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Environmental stress and genetics influence night-time leaf conductance in the C_4 grass *Distichlis spicata*

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Abstract. Growing awareness of night-time leaf conductance (g_{night}) in many species, as well as genetic variation in g_{night} within several species, has raised questions about how genetic variation and environmental stress interact to influence the magnitude of g_{night}. The objective of this study was to investigate how genotype salt tolerance and salinity stress affect g_{night} for saltgrass [*Distichlis spicata* (L.) Greene]. Across genotypes and treatments, night-time water loss rates were 5–20% of daytime rates. Despite growth declining 37–87% in the high salinity treatments (300 mM and 600 mM NaCl), neither treatment had any effect on g_{night} in four of the six genotypes compared with the control treatment (7 mM NaCl). Daytime leaf conductance (g_{day}) also was not affected by salinity treatment in three of the six genotypes. There was no evidence that more salt tolerant genotypes (assessed as ability to maintain growth with increasing salinity) had a greater capacity to maintain g_{night} or g_{day} at high salinity. In addition, g_{night} as a percentage of g_{day} was unaffected by treatment in the three most salt tolerant genotypes. Although g_{night} in the 7 mM treatment was always highest or not different compared with the 300 mM and 600 mM treatments, g_{day} was generally highest in the 300 mM treatment, indicating separate regulation of g_{night} and g_{day} in response to an environmental stress. Thus, it is clear that genetics and environment both influence the magnitude of g_{night} for this species. Combined effects of genetic and environmental factors are likely to impact our interpretation of variation of g_{night} in natural populations.

Additional keywords: genetic variation, nocturnal, salinity, saltgrass, stomatal conductance, transpiration.

Introduction

Night-time water loss in non-CAM plants occurs without simultaneous carbon gain and results in reduced predawn water status (reviewed by Caird *et al*. 2007*a*), thus, presenting a cost for plants which maintain high g_{night} throughout the night (Donovan *et al*. 2003; Caird *et al*. 2007*b*; Kavanagh *et al*. 2007). However, several benefits for night-time water loss have been proposed, including enhanced nutrient supply (Snyder *et al*. 2008), prevention of excess cell turgor (Donovan *et al*. 2003), and enhanced early morning carbon gain (Dawson *et al*. 2007). Although any proposed benefits have yet to be quantified directly, several studies have used natural variation in g_{night} both among and within species to develop adaptive hypotheses explaining the occurrence of high g_{night} (Marks and Lechowicz 2007; Christman *et al*. 2008).

Natural variation can be caused by both genetic and environmental factors as well as their interaction. A few common garden experiments have shown a genetic component to g_{night} by investigating the magnitude of g_{night} under controlled, non-stressful conditions (Caird *et al*. 2007*b*; Christman *et al*. 2008). Several other studies have exploited environmental variation to show g_{night} can be affected by environmental factors. For example, water stress (as soil drought, salinity, and high atmospheric demand) reduces g_{night} and E_{night} in many species (Rawson and Clarke 1988; Donovan *et al*. 1999; Barbour and Buckley 2007; Cavender-Bares *et al*. 2007; Dawson *et al*. 2007; Fisher *et al*. 2007; Howard and Donovan 2007; Moore *et al*. 2008). Although each of these studies has illuminated several aspects of g_{night} by focusing on either genetic or environmental effects, how these factors interact to influence g_{night} has not been directly addressed. Such interactions may confound current interpretations of variation in natural populations (Caird *et al*. 2007*a*; Dawson *et al*. 2007; Scholz *et al*. 2007).

Furthermore, how g_{night} is affected by stressful conditions, and if the effect varies as a function of stress tolerance, has not been investigated. More stress-tolerant genotypes, which can maintain growth and physiological function under stressful conditions, may have a greater capacity to regulate or maintain g_{night} because they have mechanisms to avoid or

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acclimate to the stress. For instance, mechanisms such as ion compartmentalisation or sodium (Na) exudation help salt-tolerant plants reduce the physiological effects of salinity stress and maintain physiological function at levels similar to non-stressed plants.

