RESEARCH PAPER

# Night-time conductance in $C_3$ and $C_4$ species: do plants lose water at night?

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## Abstract

**Significant night-time stomatal conductance and transpiration were found for 11 out of 17 species with a range of life histories (herbaceous annual, perennial grass, shrub, tree), photosynthetic pathways ($C_3$, $C_4$), and habitats in the western United States. Across species and habitats, higher night-time conductance and transpiration were associated with higher daytime values. The prevalence, mechanisms and ecological implications of substantial night-time water loss deserve further investigation.**

## Introduction

It is generally accepted that for $C_3$ and $C_4$ plants stomatal closure minimizes transpirational water loss ($E$) at night when there is no opportunity for carbon gain. However, there is increasing evidence that some species maintain substantial stomatal conductance ($g$) and $E$ at night. *Arabidopsis, Betula, Brassica, Chrysothamnus, Fraxinus, Picea, Rosa, Sarcobatus*, and *Tilia* all have substantial night-time $g$, based on gas exchange measurements (Winner *et al.*, 1989; Whitlow *et al.*, 1992; Wieser and Havranek, 1993; Matyssek *et al.*, 1995; Assaf and Zieslin, 1996; Donovan *et al.*, 1999; Lascève *et al.*, 1999). Sap flow measurements indicate that night-time $E$ ranges from 5–30% of daily water loss in *Actinidia, Eucalyptus, Malus, Populus, Prosopis, Salix, Taxodium*, and Dipterocarp trees, although potential capacitance effects were not addressed (Green *et al.*, 1989; Cleverly *et al.*, 1997; Hogg and

Hurdle, 1997; Becker, 1998; Benyon, 1999; Oren *et al.*, 2001). Significant night-time $g$ and $E$ changes current understanding of how these plants interact with their environment. This stomatal behaviour may have adaptive significance in explaining plant distribution and may change calculations of sap flow and modelled estimates of water budgets that assume zero night-time water loss by plants. It may also change predictions of vegetation responses to pollutants, increasing concentrations of greenhouse gases, and global climate change. The ecological implications will depend on the extent and magnitude of night-time $g$ and $E$.

The objective of this study was to determine the occurrence and magnitude of night-time $g$ and $E$ for 17 dominant species across a range of plant functional types and habitats in the western United States. It was predicted that species in water-sufficient habitats (riparian and seep habitats) would have greater night-time $g$ and $E$ than those in water-limited habitats (sagebrush scrub, pinyon-juniper woodland and warm desert habitats). It was also expected that across habitats, plants with higher daytime $g$ and $E$ as indicators of favourable plant water status and high transpiration potential would have higher night-time $g$ and E.

## Materials and methods

Gas exchange was measured for 17 species on relatively cloud-free nights in several Great Basin habitat types near Mono Lake, CA (38°5′ N, 118°5′ W; 2000±50 m elevation) and at one Mojave desert site near Owens Lake, CA (36°3′ N, 118° W; 1100 m elevation). The Great Basin habitats and the Mojave desert habitat sampled in this study are characterized by low (≅30%) summer precipitation (April to October). The majority of precipitation falls during cooler winter months (November to March) (Toft, 1995). This creates relatively high levels of available soil moisture in the spring that then decline throughout the typically dry summer months. Gas exchange

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characteristics were measured fairly late in the growing season (29 July to 11 September) and, therefore, may represent a conservative estimate of night-time rates if seasonal water availability is a limiting factor.

Leaf or shoot gas-exchange of the dominant species was measured with a LI-6400 (Li-Cor, Inc., Lincoln, NE, USA) on recently mature, fully-expanded leaves of randomly selected similar-sized plants. Night-time stomatal opening of *Arabidopsis thaliana* (Lascève *et al.*, 1997) and two desert shrubs (Donovan *et al.*, 2003) have been found to increase throughout the night. To minimize differences due to the progressive opening of stomata during the hours of darkness and to ensure that all plants were sampled without light, night-time gas exchange measurements were made 1–3 h before dawn. Daytime measurements were made 2–4 h after dawn when gas exchange rates are generally maximal in these habitats (Donovan *et al.*, 2003). Reference $CO_2$ was set to 360 µmol mol$^{-1}$. PPFD was maintained slightly above ambient (1600–1900 µmol m$^{-2}$ s$^{-1}$) during the day and there was no illumination at night. Cuvette temperature and relative humidity tracked ambient. Leaves within the cuvette were excised and scanned for leaf areas using a desktop scanner and image analysing software (Scion Corporation, 2000, Frederick, MD, USA).

