis.org) and subsequently propagated by single seed descent. Seeds were planted in 1:1 sand and fritted clay (Turface Pro-League, Profile Products, Buffalo Grove, IL, USA) to allow for maximum nutrient control and water retention. Pots consisted of a 50 ml centrifuge tube with the bottom cut off and the soil-filled tube 'planted' into the top of a soil-filled 164-ml Conetainer (Stuewe and Sons, Corvallis, OR, USA) to allow added volume for root growth; previous studies showed that roots penetrated the entire 150 ml of soil volume in the Conetainers (see Juenger et al. 2005, McKay et al. 2001). A small hole was made in the centrifuge tube cap for seeding. After seeds were planted, pots were watered and placed in the dark in a cold room at 4°C for 1 week. Pots were initially transferred to a growth chamber with a 12-h photoperiod: daytime conditions 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density (PPFD), 23°C and 65% relative humidity (RH); nighttime conditions 20°C, 55% RH. Germination dates were recorded and pots were watered daily. Approximately 7–8 days after germination, at the time of first true leaf formation, seedlings were randomly assigned to the experimental treatments.

Experimental design

The experimental design was a split-plot design with nighttime VPD_{leaf} treatment as the main plot factor and N treatments as the subplot factor. A total of five growth chambers were used in the experiment (Fig. 1). One chamber (day chamber) was maintained with constant light conditions: 330 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD, 23°C, and 65% RH; all plants were placed in this chamber for 12 h each day. Four additional chambers (night chambers) were set at constant 20°C and no light (constant dark). Two of these night chambers had 95% RH (low VPD_{leaf} treatment, <0.1 kPa) and two were set at 50% RH (high VPD_{leaf} treatment, approximately 0.7 kPa). The 95% RH level was employed to reduce E_{night} as much as possible using the growth chambers we had available, whereas the 50% RH level produced conditions which are more typical for growing *Arabidopsis* and would create a much higher level of E_{night} . Thus, we chose these levels so we could compare the typical effect, if any, of E_{night} on nutrient supply with the case where this effect was minimized as much as possible. Plants were manually switched between the day chamber and the appropriate night chamber every 12 h. The use of

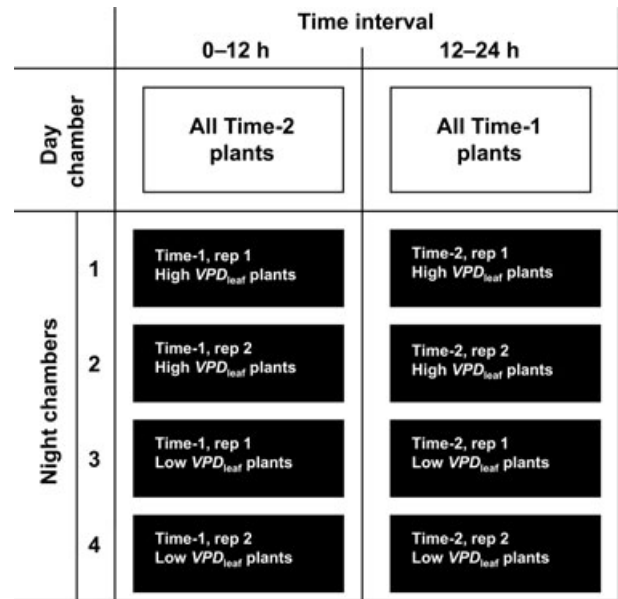


Fig. 1. Diagram illustrating the experimental design. The experiment was a split-plot design with nighttime leaf-to-air vapor pressure deficit (VPD_{leaf}) as the main plot and N treatment as a subplot factor. Each 'rep' consisted of 10 individual plants (subsamples); each night chamber thus held 10 plants per N treatment at any given time. Plants were moved between day and night chambers every 12 h so that all plants experienced daytime conditions in the same chamber. The experiment was repeated twice in time to achieve four replicates per treatment; using a 12-h photoperiod enabled the time repetitions to occur simultaneously. See text for more details of experimental design.

a single day chamber provided the most consistent daytime conditions (light, temperature, etc.) possible, and allowed two 'trials' (Time-1 and Time-2) to proceed simultaneously. That is, Time-1 plants were in the day chamber while Time-2 plants were in the night chambers, and vice versa (Fig. 1).