This study investigated the individual and interactive effects of genetic and environmental factors on g_{night} using six clonal genotypes of saltgrass [*Distichlis spicata* (L.) Greene]. Saltgrass was chosen because it is adapted to highly stressful arid and saline environments and demonstrates variation among genotypes in salt tolerance; futhermore, genotypes can be easily propagated as clones to increase replication for experiments. Due to its extreme salt tolerance, the species is used in large-scale remediation projects in arid, saline environments to stabilise soil and reduce dust and air pollution (Dahlgren *et al*. 1997; Dickey *et al*. 2005*a*, 2005*b*). The six clones included in this study were known to exhibit variable degrees of salinity tolerance (relative ability to maintain growth under high salinity) from previous trials and were obtained from a dry lake bed where the clones grew with variable success under salinity ranging from ~400 to 600 mM NaCl (Dahlgren *et al*. 1997). We tested whether these six genotypes differed in g_{night} under control conditions and in their response of g_{night} and g_{day} to salinity stress. Further, we examined the extent of salinity's effects on g_{night} and g_{day}, predicting that more salt tolerant genotypes would maintain higher g_{night} and g_{day} than less salt tolerant genotypes.

Materials and methods

Plant collection, salt tolerance and growing conditions

Thirty-eight clones of *Distichlis spicata* (L.) Greene were collected in March 2004 from locations differing in soil type and salinity on the Owens Lake playa, CA, USA. Clones were transplanted and propagated in a greenhouse at the University of California, Davis, for evaluation of salinity tolerance. In March 2005, six clones representing the maximum range of salinity tolerance observed in the 38 original clones were selected for use in this study (Table 1). Salinity tolerance of a genotype was quantified as the natural log of the ratio of plant biomass produced under high salinity (600 mM NaCl) compared with biomass produced under a low salinity (7 mM NaCl) control such that

$$\ln RR = \ln(\text{biomass}_{600\text{ mM NaCl}}/\text{biomass}_{7\text{ mM NaCl}}), \quad (1)$$

where lnRR stands for the logarithmic response ratio (Hedges *et al*. 1999). With this metric, plants that are less able to maintain biomass production as salinity increases have more negative values for lnRR, indicating lower salinity tolerance.

For this experiment, rhizomes of similar size were clipped from each of the six study clones and transplanted into a 50 : 50 sand and fritted clay mix, providing a total of 18 individuals for each genotype (clone). Plants were grown outside the greenhouse for the duration of the experiment. No rain fell during the entire study period, so treatment applications were the only source of water for the plants. One month after transplanting, three plants of each genotype were assigned randomly to one of three salinity treatments [7 (control), 300, 600 mM NaCl] in each of six blocks for a total of 108 plants (6 genotypes × 3 salinity levels × 6 blocks = 108). To allow for acclimation, the NaCl treatments were applied with increasing strength over a 2-week period until all plants were brought up to full treatment strength. Full strength salinity treatments were applied for 4 weeks before gas-exchange measurements were made. Essential nutrients were added to the salinity treatments as a 1/4-strength modified Hoagland solution (Epstein 1972).

Gas-exchange measurements

After 6 weeks of salinity treatments, daytime (beginning at solar noon) and night-time (beginning 2 h after sundown) gas exchange was measured with a LI-6400 portable photosynthesis instrument (Li-Cor Inc., Lincoln, NE, USA). Approximately 5 h before measurement, leaves were rinsed with deionised water to remove salts that had accumulated on the leaf surfaces. Measurements of daytime and night-time gas exchange on each plant were made on 2 days, with half the plants being measured during each measurement period. These two measurements repeated in time were treated as subsamples and averaged for data analysis. Additionally, each individual measurement consisted of three subsample logs made at 10 s intervals after equilibrium was reached inside the LI-6400 chamber (~2–5 min).