Interspersed empty (closed) chamber measurements, made throughout the sampling periods, provided the assessment of instrument error. Leaf area varied by species and, in part, determined measurement error; so empty chamber calculations included leaf area averaged for each of the species.

Due to the occurrence of unequal variances between species measurements and closed chamber measurements, the Welch statistic (JMP, 1997, SAS Institute) was used to determine if $g$ and $E$ of each species differed significantly ($P < 0.05$) from empty (closed) chamber values of $g$ and $E$ during the day and at night. The Welch ANOVA statistic with two levels, as used here, is equivalent to an unequal variance $t$-test with means weighted by the reciprocal of the sample variances. For each species that had significant night-time $g$ and $E$, the rates were then expressed as a percentage of daytime rates. Product–moment correlation was used to determine the relationship between mean values of night-time $g$ versus daytime $g$ and night-time $E$ versus daytime $E$ for the 11 species that had significant levels of night-time $g$ and $E$. Box-Cox transformations were used to meet the assumptions of correlation analysis.

## Results and discussion

Significant night-time $g$ and $E$ occurred in 11 out of the 17 species, including all three $C_4$ species (Fig. 1A, B, see figure legend and Table 1 for species identity). The magnitude of night-time $g$ and $E$ was not generally greater in water-sufficient habitats (riparian and seep) as opposed to water-limited habitats (sagebrush scrub, pinyon-juniper woodland, and warm desert). The lack of significant night-time $g$ and $E$ in pinyon-juniper woodland species was associated with very low daytime rates and may have reflected late-season water stress in these conifers.

Across species and habitats, the magnitude of night-time $g$ was correlated with the magnitude of daytime $g$ ($r=0.65$, $P=0.03$; Fig. 1E). Parallelling this pattern, night-time $E$ at the leaf/shoot level was also correlated with daytime $E$ when both were measured under ambient temperature and relative humidity conditions ($r=0.81$, $P=0.002$; Fig. 1F). These positive correlations support the hypothesis that night-time stomatal opening and transpirational water loss

are greater when species have a high daytime potential for $g$ and $E$ and sufficient water availability to express that potential.

For species where night-time $g$ was significantly greater than empty chamber measurements (solid bars in Fig. 1A), night-time $g$ was generally greater than 20% of daytime $g$ (Fig. 1C). For a given total conductance, the actual transpiration at night depends on the driving gradient between the leaf and the atmosphere. Again for species where night-time $E$ was significantly greater than empty chamber measurements (solid bars in Fig. 1B), night-time $E$ as a percentage of daytime $E$, though measured in a cuvette on a leaf area basis, but under ambient temperature and relative humidity conditions, was not trivial and exceeded 10% of daytime $E$ in all 11 species and was greater than 15% in 7 species (Fig. 1D). Although inserting leaves into a gas exchange cuvette can lead to an overestimation of $E$ due to increased boundary layer conductance, significant overestimation of $E$ and $g$ is unlikely in this system because of generally small leaf sizes and inherently large boundary layer conductances (both at leaf and canopy level). In addition, the species in this study are well coupled to the atmosphere during both the day and night due to wide plant spacing and discontinuous canopies. Thus chamber effects on $E$ are likely to be minimal in this study.