Each night chamber (VPD_{leaf} treatment) held 10 plants per N treatment, which were treated as subsamples and averaged as a single replicate. Thus, in each trial the four night chambers provided two replicates per VPD_{leaf} treatment, and using two trials increased replication of each VPD_{leaf} treatment to four.

Watering occurred only when plants were placed in the day chamber so that humidity in the night chambers was not affected by watering; preliminary trials had indicated that watering substantially changed night humidity in the chambers. Pots were brought to field capacity at each watering. Each time plants were transferred to the day chamber, they were randomly arranged to minimize positional effects within that chamber. Temperature, RH, and PPFD in the growth chambers were monitored with portable 'weather' stations with calibrated sensors (HMP45D, Vaisala, Inc,

San Jose, CA, USA; LI-190, LI-COR, Inc., Lincoln, NE, USA) and a CR10x datalogger (Campbell Scientific, Inc, Logan, UT, USA).

The two N treatments began at the same time as the nighttime VPD_{leaf} treatments. N treatments were modified 1/4-strength Hoagland's solution containing either high or low amounts of N in solution. Only nitrate (10 vs 2.5 mM, NO₃⁻) and Ca (5 vs 1.25 mM) concentrations differed in the solutions. The lower nitrate concentration was previously shown to produce N limitation in *Arabidopsis* and the higher concentration assured sufficient N for these plants (Loudet et al. 2003). Concentrations for all other nutrients were: 1.5 mM K; 0.5 mM P; 0.75 mM S; 0.25 mM Mg; 12.5 μM Cl; 6.25 μM B; 0.5 μM Mn; 0.5 μM Zn; 0.125 μM Cu; 0.125 μM Mo; 125 μM Fe. Fertilization was provided in 10 ml additions three times a week on alternate days after watering. Observation as well as previous experiments using this setup indicated nutrients were uniformly available throughout the rooting zone.

Gas exchange measurements

Whole-rosette gas exchange was measured with a LI-6400 portable photosynthesis system (LI-COR, Inc., Lincoln, NE, USA) and a custom-made whole-plant *Arabidopsis* cuvette designed to seal around the top of the centrifuge tubes used for plant growth (described in Juenger et al. 2005, McKay et al. 2001). The small hole in the cap of the tube was blocked by the rosette, and previous tests indicated that there is no effect of soil water loss or soil respiration on rosette gas exchange measurements. A thermocouple within the chamber measured leaf temperature for one fully expanded leaf within the rosette. After the gas exchange measurements were completed for an individual plant, total rosette leaf area was obtained from a digital photograph of the canopy and image analysis using Scion Image Software program (Scion Corporation, Frederick, MD, USA).

We measured the effect of nighttime VPD_{leaf} and N treatments on daytime gas exchange [assimilation (*A*), leaf conductance (*g*_{day}), and transpiration (*E*_{day})], for all treatment combinations, but nighttime gas exchange could only be measured for the high VPD_{leaf} treatment. The LI-6400 cannot be reliably operated at the 95% RH associated with the low VPD_{leaf} treatment; condensation will occur within the analyzer beginning at 85% humidity, which makes measurements under such conditions unreliable (see LI-6400 manual). For the low VPD_{leaf} treatment, *g*_{night} and *E*_{night} were estimated from VPD_{leaf} response curves (see

next paragraph). For gas exchange measurements, LI-6400 cuvette conditions were 400 mmol s⁻¹ flow rate and 400 μmol mol⁻¹ CO₂, with temperature and RH tracking ambient growth chamber conditions. Light in the cuvette was 300 μmol m⁻² s⁻¹ PPFD for daytime measurements and 0 μmol m⁻² s⁻¹ PPFD for nighttime measurements. Measurements were made over two consecutive 24-h periods so that each trial (Time-1 and Time-2) had daytime and nighttime measurements performed twice. Three different plants from each time-trial × rep × VPD_{leaf} × N treatment combination were sampled at each measurement period, giving six total measurements per time-trial × rep × VPD_{leaf} × N treatment combination, which were treated as subsamples and averaged as a single replicate.