Table 1. Relative salt tolerance of the six *Distichlis* genotypes and the effect of salinity on leaf Na concentration and exudation rate of mature leaves from each genotype

Data are means ± s.e. ($n = 6$). Relative salt tolerance was quantified using the logarithmic response ratio (ln RR; Hedges *et al*. 1999) as the natural log of the ratio of plant biomass produced under high (600 mM) salinity compared with biomass produced under low (7 mM) salinity control conditions. In this metric more negative values indicate lower salinity tolerance

Clone no.	Relative salt tolerance (ln RR)	Leaf Na concentration (g kg^{-1})			Leaf Na exudation rate (nmol m^{-2} s^{-1})		
		7 mM	300 mM	600 mM	7 mM	300 mM	600 mM
38	–2.00	3.9 ± 0.3	9.6 ± 0.8	22.1 ± 4.1	79 ± 7	214 ± 12	233 ± 25
33	–1.83	4.1 ± 0.3	13.8 ± 4.6	24.5 ± 4.9	145 ± 21	290 ± 44	445 ± 31
2	–1.79	5.0 ± 0.3	8.8 ± 0.5	24.4 ± 5.0	84 ± 5	238 ± 26	200 ± 16
24	–1.43	4.0 ± 0.3	12.5 ± 0.8	17.8 ± 3.1	97 ± 7	327 ± 36	308 ± 22
23	–1.42	4.4 ± 0.3	11.8 ± 1.4	29.4 ± 3.4	143 ± 20	408 ± 34	337 ± 46
12	–1.37	4.4 ± 0.3	9.1 ± 0.4	14.5 ± 1.6	80 ± 5	213 ± 8	213 ± 23

Li-Cor chamber conditions tracked ambient VPD and were set at 370 μmol mol^{-1} CO_2 for day and night measurements. Over the 4 days and nights of measurement, temperatures averaged 35.3 ± 0.1°C and 19.7 ± 0.5°C during the daytime and night-time, respectively, and VPD_{leaf} averaged 3.6 ± 0.2 kPa and 1.2 ± 0.0 kPa during the daytime and night-time, respectively. Photosynthetic photon flux density (PPFD) inside the chamber was maintained at 1000 μmol m^{-2} s^{-1} with a LI-6400 red-blue light source during daytime measurements. During night-time measurements, a headlamp with a green safe-light with intensity not detectable with a LI-190 quantum sensor (0 μmol m^{-2} s^{-1} PPFD) was used to avoid promoting stomatal opening. There is no evidence from previous trials that the very low intensity of green light caused any change in stomatal aperture; nonetheless, direct illumination of leaves was avoided and the headlamp was primarily used for operating the LI-6400. Instantaneous water use efficiency (WUE) was calculated as A/g_{day}. Both g_{night} and E_{night} were significantly greater than empty chamber measurements for all genotypes and salinity treatments ($P < 0.001$). After measurement, leaves were harvested and taped flat to paper for leaf area determination. Leaf area measurements were made using the WinRhizo Pro software package (Regent Instruments Inc., Saint-Foy, Quebec, Canada).

Leaf Na exudation rates, biomass, leaf Na and leaf N

Following gas-exchange measurements, an additional leaf from each plant was marked and rinsed with deionised water to remove any exuded salt from the leaf surface. After 48 h, leaves were collected and placed in vials with 10 mL of deionised water and gently shaken so that the exuded salts would be dissolved off the leaf surfaces. Na concentration in the solutions was then measured by atomic emission spectroscopy (AAnalyst 200, Perkin-Elmer, Wellesley, MA, USA). Na exudation rates were expressed on a leaf area basis. Aboveground biomass was harvested following Na exudation measurements.

Aboveground biomass was clipped at the soil surface, triple rinsed with deionised water, dried at 60°C, and weighed. Reproductive tissues were separated from vegetative tissues to determine inflorescence number and biomass. Leaf tissue was ground to a fine powder for N and Na analysis. Leaf N was determined by micro-Dumas combustion with a Carlo Erba NA1500 elemental analyser (Milan, Italy). Leaf Na samples were dry-ashed at 475°C for 4 h, dissolved in 1 N HCl and then analysed by atomic emission spectroscopy.

Statistical analysis

The main effects of genotype and salinity and their interaction (salinity × genotype) were assessed for gas exchange traits, biomass and leaf chemistry with ANOVA (SAS 2001). Assumptions of ANOVA were evaluated using the Shapiro-Wilk test for normality and Levene's test for homogeneity of variance. When these assumptions were not met, data were weighted by the inverse of the variance (Neter *et al.* 1990). Pearson correlations were used to evaluate correlations among gas exchange characteristics and correlations between salt-tolerance and other plant characteristics. Mean genotype values for each treatment were used when analysing correlations.

