

Inter- and intra-specific variation in nocturnal water transport in *Eucalyptus*

NATHAN G. PHILLIPS,^{1,2,5} JAMES D. LEWIS,³ BARRY A. LOGAN⁴ and DAVID T. TISSUE¹

¹ Centre for Plants and the Environment, University of Western Sydney, Richmond, NSW 2753, Australia

² Department of Geography and Environment, Boston University, 675 Commonwealth Avenue, Boston, MA 02215, USA

³ Department of Biological Sciences, Fordham University, 441 E. Fordham Rd, Bronx, NY 10458, USA

⁴ Biology Department, Bowdoin College, 6500 College Station, Brunswick, ME 04011, USA

⁵ Corresponding author (nathan@bu.edu)

Received June 24, 2009; accepted January 25, 2010; published online March 23, 2010

Summary To identify environmental and biological drivers of nocturnal vapour exchange, we quantified intra-annual, intra- and inter-specific variation in nocturnal water transport among ecologically diverse *Eucalyptus* species. We measured sap flux (J_s) and leaf physiology (leaf surface conductance (g_s), transpiration (E) and water potential (Ψ_l)) in three to five trees of eight species. Over 1 year, nocturnal J_s ($J_{s,n}$) contributed 5–7% of total J_s in the eight species. The principal environmental driver of $J_{s,n}$ was the product of atmospheric vapour pressure deficit (D) and wind speed (U). Selected observations suggest that trees with higher proportions of young foliage may exhibit greater $J_{s,n}$ and nocturnal g_s ($g_{s,n}$). Compared with other tree taxa, nocturnal water use in *Eucalyptus* was relatively low and more variable within than between species, suggesting that (i) *Eucalyptus* as a group exerts strong nocturnal stomatal control over water loss and (ii) prediction of nocturnal flux in *Eucalyptus* may depend on simultaneous knowledge of intra-specific tree traits and nocturnal atmospheric conditions.

Keywords: conductance, *Eucalyptus*, nocturnal, phenology, stomata, transpiration.

Introduction

The nocturnal physiology of plants is a topic of continuing interest (Phillips et al. 2007). Evidence has accumulated that nocturnal vapour exchange is a common phenomenon (Dawson et al. 2007) and has been considered to be potentially significant for plant and ecosystem carbon relations (e.g., Turnbull et al. 2004, Whitehead et al. 2004, Barbour et al. 2005, Seibt et al. 2007), water relations and surface hydrology (Oren et al. 1999, Daley and Phillips 2006, Cuntz et al. 2007, Dawson et al. 2007, Oishi et al. 2008, Zeppel et al. 2008) and plant nutrient relations (e.g., Scholz et al. 2007).

Although it is a long-recognized phenomenon (Darwin 1898, Levitt 1967, Gindell 1970), the ecological significance of nocturnal stomatal opening is unclear. Results from some studies are consistent with the general hypothesis that nocturnal vapour exchange is linked to resource acquisition (Dawson et al. 2007) and is a trait upon which natural selection may operate (Christman et al. 2008). Yet several aspects of nocturnal vapour exchange remain puzzling. One key unknown is the degree of intra- vs inter-specific variation in nocturnal vapour exchange among co-occurring trees and/or species. Daley and Phillips (2006) observed large nocturnal leaf surface conductance ($g_{s,n}$) in all individuals of one tree species, but not in all individuals of two other tree species within the same plot, although all trees experienced very similar micro-environmental conditions. That study could not conclusively identify ecological or physiological factors that contributed to dramatically different inter-specific nocturnal behaviours, and little is known about whether this substantial inter-specific variation in nocturnal physiology—and lack of intra-specific variation—is an exceptional case or not.

To further investigate intra- and inter-specific variation in nocturnal vapour exchange, here we compared diurnal and nocturnal sap flux and vapour exchange of eight contrasting tree species of *Eucalyptus*, ranging from riverine phreatophytes to xeric woodland species (Table 1), which were grown in a common plantation. These eight species belong to the subgenus *Symphyomyrtus*, which contains generally more xeric species than the subgenus *Monocalyptus* (Noble 1989). One conspicuous aspect of intra-specific variation in *Eucalyptus* is foliar phenology, which can be highly asynchronous among individuals (Jacobs 1955). Physiological changes with leaf age have long been recognized (Field 1985), but little is known about differences in nocturnal physiology with leaf age. Our objectives were to (i) evaluate the magnitude of nocturnal water loss within and between species of *Eucalyptus* on a seasonal basis, (ii) identify meteorological drivers of nocturnal vapour exchange and (iii) de-

Table 1. Typical growth characteristics and distribution of eucalypt species.