Because the LI-6400 could not be operated at the low VPD_{leaf} treatment humidity level, we used the relationship between VPD_{leaf} and *g*_{night} to estimate *E*_{night} and thereby confirm that the treatments were successful at producing two very different levels of *E*_{night}. The effect of VPD_{leaf} on *g*_{night} and *E*_{night} was determined on the night preceding harvest of the experiment. At the beginning of the night (dark) period, six high-N-treatment plants from each of the VPD_{leaf} treatments (12 plants total) were randomly rearranged into three dark growth chambers, each with the same temperature (20°C) but different RH (approximately 40, 60, 80% RH). Plants and stomata were allowed to adjust for 2 h before gas exchange measurements were made with the LI-6400 with cuvette conditions set the same as for nighttime measurements described above, including RH and temperature tracking ambient conditions for each growth chamber. Following a set of measurements early in the night, the 12 plants were reassigned randomly so that each plant would be subjected to a new RH level and thus new VPD_{leaf}. This second time period for measurements was employed to account for effects of potentially confounding endogenous circadian rhythms or overnight recovery of plant water status separate from the effects of VPD_{leaf} on *g*_{night}. After a further 2 h for stomatal adjustment, gas exchange measurements were made. Overall, this provided two sets of 12 measurements of *g*_{night} and *E*_{night}, with VPD_{leaf} in the cuvette ranging from approximately 0.4 to 1.3 kPa. The relationship of *g*_{night} to VPD_{leaf} was fitted with the modified Lohammar's function:

$$g_{\text{night}} = -m \times \ln \text{VPD}_{\text{leaf}} + b$$

as in Oren et al. (1999). The relationship of *g*_{night} and *E*_{night} as

$$E_{\text{night}} = \text{VPD}_{\text{leaf}} \times g_{\text{night}}$$

can thus be rearranged to:

$$E_{\text{night}} = e^{(b/m)} \times g_{\text{night}} \times e^{-(1/m)g_{\text{night}}}.$$

g_{night} and E_{night} for our low and high VPD_{leaf} treatments (approximately 0.1 and 0.7 kPa, respectively) were calculated from these relationships once m and b were fitted to the data.

Growth and nutrition measures

Plants were harvested just prior to bolting, following 3 weeks of nighttime VPD_{leaf} treatments. Each rosette was weighed for fresh biomass, dried at 60°C, and weighed for dry biomass ($n = 4$, with 10 subsamples per replicate). Roots from two plants of each time-trial \times rep \times VPD_{leaf} \times N treatment combination were extracted from the soil, cleaned, dried at 60°C, and weighed. Root mass ratio was calculated as root dry weight over total (root plus shoot) dry weight. Rosette or total biomass prior to bolting were used as proxies for plant fitness because these traits are good indicators of potential seed production in *Arabidopsis*, especially within a single genotype (Korves et al. 2007, Mitchell-Olds 1996, J.K. McKay *pers. comm.*). Two fully expanded leaves from each plant were ground and analyzed for total leaf N (%) and carbon isotope composition ($\delta^{13}\text{C}$, ‰) at the UC Davis Stable Isotope Facility (<http://stableisotopefacility.ucdavis.edu>). Leaf $\delta^{13}\text{C}$ reflects water use efficiency integrated over the lifetime of the leaf (Ehleringer et al. 1992, Farquhar et al. 1989), with higher (less negative) values indicating greater water use efficiency. Remaining leaves were combined and ground for leaf cation content. Ground tissue was dry-ashed at 475°C for 4 h, digested with 1 M HCl, and the extract analyzed for cations (Ca, K) by atomic emission spectroscopy (AAAnalyst 200, Perkin-Elmer, Wellesley, MA, USA).

Statistics

The experimental design called for a split-plot analysis with nighttime VPD_{leaf} treatment as the main plot factor and N treatment as the subplot factor. Daytime gas exchange characters (g_{day} , E_{day} , A) and plant growth/nutrition variables (shoot and root biomass, root mass ratio, $\delta^{13}\text{C}$, and leaf N and cation contents) were compared using 4-way ANOVAs (PROC GLM, SAS Institute, Inc., Cary, NC, USA) with time, VPD_{leaf} treatment, N treatment, and night chamber as fixed effects. Nighttime gas exchange parameters (g_{night} , E_{night}), which were only measured for the high VPD_{leaf} treatment, were compared using 3-way ANOVAs with time, night chamber, and N treatment as fixed effects.

Due to the split-plot design, night chamber was nested within VPD_{leaf} treatment and the error term for the effect of VPD_{leaf} was the between night chamber variation. The regressions for VPD_{leaf} and g_{night} were performed separately on data from the early and late measurement periods and compared in SAS using PROC SYSLIN.