Results

Biomass and salinity tolerance

Aboveground biomass decreased in each genotype with increasing salinity, but the magnitude of this decrease differed among genotypes ($P < 0.001$; Fig. 1). For example, as salinity increased from 7 mM NaCl in the control treatment to 600 mM NaCl, biomass decreased to ~1/4 in genotype 12, the most salt-tolerant genotype, but biomass decreased to less than 1/7 in genotype 38, the least salt-tolerant genotype (Table 1; Fig. 1). In all but genotype 23, growth was significantly reduced in the 300 mM treatment relative to control; only at 600 mM was growth significantly lower in that genotype.

Of the four genotypes producing inflorescences (genotypes 2, 12, 24, 38) during the experiment, only three (2, 12, 38) produced significant numbers. In these genotypes, salinity decreased inflorescence number per plant and total inflorescence biomass per plant, and this decrease varied among genotypes ($P < 0.0001$ and $P < 0.0001$, respectively). Although leaf Na exudation rates increased with salinity and varied by genotype ($P < 0.0001$), higher leaf Na concentrations or leaf Na exudation rates did not correspond to greater salt tolerance among genotypes ($r < 0.31$, $P > 0.56$ and $r < 0.46$, $P > 0.35$ for all treatments, respectively; Table 1).

Genotype and salinity effects on gas exchange

Salinity treatment and genotype significantly affected g_{night} ($P = 0.003$ and $P = 0.04$, respectively; Fig. 2), but there was no significant interaction between genotype and salinity ($P = 0.24$). In four of the six genotypes, g_{night} was not significantly affected by increasing salinity. In the two genotypes (33 and 24) in which g_{night} was affected, g_{night} decreased between 32 and 41% with increasing salinity.

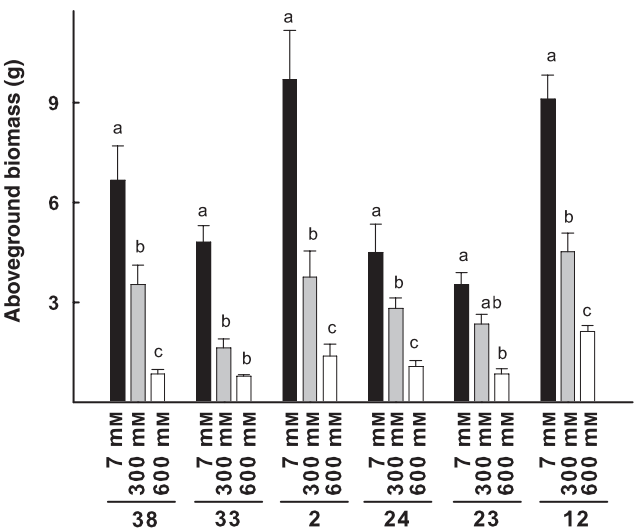

Fig. 1. Effect of soil salinity on total aboveground biomass of the six *Distichlis* genotypes (mean ± s.e., $n = 6$). Genotypes are arranged in order of increasing salinity tolerance (Table 1). Different letters denote significant differences among treatments within genotypes ($P = 0.05$).