Substantial night-time water loss is unexpected for these $C_3$ and $C_4$ plants. It was predicted that the occurrence and magnitude of night-time $g$ and $E$ would be greater in wetter habitats than in water-limited habitats. Contrary to this initial prediction, habitat differences seemed to have little effect on the occurrence or magnitude of night-time water loss (Fig. 1A, B). The occurrence of night-time $g$ and $E$ in all surveyed $C_4$ species was also unexpected, because evolution of the $C_4$ photosynthetic pathway is generally thought to be a response to low atmospheric $CO_2$ concentration, as well as an important adaptive mechanism to hot, dry climates (Waller and Lewis, 1979). It was found that there was as much variability comparing species in the same genus, as there was when comparing across the various habitats. Specifically, *Salix exigua* exhibited significant night-time $g$ and $E$ in contrast to *S. lutea* which did not. *Populus balsamifera* ssp. *trichocarpa* also had significant night-time $g$ and $E$ in contrast to *P. tremuloides*. The lack of night-time $g$ and $E$ for *P. tremuloides* in the current study is in contrast to results from Hogg and Hurdle (1997) where night-time sap flow was linearly related to vapour pressure deficit (*VPD*). The lack of significant night-time $g$ and $E$ in the measurement of *P. tremuloides* may have been due to a temporal trend of greater stomatal opening later in the sampling period, which increased the variance of the plant gas exchange measurements and prevented the detection of significant differences between plant and empty chamber measurements (Fig. 1A, B). Substantial night-time $g$ and $E$ has
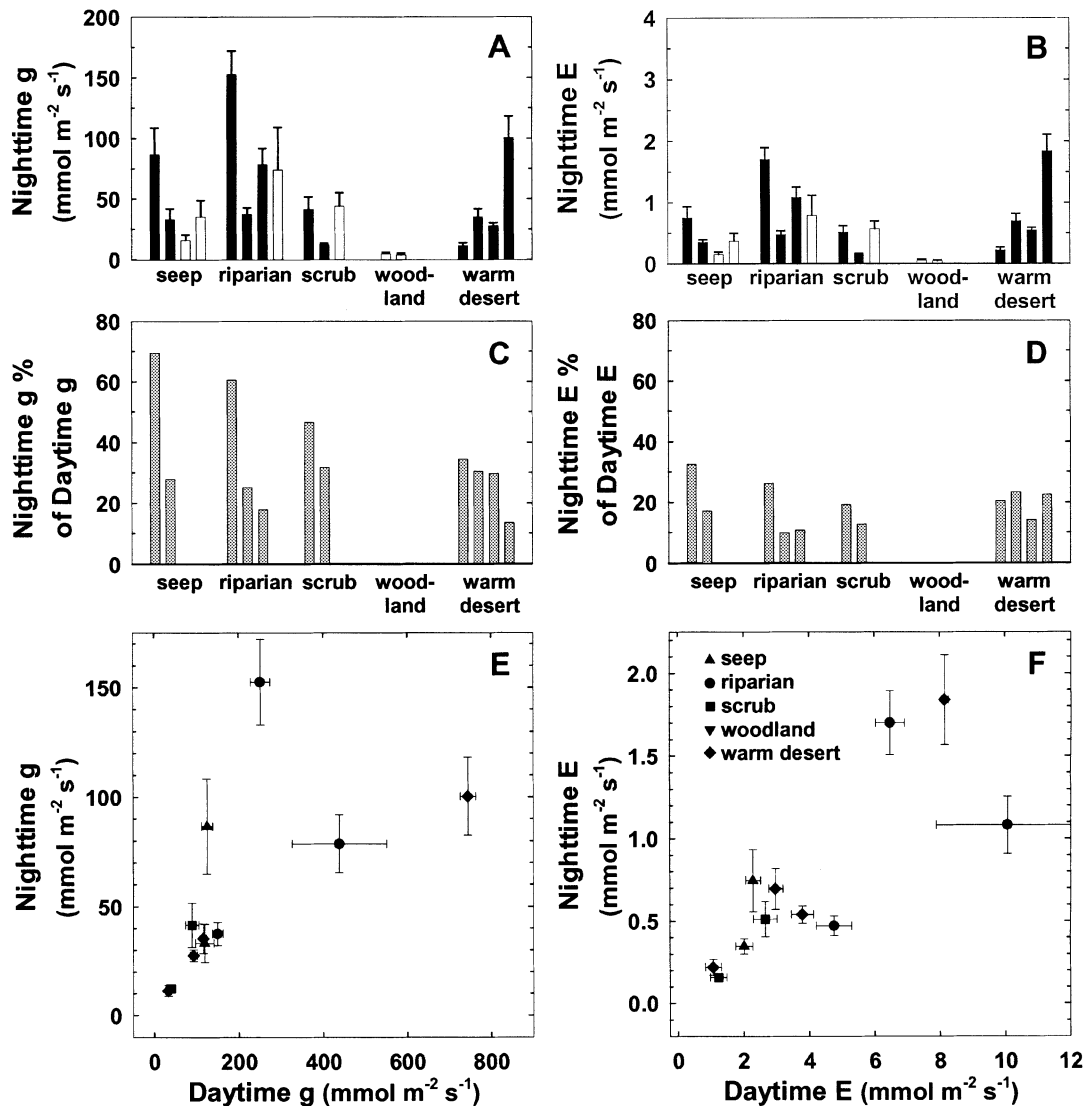
**Fig. 1.** (A) Mean night-time stomatal conductance ($g$) $\pm 1$ SE and (B) mean night-time transpiration ($E$) $\pm 1$ SE for all 17 species surveyed ($n=5$ except warm desert $n=4$). Closed bars represent means that were significantly greater ($P < 0.05$) than closed, empty chamber means, while open bars were not significantly greater than closed, empty chamber means. (C) Night-time $g$ expressed as percentage of daytime $g$ and (D) night-time $E$ expressed as percentage of daytime $E$ for the 11 species in (A) and (B) where night-time $g$ and $E$ were significantly greater than closed, empty chamber means. The bars in (A–D) represent the species in the following order: Seep habitat; *Leymus cinereus, Shepherdia argentea, Rhus trilobata, Salix lutea*; Riparian habitat; *Populus balsamifera* ssp. *trichocarpa, Rosa woodsii, Salix exigua, Populus tremuloides*; Sagebrush scrub habitat; *Purshia tridentata, Artemisia tridentata, Chrysothamnus nauseosus*; Pinyon-juniper woodland habitat; *Juniperus osteosperma, Pinus monophylla*; Warm desert habitat; *Atriplex confertifolia, Distichlis spicata, Atriplex parryi, Helianthus annuus*. Species were ordered within habitat by the values from (C). The relationships between (E) night-time and daytime $g$ and (F) night-time and daytime $E$ under ambient conditions. Each point (mean $\pm 1$ SE, $n=5$ except warm desert $n=4$) represents one of the 11 species where night-time rates differed significantly from closed, empty chamber means ($P < 0.05$).