Results

g_{night} and E_{night} responses to VPD_{leaf} treatments

Nighttime gas exchange was not measured for plants in the low VPD_{leaf} treatment because the LI-6400 cannot be operated at very high humidity. To provide a basis for estimating differences in E_{night} between VPD_{leaf} treatments, we determined the relationships between nighttime VPD_{leaf} and g_{night} for plants in each VPD_{leaf} treatment. In response to experimentally decreasing VPD_{leaf} at night, g_{night} increased but the change in g_{night} did not compensate for the reduction in VPD_{leaf} and E_{night} still decreased (Fig. 2). The relationship of g_{night} with VPD_{leaf} did not differ between measurement periods for plants within the high or low VPD_{leaf} treatments [$F(1,8) = 0.16$, $P = 0.70$ and $F(1,8) = 0.00$, $P = 0.95$, respectively]. The relationship for g_{night} and VPD_{leaf} was also not different between background VPD_{leaf} treatments during the early measurement period [$F(1,8) = 0.22$, $P = 0.65$], but there was a marginally significant difference during the late measurement period [$F(1,8) = 5.42$, $P = 0.05$], with plants from the high VPD_{leaf} treatment showing greater sensitivity (steeper slope) to VPD_{leaf} than plants from the low VPD_{leaf} treatment.

To confirm differences in E_{night} between VPD_{leaf} treatments, we calculated values of g_{night} and E_{night} for both VPD_{leaf} treatments using the relationships obtained during the first measurement period, which coincided with the times of actual gas exchange measurements performed on high VPD_{leaf} treatment plants. Using the modified Lohammar's relationship for g_{night} vs VPD_{leaf} from each VPD_{leaf} treatment, respectively, we calculate that plants in the low VPD_{leaf} treatment had g_{night} approximately $0.171 \text{ mol m}^{-2} \text{ s}^{-1}$ while we estimate g_{night} was $0.090 \text{ mol m}^{-2} \text{ s}^{-1}$ for high VPD_{leaf} treatment plants, which is slightly higher than actual measured values ($0.076 \text{ mol m}^{-2} \text{ s}^{-1}$; Table 1). From these approximations of g_{night} , we estimate E_{night} in the low VPD_{leaf} treatment was $0.11 \text{ mol m}^{-2} \text{ s}^{-1}$ and that in the high VPD_{leaf} treatment was $0.60 \text{ mmol m}^{-2} \text{ s}^{-1}$. Therefore, although g_{night} was approximately 89% higher in the low VPD_{leaf} treatment, E_{night} was still approximately 81% lower compared to the high VPD_{leaf} treatment. Even if a linear fit was used for g_{night} vs VPD_{leaf} for each VPD_{leaf} treatment—a physiologically unlikely

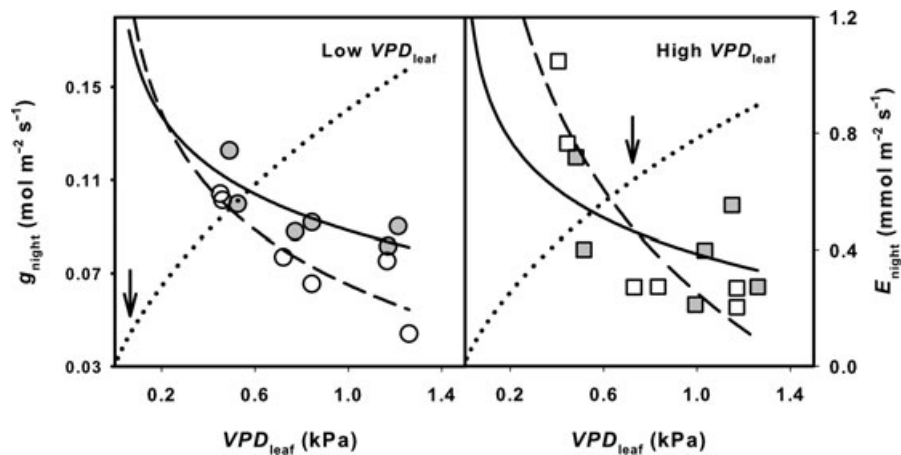


Fig. 2. Response of leaf conductance (g_{night} ; solid and dashed lines for early and late data, see below) and transpiration (calculated E_{night} for the early data, dotted line) to leaf-to-air vapor pressure deficit (VPD_{leaf}) in *Arabidopsis*. Six plants from each VPD_{leaf} treatment were each measured twice during the night, each time after 2-h exposure to a different VPD_{leaf} (circles are for low VPD_{leaf} plants; squares are for high VPD_{leaf} plants). Filled symbols are from measurements early in the night; open symbols are from measurements late in the night. Solid lines represent Lohammar function fits for data obtained at the early (first) measurement and dashed lines represent fits from the late (second) measurement period. The dotted line is E_{night} calculated from the Lohammar function for g_{night} from the first measurement period. Arrows indicate values of E_{night} corresponding to each VPD_{leaf} treatment.