Species (common name)	Height (m)	d.b.h. (m)	Altitude (m)	Rainfall (mm a ⁻¹)	Habitat/location
<i>E. argophloia</i> (Scrub/White Gum)	to 40	1	300–340	700	Woodland or open forest
<i>E. camaldulensis</i> (River Red Gum)	to 20	1–2	20–700	250–600	Riverine phreatophyte
<i>E. dunnii</i> (Dunn's White Gum)	50	1–1.5	300–750	1000–1750	Forest species of fertile valleys and lower slopes
<i>E. globulus</i> (Maiden's Gum)	30–75	to 2.5	200–900	800–1200	Forests of coastal escarpments
<i>E. grandis</i> (Flooded Gum)	45–75	1.2–2	0–1100	1000–3500	Coastal flats, slopes of fertile valleys
<i>E. occidentalis</i> (Swamp Yate)	10–20	to 0.5	0–300	300–800	Woodland alluvial flats subject to flooding
<i>E. sideroxylon</i> (Red Ironbark)	10–25	1	0–1000	1000–1750	Inland xeric species
<i>E. tereticornis</i> (Forest Red Gum)	20–50	2	0–1000	650 to >3000	Open forests or scattered trees on alluvial flats

Table reproduced from Sefton (2003).

termine whether any differences in the magnitude of nocturnal water loss within or between species reflected ecological and/or physiological attributes of those trees and/or species.

Materials and methods

Site description

Research was conducted at the Hawkesbury Forest Experiment site, a climate change research facility on the campus of the University of Western Sydney, Richmond NSW, Australia. Within this facility, we made measurements in the Sefton Plantation (33°36'20.3" S, 150°44'11.5" E), a 1.3-ha forest plantation which was established in April 2000 (Sefton 2003); trees were between 7 and 8 years old during the study period (November 2007–July 2008). Twenty individuals of each of eight *Eucalyptus* species (*E. argophloia* Blakely, *E. camaldulensis* Dehnh., *E. dunnii* Maiden, *E. globulus* Labill. subsp. *maidenii* (F. Muell) Kirkpatr., *E. grandis* W. Hill ex Maiden, *E. occidentalis* Endl., *E. sideroxylon* Cunn. ex Woolls subsp. *sideroxylon* and *E. tereticornis* Smith) were initially planted in adjoining 10 × 16 m plots, which were located within a 40 × 48 m plantation block. Three to six healthy trees of each species were used for bole sap flow measurements (Table 2), and of these sap flow trees, the crowns of three to four trees were accessed by towers for leaf vapour exchange measurements.

Soil at this site is classified as a Blackendon sand extending to 0.9 m depth, underlain by a clay hardpan. Elevation of the site is 25 m a.s.l. Mean annual temperature is 17 °C; the maximum and minimum mean monthly temperatures are 29 and 3 °C, respectively. Mean annual rainfall is 801 mm. Spring 2007 through winter 2008 was abnormally wet, with 19% more accumulated rainfall between 1 November 2007 and 31 July 2008 than the long-term average (Australian Government Bureau of Meteorology, Richmond—UWS Hawkesbury Station; <http://www.bom.gov.au>) and 63% more than the long-term median rainfall. An important consequence of the unusually wet 2007–08 period for this study was that soil moisture was high and unlikely to generate large or persistent plant water stress.

Sap flux (J_s)

J_s was measured in boles using thermal dissipation probes as described in Granier (1985, 1987). In determining J_s , we chose to maximize sampling of hydro-active sapwood in the greatest number of trees, rather than scaling J_s within individual trees (Phillips et al. 1996). Therefore, a single 20-mm long probe was installed into each tree in the outermost hydro-active sapwood in each bole. Previous data on *Eucalyptus* suggest that this sapwood sampling strategy measured the region of highest sap flux density (Bamber 1985, Wilkins 1991, Zang et al. 1996, Benyon 1999). Electrical current sent to the sensors was adjusted to dissipate ca. 200 mW of heat into hydro-active sapwood; sensors were shielded from solar radiation using Styrofoam and reflective bubble wrap insulation.

Twenty-four trees (i.e., three trees from each of eight species) were equipped with sap flow sensors on 5 November 2007; sampling was increased to 38 trees on 12 December 2007 (Table 2). Voltages from sap flow sensor thermocouples were read every 30 s and averaged and recorded every 2–5 min with a CR3000 data logger connected to two AM416 multiplexers (Campbell Scientific Inc., Logan, UT, USA). These voltages, proportional to temperature differences between heated and unheated probes in each tree, were used to estimate sap flux density (J_s , g m⁻² s⁻¹) using the empirical calibration described in Granier (1987) and re-evaluated by Clearwater et al. (1999). Data used for 24-h sap flux analysis (i.e., 24-h periods where the majority of sensors were fully operational) included the following number of days in each season: (i) spring (21 days; 21 September 2007–20 December 2007), (ii) summer (57 days; 21 December 2007–20 March 2008), (iii) autumn (41 days; 21 March 2008–20 June 2008) and (iv) winter (22 days; 21 June 2008–20 September 2008).