been documented for *Chrysothamnus nauseosus* at another Great Basin site near Mono Lake (Donovan *et al.*, 2003), yet this species did not exhibit significant night-time *g* and *E* in the present study, probably because this species was sampled late in the growing season at a site with greater depth to groundwater than the site used by Donovan *et al.* (2003). Reduced water availability may have prevented this species from expressing its potential for night-time transpiration. Although the water status in the current

study was not measured, plants are generally expected to have stomata closed at night regardless of plant water status. Stomata are known to close in response to declines in bulk leaf water potential (Lösch and Tenhunen, 1981). The fact that some of the plants in this study were probably water stressed, yet still exhibited night-time *g* and *E* in complete darkness, indicates that the magnitude of *g* and *E* under well-watered conditions could be greater. These data suggest that the controls on night-time *g* and *E* are more

**Table 1.** *Species surveyed, habitats within which species were sampled, photosynthetic pathway, life history, and the occurrence of night-time stomatal conductance (g) and transpiration (E) that differed significantly from closed, empty chamber measurements (P < 0.05)*

Nomenclature follows *The Jepson manual: higher plants of California* (Hickman, 1993). Species are listed in the same order as in Fig. 1A–D and within habitat the order is based on the values given in Fig. 1C.

| Species | Habitat | Photosynthetic pathway | Life history | Night-time $g$ and $E$ |
|---|---|---|---|---|
| *Leymus cinereus* | Seep | $C_3$ | Grass | Yes |
| *Shepherdia argentea* | Seep | $C_3$ | Shrub | Yes |
| *Rhus trilobata* | Seep | $C_3$ | Shrub | No |
| *Salix lutea* | Seep | $C_3$ | Shrub | No |
| *Populus balsamifera* ssp. *trichocarpa* | Riparian | $C_3$ | Tree | Yes |
| *Rosa woodsii* | Riparian | $C_3$ | Shrub | Yes |
| *Salix exigua* | Riparian | $C_3$ | Shrub | Yes |
| *Populus tremuloides* | Riparian | $C_3$ | Tree | No |
| *Purshia tridentata* | Sagebrush scrub | $C_3$ | Shrub | Yes |
| *Artemisia tridentata* | Sagebrush scrub | $C_3$ | Shrub | Yes |
| *Chrysothamnus nauseosus* | Sagebrush scrub | $C_3$ | Shrub | No |
| *Juniperus osteosperma* | Pinyon-juniper woodland | $C_3$ | Tree | No |
| *Pinus monophylla* | Pinyon-juniper woodland | $C_3$ | Tree | No |
| *Atriplex confertifolia* | Warm desert | $C_4$ | Shrub | Yes |
| *Distichlis spicata* | Warm desert | $C_4$ | Grass | Yes |
| *Atriplex parryi* | Warm desert | $C_4$ | Shrub | Yes |
| *Helianthus annuus* | Warm desert | $C_3$ | Annual | Yes |

complicated than initially predicted based on habitat or photosynthetic pathway.