Table 1. Whole-plant gas exchange characteristics, dry biomass of shoot and root, root mass ratio, $\delta^{13}\text{C}$, and leaf nutrient content for *Arabidopsis* plants grown with high or low nighttime leaf-to-air vapor pressure deficit (VPD_{leaf}) treatments and high or low nitrogen (N) treatments during their entire vegetative growth phase [data are means (SE), $n = 4$]. Abbreviations: assimilation (A), daytime leaf conductance (g_{day}), daytime transpiration (E_{day}), nighttime leaf conductance (g_{night}), and nighttime transpiration (E_{night}). Superscript letters denote significant differences between means within rows ($P < 0.05$). *Asterisked values are predicted values based on the VPD_{leaf} response curves shown in Fig. 2.

	High N		Low N	
	High night VPD_{leaf}	Low night VPD_{leaf}	High night VPD_{leaf}	Low night VPD_{leaf}
A ($\text{mmol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	8.48 (0.62)	8.65 (0.44)	8.61 (0.40)	8.19 (0.26)
g_{day} ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	0.178 (0.011)	0.177 (0.007)	0.193 (0.008)	0.167 (0.015)
E_{day} ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	1.83 (0.11) ^a	1.84 (0.06) ^a	2.38 (0.12) ^b	1.98 (0.14) ^a
g_{night} ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	0.076 (0.009)	0.171*	0.063 (0.004)	0.171*
E_{night} ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	0.76 (0.06)	0.11*	0.74 (0.02)	0.11*
$g_{\text{night}}/g_{\text{day}}$ (%)	44.3 (7.0) ^a	96.6*	33.5 (1.8) ^b	102.4*
$E_{\text{night}}/E_{\text{day}}$ (%)	43.8 (2.4) ^a	6.0*	32.0 (0.5) ^b	5.6*
Shoot biomass (g)	0.033 (0.006) ^a	0.033 (0.004) ^a	0.013 (0.002) ^b	0.013 (0.001) ^b
Root biomass (g)	0.014 (0.003) ^a	0.012 (0.002) ^a	0.008 (0.001) ^b	0.008(0.001) ^b
Root mass ratio	0.30 (0.01) ^a	0.27 (0.03) ^a	0.37 (0.04) ^b	0.36 (0.04) ^b
Leaf $\delta^{13}\text{C}$ (%)	-32.10 (0.13)	-32.10 (0.21)	-32.35 (0.21)	-32.23 (0.17)
Leaf N (%)	4.81 (0.39) ^a	4.84 (0.38) ^a	3.30 (0.27) ^b	3.43 (0.25) ^b
Leaf Ca (%)	2.62 (0.13) ^a	2.57 (0.08) ^a	1.48 (0.05) ^b	1.46 (0.06) ^b
Leaf K (%)	4.64 (0.22) ^a	5.06 (0.49) ^a	3.49 (0.22) ^b	3.70 (0.19) ^b

response, but also a more modest extrapolation from the data in Fig. 2—the difference in E_{night} between treatments is at least 50%. Thus, we are confident our treatments were successful at producing two very different levels of E_{night} .

Experimental design factors

The factor night chamber (VPD_{leaf}) was significant in the analyses of leaf N ($F = 6.88$, $P = 0.02$), shoot dry

weight ($F = 5.48$, $P = 0.03$), g_{day} ($F = 7.85$, $P = 0.01$), and E_{day} ($F = 4.85$, $P = 0.04$). Time was only significant in the analyses of g_{night} ($F = 16.18$, $P = 0.02$), leaf Ca ($F = 8.78$, $P = 0.02$), and leaf N ($F = 26.74$, $P < 0.001$).