Estimates of sap flux using the Granier method are referenced to a 'zero baseline' value of temperature difference between a heated and reference probe. This baseline was set on nights when atmospheric vapour pressure deficit (D) was zero for several hours, so that there was no driving force for sap flux. Zero baselines on these nights were set as late into the night as dew point persisted, after baselines reached stable values.

Table 2. Biometric information on trees used in this study.

Species	Number of sap flow trees	Diameter at 1.3 m (cm)	Height (m)
<i>E. argophloia</i>	5	13.3 (2.2)	9.9 (0.8)
<i>E. camaldulensis</i>	5	13.5 (2.7)	10.8 (0.7)
<i>E. dunnii</i>	5	17.2 (3.0)	12
<i>E. globulus</i>	4	17.0 (2.5)	13
<i>E. grandis</i>	6	16.1 (2.4)	14.2 (1.1)
<i>E. occidentalis</i>	3	6.5 (1.7)	6.0 (0.4)
<i>E. sideroxylon</i>	5	9.8 (1.3)	7.7 (0.6)
<i>E. tereticornis</i>	5	12.1 (1.9)	11

Measurements were made in May 2008. Values are mean (\pm SD). Heights for *E. dunnii*, *E. globulus* and *E. tereticornis* are based on visual estimates.

Leaf physiology

Two 24-h campaigns (2 and 11 January 2008) were conducted to directly evaluate diurnal and nocturnal leaf physiology. Aluminium crown access towers (6–11 m tall) with work platforms were erected to access three to four trees of six *Eucalyptus* species: *E. argophloia* (four trees), *E. camaldulensis* (three), *E. dunnii* (four), *E. grandis* (four), *E. sideroxylon* (three) and *E. tereticornis* (three). From each tree, two healthy, fully expanded, upper crown leaves were chosen for repeated measurements of leaf surface conductance (g_s , $\text{mol m}^{-2} \text{s}^{-1}$) and transpiration (E , $\text{mmol m}^{-2} \text{s}^{-1}$). Vapour exchange measurements were conducted every ca. 3 h using four LI-6400 Portable Photosynthesis Systems (LICOR, Inc., Lincoln, NE, USA) commencing at 07:00 and ending at 04:00 the following morning. The same two leaves were used for measurement every 3 h and during both dates, except for rare occasions when leaf damage necessitated using a new leaf. Generally, mature leaves were measured, which were dark green with a dull sheen. Occasionally, vapour exchange of fully expanded, young, bright green and glossy young leaves was measured for comparison. During each measurement period, cuvette block temperatures and photosynthetically active radiation (Q , $\mu\text{mol m}^{-2} \text{s}^{-1}$) levels were maintained at current ambient conditions for the site; reference $[\text{CO}_2]$ was set to $400 \mu\text{mol mol}^{-1}$.

At night, to the extent that E and D are small, measurement of their ratio to compute g_s is prone to error. To minimize error during conditions of low E and D at night, the cuvette air flow rate was reduced to $300 \mu\text{mol s}^{-1}$ (from daytime values of $500 \mu\text{mol s}^{-1}$), sample and reference cells were 'matched' prior to each leaf $g_{s,n}$ measurement, $g_{s,n}$ readings were recorded after leaves stabilized within the leaf cuvette for at least 5 min and five subsample measurements of leaf vapour exchange separated in time by 10 s each were made on each leaf.

Nocturnal leaf vapour conductance could be partially due to cuticular conductance (g_{cut}). To estimate g_{cut} in *Eucalyptus*, we conducted leaf water loss measurements on five mature, sun-grown, fully expanded leaves from saplings of each

of eight *Eucalyptus* species (*E. cladocalyx*, *E. crebra*, *E. dunnii*, *E. grandis*, *E. melliodora*, *E. saligna*, *E. sideroxylon* and *E. tereticornis*). The saplings were 1.5 years old and also located at the Hawkesbury Forest Experiment. Leaf mass loss was monitored over time, beginning immediately after leaf cutting, until sharp curvilinear declines in mass, presumably associated with stomatal closure, approached a linear phase of mass loss, reflecting cuticular loss in addition to any residual stomatal water loss. Vapour conductance was computed from leaf water loss and laboratory vapour pressure deficit as:

$$g_{\text{cut}} = E_{\text{cut}} PD^{-1}$$

where E_{cut} is mass loss per projected leaf area ($\text{mol m}^{-2} \text{s}^{-1}$) and P is atmospheric pressure (kPa). This equation is valid when leaf temperature is close to air temperature, which was likely in the laboratory setting.