Night-time stomatal opening appears to be a potentially widespread behaviour in plants, which leads to questions about the relative costs and/or benefits of night-time water loss. There may be no substantial water 'cost' of incomplete night-time stomatal closure, although this seems unlikely for the dry habitat species in this study. In these Great Basin and Mojave desert habitats, *VPD*s can fluctuate by 4 kPa over the course of an average day (KA Snyder, unpublished data). Species that keep stomata open at high *VPD*s may have reduced capacity to close stomata when *VPD*s and thus transpirational water loss are low. If night-time *VPD*s rarely create conditions necessary to drive substantial night-time *E*, and if water availability to these species is high, the water 'cost' of incomplete stomatal closure may be low. This would be consistent with the observed positive correlation between night-time and daytime rates. Low night-time *VPD*s would be expected to allow greater stomatal opening than during the day, as would higher plant water potential at night. However, darkness typically over-rides these signals. In general, a positive relationship between night-time sap flow and *VPD* has been found in several mesic tree species (Hogg and Hurdle, 1997; Benyon, 1999; Oren *et al.*, 2001). The role of *VPD*s in driving night-time sap flow is currently being investigated.

Alternatively, there may be potential physiological benefits to night-time *E*, such as improved nutrient acquisition. Continued *E* at night would increase total daily mass flow of water to roots, which is important for supply of $NO_3^-$ (Nye and Tinker, 1977; Barber, 1995;

McDonald *et al.*, 2002). Another potential benefit of night-time *E* may be the recovery of hydraulic conductance from partial xylem cavitation events that occur during more stressful daytime conditions. For example, (Zwieniecki *et al.*, 2001) found that perfusing stems with ionic solutions and water produced rapid and reversible changes in xylem hydraulic conductance and conductance was greatest when ionic concentrations were similar to natural xylem sap. Their data suggest active, plant-mediated control of water flow through ion regulation, which may require continued *E* during the dark hours. Lastly, for plants that accumulate substantial concentrations of ions and compatible solutes, night-time leaf water loss may be necessary to prevent excess leaf turgor when substrate water potentials become more favourable (Donovan *et al.*, 1999, 2001).

Many current sap flow systems assume night-time water loss is zero. These data indicate, however, that continuous measurement of sap flow throughout the night may be necessary to measure whole plant water loss accurately. The magnitude of water loss at night is potentially not trivial when scaled to stand and ecosystem levels. If potential changes in climate create warmer, drier conditions at night (increased *VPD*s) and/or reduce water availability within the plant-rooting zone this may change the competitive abilities of species with incomplete night-time stomatal closure. Hydrometeorological models based on current and future climates may need to modify algorithms of plant water use, particularly if this phenomenon of night-time water loss is widespread in warm, dry environments, such as the warm desert, with high night-time *VPD*s.

Night-time stomatal opening will have effects beyond those mediated through water transport and water loss. Ozone pollution can have detrimental effects on plant growth and studies of ozone uptake found stomata were open at night and the uptake of ozone continued during the hours of darkness (Winner *et al.*, 1989; Wieser and Havranek, 1993; Matyssek *et al.*, 1995). Although total ozone uptake was reduced by partial stomatal closure at night, the detrimental effects on the growth of *Betula pendula* were also greater at night than during the day due to the lack of photosynthetic electron transport required to detoxify reactive oxygen species (Matyssek *et al.*, 1995).

Substantial night-time *g* and *E* were found in plants representing a range of life histories (herbaceous annual, perennial grass, shrub, and tree) photosynthetic pathways ($C_3$, $C_4$) and habitats, suggesting that it may be widespread in plants. This behaviour may have ecological consequences for a variety of species and habitats in other regions. The extent of night-time stomatal opening and water loss, its physiological and ecological relevance, and proximate controls deserve further study.

## Acknowledgements

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