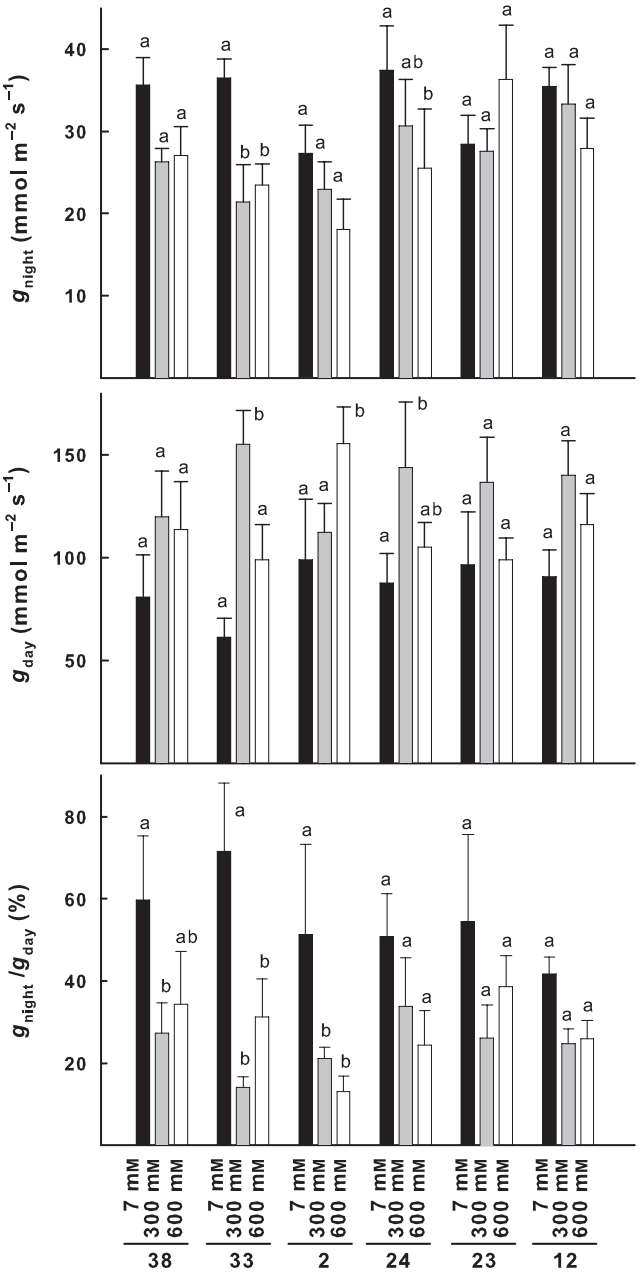

Fig. 2. Effect of soil salinity on night-time leaf conductance (g_{night}), daytime leaf conductance (g_{day}) and g_{night} as percent of g_{day} for the six *Distichlis* genotypes (mean ± s.e., $n = 6$). Genotypes are arranged in order of increasing salinity tolerance (Table 1). Different letters denote significant differences among treatments within genotypes ($P = 0.05$).

For daytime measurements (Fig. 2), salinity affected g_{day} in three of the genotypes ($P = 0.01$), but genotypes did not differ in g_{day} within salinity treatments ($P = 0.88$). There was no significant genotype by treatment interaction ($P = 0.43$). In three genotypes (33, 2, 24), daytime gas exchange showed some degree of salt stimulation, increasing ≈13–153% between the 7 mM control and 300 mM treatments (Fig. 2). Only one genotype (2) had g_{day} stimulated by the 600 mM treatment.

Among genotypes and treatments, g_{night} and E_{night} as a percentage of g_{day} and E_{day} ranged between 10–70% and 5–20%, respectively. In all genotypes g_{night} as a percentage of g_{day} was always highest in the 7 mM treatment ($P < 0.0001$; Fig. 2), but it did not differ among genotypes ($P = 0.64$). In the three most salt tolerant genotypes (24, 23, 12), g_{night} as a percentage of g_{day} was not affected by salinity, but in the three least tolerant genotypes, g_{night} as a percentage of g_{day} was significantly reduced by salinity. Instantaneous WUE was affected by the treatments ($P = 0.03$) but was not different among genotypes ($P = 0.20$).

Correlation of g_{night} with salt tolerance and other gas exchange traits

No significant trend between g_{night} and mean salinity tolerance among genotypes was observed in any treatment ($r = –0.03$, $P = 0.96$; $r = 0.13$, $P = 0.70$; and $r = 0.23$, $P = 0.57$, for the 7, 300 and 600 mM treatments, respectively; Table 1; Fig. 2). Instantaneous WUE and A also were not correlated with salt tolerance in any treatment ($r < 0.17$, $P > 0.75$ and $r < 0.60$, $P > 0.20$ for all treatments, respectively). Within treatments, g_{night} was negatively correlated with A in the 7 and 600 mM treatments ($r = –0.93$, $P = 0.01$ and $r = –0.96$, $P = 0.003$, respectively), but not in the 300 mM treatment ($r = 0.02$, $P = 0.98$; Fig. 3). However, g_{night} was not correlated with g_{day} within any treatment ($r = –0.67$, $P = 0.14$; $r = 0.16$, $P = 0.76$; and $r = –0.69$, $P = 0.13$ for the 7, 300 and 600 mM treatments, respectively; Fig. 3).