Effects of nighttime VPD_{leaf} treatments on growth, nutrition, and gas exchange

Contrary to our expectation, long-term nighttime VPD_{leaf} treatments had no effect on shoot or root biomass, leaf

area, root mass ratio, $\delta^{13}\text{C}$, or leaf nutrient content of *Arabidopsis* plants just prior to bolting [all $F(1,2) < 3.71$, $P > 0.19$; Table 1]. The nighttime VPD_{leaf} treatments also had no effect on A or g_{day} [$F(1,2) = 0.18$, $P = 0.71$ and $F(1,2) = 0.45$, $P = 0.57$, respectively], but E_{day} was increased in low N plants subjected to the high nighttime VPD_{leaf} treatment compared to the other three treatment combinations [$F(1,9) = 6.39$, $P = 0.03$].

Effects of N treatments on growth, nutrition, and gas exchange

Leaf N, Ca, and K were all significantly lower in plants in the low N than the high N treatment [all $F(1,9) > 14.49$; all $P < 0.005$] (Table 1). Leaf N remained relatively high in the low N treatment (approximately 3.4% compared with approximately 4.8% in the high N treatment). Despite this, low N treatment plants demonstrated N limitation, having 60% lower shoot and 43% lower root biomass than high N treatment plants [$F(1,9) = 59.44$, $P < 0.0001$ and $F(1,9) = 9.70$, $P = 0.01$, respectively]. Root mass ratio was 23–33% greater in low N than high N treatment plants [$F(1,9) = 5.83$, $P = 0.04$]. $\delta^{13}\text{C}$ was not affected by N treatment [$F(1,9) = 1.77$, $P = 0.22$].

N treatment had no effect on A or g_{day} [$F(1,9) = 0.15$, $P = 0.70$ and $F(1,9) = 0.09$, $P = 0.77$, respectively], although there was the interaction with VPD_{leaf} treatment mentioned above for E_{day} . N treatment had no effect on g_{night} or E_{night} [$F(1,4) = 5.19$, $P = 0.08$ and $F(1,4) = 0.28$, $P = 0.63$, respectively]. However, both g_{night} and E_{night} as percentages of g_{day} and E_{day} , respectively, averaged approximately 25% lower in low N treatment plants due to the differences in g_{night} and E_{day} [$F(1,4) = 4.87$, $P = 0.09$ and $F(1,4) = 21.43$, $P < 0.01$, respectively].

Discussion

One of the proposed implications of maintaining open stomata during the night is that E_{night} may increase nutrient supply by increasing mass flow movement of water plus dissolved nutrients (e.g., NO_3^-) toward plant roots, essentially extending E_{day} 's role in supplying nutrients into a 24-h mechanism (Caird et al. 2007a, Dawson et al. 2007, Scholz et al. 2007, Snyder et al. 2003, 2008). This role for E_{night} could increase plant nutrient uptake and ultimately enhance plant growth and fitness, especially for plants that are more nutrient than water-limited. However, in this study, experimentally reducing E_{night} throughout the vegetative growth period of *Arabidopsis* had no effect on final leaf nutrient content or fitness estimated from vegetative biomass. Thus, in *Arabidopsis*, there does not appear to be any benefit to

transpiring at night in terms of improved plant nutrition or growth, even for N-limited plants.

One possible explanation is that E_{night} 's effect on nutrient supply was negligible. Even with high E_{night} , the amount of water flux was substantially lower than during the day. Some researchers have argued that increased mass flow is an unimportant aspect of plant nutrition because observed trends between magnitude of transpiration and nutrient uptake only occur at unnaturally high soil nutrient concentrations, and uptake at natural levels is unrelated to water movement into and through the plant (Grubb 1977, Schulze and Bloom 1984). Although transpiration can affect nutrient movement in soil (Nye and Tinker 1977), for many nutrients there is little experimental evidence that demonstrates how changing transpiration rate actually translates into altered uptake (Conroy and Hocking 1993, McDonald et al. 2002). Here, the small rooting volume combined with relatively high nutrient concentrations may have allowed diffusion to maintain adequate supply of nutrients and thereby masked the contribution of E_{night} -driven bulk flow supply. Depletion around roots as would have happened during the night in the low VPD_{leaf} treatment may not have been to a level low enough to reduce nitrate uptake, and thus the decreased concentrations still left root uptake mechanisms functioning at high rates.