Discussion

The genotypes of saltgrass studied here exhibited genetic variation in g_{night} and, as expected, salinity reduced g_{night} similarly to water deficit treatments applied to other species (wheat, Rawson and Clarke 1988; *Quercus*, Cavender-Bares *et al*. 2007; *Helianthus*, Howard and Donovan 2007). However, reduction in g_{night} with increasing salinity stress was only observed in two genotypes, and g_{day} was affected by salinity in only three of the six genotypes, despite substantial reductions in growth in the higher salinity treatments in all six genotypes. Saltgrass is an extremely salt-tolerant species which is not uncommon in environments where soil salinity is at the upper range of our treatments. Thus, the lack of a stomatal response to such high salinity levels in many genotypes is quite surprising.

Higher salt tolerance was expected to correspond with greater ability to maintain g_{night} at higher salinity levels, but this was not observed (Table 1; Fig. 2). Leaf Na and Na exudation rate were similarly uncorrelated with salt tolerance or the magnitude of g_{night}. It is possible that the low number of genotypes used in the study may limit our ability to detect a correlation between magnitude of g_{night} and degree of salt tolerance. However, the dramatic differences between genotypes in the effects of salinity on vegetative growth (Fig. 1) and inflorescence production suggests that the lack of trend is not simply due to a small number of genotypes. We note that g_{night} as a percent of g_{day} was not affected by salinity in the three most salt tolerant genotypes, whereas it was reduced 29–52% in the three least salt tolerant genotypes.

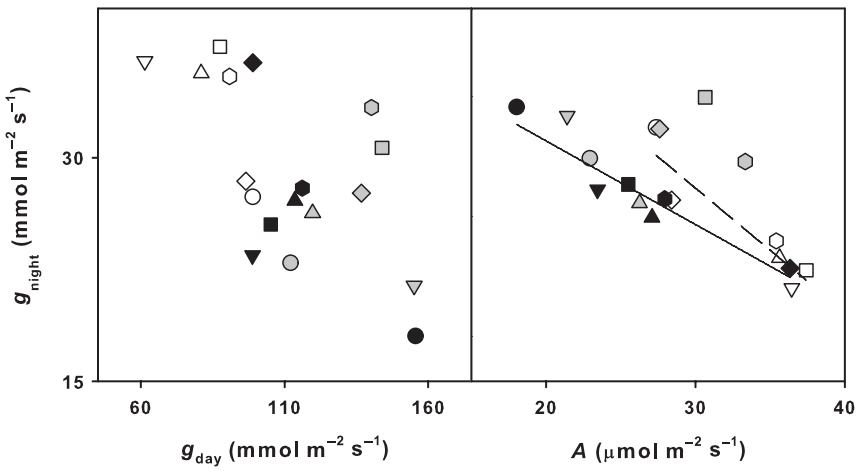

Fig. 3. Night-time leaf conductance (g_{night}) *v*. daytime leaf conductance (g_{day}) and photosynthesis (A) for six *Distichlis* genotypes in three salinity treatments. Symbols are means ± s.e. ($n = 6$) for each genotype (denoted by shape) with treatments represented as follows: open, 7 mM NaCl; grey, 300 mM NaCl; black, 600 mM NaCl. Lines represent significant correlations between A and g_{night} for the 7 mM (dashed) and the 600 mM (solid) treatments.

Although growth in all genotypes decreased with increasing salinity (Fig. 1), g_{day} was stimulated by the 300 mM treatment compared with the control and remained very similar to control plants even in the 600 mM treatment in three genotypes. Stimulation of g_{day} by salinity is not uncommon in halophytes, although most typically experience a reduction in g_{day} at some level of salinity (Ungar 1991). In contrast with the stimulation of g_{day}, g_{night} was reduced at the higher salinity levels in two of these genotypes. The opposing trends found here may be unique to halophytes subjected to salinity treatments, although the separate regulation of g_{day} and g_{night} may extend to other species and environmental conditions.

If separate regulation of g_{day} and g_{night} is possible it would imply that high g_{night} is not necessarily linked to high g_{day}. Strong, positive correlations between g_{night} and g_{day} have been observed among species (Snyder *et al*. 2003; Jordan *et al*. 2004) as well as among accessions of a single species (Christman *et al*. 2008). The reason for the relationship is unclear, though suggested possibilities for the g_{day}-g_{night} relationship at least among accessions of a single species include factors such as stomatal size and density. The use of near isogenic lines (NILs) has demonstrated that specific genetic factors can disrupt the g_{day}-g_{night} relationship (Christman *et al*. 2008), but how an environmental stress can affect the relationship in a more short-term manner has not previously been investigated. Here, we found no relationship between g_{night} and g_{day} among the six genotypes within any of the salinity treatments (Fig. 3).

It also has been suggested that the g_{day}-g_{night} relationship is due to daytime photosynthate production, with high leaf starch content being correlated with higher g_{night} in *Vicia faba* (Easlon and Richards 2008). Marks and Lechowicz (2007) correlated higher sap flow with higher leaf nitrogen and dark respiration across 21 temperate tree species, suggesting that g_{night} may have a role in dark respiration. However, in this study we found a negative relationship between g_{night} and A in the 7 and 600 mM treatments (Fig. 3), suggesting that higher photosynthate production during the day did not strictly influence stomatal opening under salinity stress.

Leaf conductance is a combination of two parallel conductance components, one actively regulated by the plant in the short-term (stomatal conductance, $g_{stomatal}$) and one not (cuticular conductance, $g_{cuticular}$). Although $g_{cuticular}$ was not directly measured in this study, typical values in other species range from 4–20 mmol m^{-2} s^{-1} (see Caird *et al*. 2007*a*). In the four genotypes in which increasing salinity did not decrease g_{night}, stomata may have been closed as much as is possible and thus the values of g_{night} measured may largely reflect cuticular water loss in these species. However, all but one of these genotypes also showed no effect of salinity on g_{day}, suggesting that the lack of effect may not have simply been due to inability to further close stomata at night.

This study combines an investigation of within-species variation in g_{night} with an examination of the effects of environmental stress on g_{night}. Our results show that in saltgrass g_{night} can apparently be regulated and reduced even when g_{day} is increased or not affected by stress. The variable effects of salinity stress across the six genotypes highlights the importance of recognising that interpretations of naturally occurring variation either among or within species can be confounded by interactions between genotypes and environments. These results suggest that studies exploiting environmentally-induced variation need to specifically consider genetic variation among populations, possible interactions with environmental stress, and consequences of these effects on the various implications of g_{night}.

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References

Barbour MM, Buckley TN (2007) The stomatal response to evaporative demand persists at night in *Ricinus communis* plants with high nocturnal conductance. *Plant, Cell & Environment* **30**, 711–721. doi: 10.1111/j.1365-3040.2007.01658.x

Caird MA, Richards JH, Donovan LA (2007*a*) Night-time stomatal conductance and transpiration in C_3 and C_4 plants. *Plant Physiology* **143**, 4–10. doi: 10.1104/pp.106.092940

Caird MA, Richards JH, Hsiao TC (2007*b*) Significant transpirational water loss occurs throughout the night in field-grown tomato. *Functional Plant Biology* **34**, 172–177. doi: 10.1071/FP06264

Cavender-Bares J, Sack L, Savage J (2007) Atmospheric and soil drought reduce nocturnal conductance in live oaks. *Tree Physiology* **27**, 611–620.

Christman MA, Richards JH, McKay JK, Stahl EA, Juenger TE, Donovan LA (2008) Variation among *Arabidopsis thaliana* accessions in night-time leaf conductance and correlations with ecophysiological and environmental characters. *Plant, Cell & Environment* **31**, 1170–1178. doi: 10.1111/j.1365-3040.2008.01833.x

Dahlgren RA, Richards JH, Yu Z (1997) Soil and groundwater chemistry and vegetation distribution in a desert playa, Owens Lake, California. *Arid Soil Research and Rehabilitation* **11**, 221–244.

Dawson TE, Burgess SSO, Tu KP, Oliveira RS, Santiago LS, Fisher JB, Simonin KA, Ambrose AR (2007) Night-time transpiration in woody plants from contrasting ecosystems. *Tree Physiology* **27**, 561–575.