Alternatively, it is possible that transpiration-independent mechanisms dominated in maintaining nutrient flux to and through the plants, allowing low nighttime VPD_{leaf} treatment plants to attain the same level of leaf nutrients as their nighttime transpiring counterparts. For example, transpiration-independent water flow (e.g., growth water, 'Munch's counterflow,' and root pressure) was sufficient to maintain mineral transport and growth in solution culture-grown *Helianthus* when transpiration was completely separated from nutrient supply. In larger plants, such as tall trees and large shrubs, transpiration-driven water flux may be more important in distributing nutrients upward through the plant because larger fluxes would be necessary to support mineral supply to all areas. However, based on their results with *Helianthus*, Tanner and Beevers (2001) calculated that transpiration-independent mechanisms would still be sufficient in driving water and nutrient flow, even in tall trees.

In a recent study with desert shrubs, Snyder et al. (2008) observed greater short-term ^{15}N uptake in nighttime transpiring plants compared with plants where E_{night} was suppressed by 'bagging' and humidifying

plant canopies. However, they also found evidence of a net flux of water away from roots (due to hydraulic lift) at the injection site of the ^{15}N label, suggesting that transpiration-driven mass flow was not the mechanism behind the effect. Rather, effects of the bagging treatment on daytime gas exchange (via changes in CO_2 concentration and air temperature inside bags) or other processes were cited as possible causes. Longer-term effects of manipulating E_{night} on plant nutrition, as in our study, were not assessed in Snyder et al. (2008) and remain to be tested for other species.

The effects of plant nutritional status on g_{night} vary among species (reviewed in Caird et al. 2007a; see also Howard and Donovan 2007, Ludwig et al. 2006, Scholz et al. 2007), making it unclear if any species actively regulate g_{night} to enhance E_{night} and, consequently, nutrient supply. Here, g_{night} was not different in the low N compared to the high N treatment, contrary to the hypothesis that plants may regulate g_{night} to enhance nutrient supply. However, compensation for insufficient supply of N by reduced shoot growth and greater root mass ratio maintained relatively high leaf N concentration (3.3%) in the low N treatment, which may explain why a difference in g_{night} between the N treatments was not observed. It therefore remains inconclusive (1) if plants actually regulate g_{night} by sensing the need to increase nutrient uptake, (2) if enhanced nutrient supply is a passive benefit unconnected to regulation of g_{night} , or (3) if nutritional status affects stomatal regulation in a manner totally unrelated to any potential benefits provided by undergoing E_{night} .

In this study, we tested how *Arabidopsis* stomata responded to nighttime VPD_{leaf} conditions (Fig. 2) by measuring g_{night} and E_{night} in plants at various VPD_{leaf} conditions at two separate times during the night. As has been suggested by a number of observational studies of nighttime sap flux (Benyon 1999, Bucci et al. 2004, Cavender-Bares et al. 2007, Daley and Phillips 2006, Dawson et al. 2007, Fisher et al. 2007, Kavanagh et al. 2007, Marks and Lechowicz 2007, Moore et al. 2008) and confirmed by Barbour and Buckley (2007), we found that g_{night} declined in response to increasing nighttime VPD_{leaf} . Here, using experimental manipulations that isolated the VPD_{leaf} response from potentially confounding endogenous circadian rhythms or overnight recovery of plant water status, we did not find a difference in the VPD_{leaf} response due to time of measurement (early vs late in the night period). However, we did observe greater sensitivity of g_{night} to VPD_{leaf} in plants from the high VPD_{leaf} treatment, but only during the late (second) measurement period.

Thus, the question remains: why do plants transpire at night? For *Arabidopsis*, it may simply be that there is no significant cost to E_{night} when water is not limiting. However, it is important to note that this study only addressed whether E_{night} provided a benefit by affecting plant nutrition, and there are several additional aspects of g_{night} and E_{night} not tested by this study which may affect plant growth and fitness. For example, nighttime water loss could play a role in nighttime recovery from cavitation (Snyder et al. 2003) or, under certain conditions, prevention of excess cell turgor (Donovan et al. 2003). Some evidence also suggests a role for E_{night} in supplying guard cells with carbohydrates from starch breakdown in mesophyll cells (Easlon and Richards 2009). Perhaps future investigations utilizing additional species and situations (e.g., where tradeoffs may be important) and looking at additional proposed physiological or ecological benefits will provide a more definitive answer to the question of g_{night} and/or E_{night} 's functional roles.

Acknowledgements – This research was supported by an NSF graduate research fellowship [M. A. (Caird) Christman], NSF grant IBN-0416581 (J.H. Richards), and the California Agricultural Experiment Station. The assistance of Rebecca Hundt and Joshua Christman in moving plants among chambers is greatly appreciated.

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