Dickey J, Hall M, Madison M, Smesrud J, Griswold M, *et al*. (2005*a*) Stabilizing Owens Dry Lake surface with irrigated saltgrass. Part 1: Existing conditions and the challenges of establishing saltgrass on the playa. *Ecesis* **15**, 1–9.

Dickey J, Hall M, Madison M, Smesrud J, Griswold M, *et al*. (2005*b*) Stabilizing Owens Dry Lake surface with irrigated saltgrass. Part II: The managed vegetation project. *Ecesis* **15**, 1–9.

Donovan LA, Grisé DJ, West JB, Pappert RA, Alder NN, Richards JH (1999) Predawn disequilibrium between plant and soil water potentials in two cold-desert shrubs. *Oecologia* **120**, 209–217. doi: 10.1007/s004420050850

Donovan LA, Richards JH, Linton MJ (2003) Magnitude and mechanisms of disequilibrium between predawn plant and soil water potentials. *Ecology* **84**, 463–470. doi: 10.1890/0012-9658(2003)084[0463:MAMODB]2.0.CO;2

Easlon HM, Richards JH (2008) Photosynthesis affects following night leaf conductance in *Vicia faba*. *Plant, Cell and Environment*, in press. doi: 10.1111/j.1365-3040.2008.01895x

Epstein E (1972) 'Mineral nutrition of plants: principles and perspectives.' (Wiley: New York)

Fisher JB, Baldocchi DD, Misson L, Dawson TE, Goldstein AH (2007) What the towers don't see at night: nocturnal sap flow in trees and shrubs at two AmeriFlux sites in California. *Tree Physiology* **27**, 597–610.

Hedges LV, Gurevitch J, Curtis PS (1999) The meta-analysis of response ratios in experimental ecology. *Ecology* **80**, 1150–1156.

Howard AR, Donovan LA (2007) *Helianthus* night-time conductance and transpiration respond to soil water but not nutrient availability. *Plant Physiology* **143**, 145–155. doi: 10.1104/pp.106.089383

Jordan GJ, Brodribb TJ, Loney PE (2004) Water loss physiology and the evolution within the Tasmanian conifer genus *Athrotaxis* (Cupressaceae). *Australian Journal of Botany* **52**, 765–771. doi: 10.1071/BT04029

Kavanagh KL, Pangle R, Schotzko AD (2007) Nocturnal transpiration causing disequilibrium between soil and stem predawn water potential in mixed conifer forests of Idaho. *Tree Physiology* **27**, 621–629.

Marks CO, Lechowicz MJ (2007) The ecological and functional correlates of nocturnal transpiration. *Tree Physiology* **27**, 577–584.

Moore GW, Cleverly JR, Owens MK (2008) Nocturnal transpiration in riparian *Tamarix* thickets authenticated by sap flux, eddy covariance and leaf gas exchange measurements. *Tree Physiology* **28**, 521–528.

Neter J, Wasserman W, Kutner MH (1990) 'Applied linear statistical models: regression, analysis of variance, and experimental design.' (Irwin: Homewood, IL)

Rawson HM, Clarke JM (1988) Nocturnal transpiration in wheat. *Australian Journal of Plant Physiology* **15**, 397–406.

SAS (2001) 'SAS/STAT user's guide. Version 8.' (SAS Institute: Cary, NC)

Scholz FG, Bucci SJ, Goldstein G, Meinzer FC, Franco AC, Miralles-Wilhelm F (2007) Removal of nutrient limitations by long-term fertilization decreases nocturnal water loss in savanna trees. *Tree Physiology* **27**, 551–559.

Snyder KA, Richards JH, Donovan LA (2003) Night-time conductance in C_3 and C_4 species: do plants lose water at night? *Journal of Experimental Botany* **54**, 861–865. doi: 10.1093/jxb/erg082

Snyder KA, James JJ, Richards JH, Donovan LA (2008) Does hydraulic lift or night-time transpiration facilitate nitrogen acquisition? *Plant and Soil* **306**, 159–166. doi: 10.1007/s11104-008-9567-7

Ungar IA (1991) 'Ecophysiology of vascular halophytes.' (CRC Press: Boca Raton)

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