Bulk leaf water potential (Ψ_l , MPa) was measured at mid-day (ca. 13:00–14:00) and pre-dawn the following day (ca. 02:00–03:00) during 2–3 and 11–12 January 2008. Leaves adjacent to vapour exchange leaves were detached, placed in a foil-laminate bag (PMS Instrument Company, Corvallis, OR, USA) and transferred to the ground for immediate bulk leaf water potential measurement with a Scholander-style pressure chamber (PMS Instrument Company, Corvallis, OR, USA). Additionally, on the evening preceding each pre-dawn measurement, a second leaf was sealed into a foil-laminate bag while still attached to the tree to induce a saturating vapour environment in the immediate vicinity of that leaf and to promote equilibration of leaf with twig xylem water potential. During pre-dawn measurements of Ψ_l , water potential of bagged and unbagged leaves were compared to evaluate whether nocturnal evaporative demand promoted leaf transpiration and consequent disequilibrium of leaf water potential with twig water potential in unbagged leaves.

Meteorological data

Meteorological data were collected at a weather station within the Hawkesbury Forest Experiment site, located ca. 300 m away from the Sefton Plantation. Air temperature (T_a , °C) and relative humidity (h , %) were measured with a HMP45C probe (Vaisala Inc., Helsinki, Finland). Photosynthetically active radiation (Q , $\mu\text{mol m}^{-2} \text{s}^{-1}$) was measured with a LI190SA quantum probe (LICOR, Lincoln, NE, USA). Wind speed (U , in metres per second) was measured with a cup anemometer mounted in an exposed area at 4 m height. All sensors were sampled with an input/output module (SIOX S12; Telefrang AB, Göteborg, Sweden), and data were downloaded onto a personal computer. The base sampling rate was 60 s and meteorological data were averaged every 5 min. Vapour pressure deficit (D) was computed from air temperature and relative humidity using a polynomial equation.

Table 3. Total daily sap flux (24 h, $\text{Mg H}_2\text{O m}^{-2}$ sapwood d^{-1}) and nocturnal percentages (% night) of total daily sap flux and corresponding nocturnal meteorological variables by season. Values in parentheses are standard deviations (and coefficients of variation (COV) where specified).

Species	Season				
	Spring	Summer	Autumn	Winter	Annual
<i>E. argophloia</i> —24-h	4.1 (1.3)	7.2 (0.9)	5.2 (1.2)	3.9 (1.0)	5.1 (1.5)
% night	6.1 (4.3)	5.2 (2.2)	6.2 (2.6)	1.9 (0.9)	4.9 (2.0)
<i>E. camaldulensis</i> —24-h	3.6 (1.6)	5.9 (1.0)	3.2 (1.2)	1.3 (0.4)	3.5 (1.9)
% night	5.7 (3.6)	4.9 (1.6)	8.9 (3.9)	6.2 (2.4)	6.4 (1.7)
<i>E. dunni</i> —24-h	4.0 (1.5)	6.3 (1.6)	3.6 (2.4)	3.9 (1.0)	4.5 (1.2)
% night	7.0 (4.0)	5.8 (2.3)	14.2 (7.1)	2.0 (0.7)	7.3 (5.1)
<i>E. globulus</i> —24-h	4.0 (1.3)	6.6 (1.2)	3.0 (1.6)	4.0 (1.3)	4.4 (1.5)
% night	4.6 (3.2)	5.3 (2.3)	8.4 (3.9)	1.7 (0.3)	5.0 (2.8)
<i>E. grandis</i> —24-h	4.4 (1.4)	6.3 (2.1)	4.1 (1.2)	4.7 (1.3)	4.9 (1.0)
% night	5.4 (2.8)	7.6 (4.3)	7.7 (2.9)	1.7 (0.7)	5.6 (2.8)
<i>E. occidentalis</i> —24-h	3.4 (1.6)	4.7 (1.4)	3.1 (1.3)	1.2 (0.4)	3.1 (1.4)
% night	7.8 (3.8)	6.2 (3.0)	7.6 (3.9)	6.4 (1.9)	7.0 (0.8)
<i>E. sideroxylon</i> —24-h	4.6 (1.8)	7.1 (2.8)	2.5 (1.4)	2.7 (0.6)	4.2 (2.1)
% night	8.8 (4.7)	6.5 (3.6)	9.9 (3.6)	3.6 (2.6)	7.2 (2.8)
<i>E. tereticornis</i> —24-h	3.8 (1.8)	6.2 (1.3)	3.4 (1.5)	2.5 (0.7)	4.0 (1.6)
% night	7.3 (6.1)	5.3 (1.9)	10.6 (6.2)	3.5 (2.0)	6.7 (3.0)
Average—24-h	4.0	6.3	3.5	3.0	4.2
Intra-specific—24-h (SD, COV)	(1.5, 38)	(1.5, 24)	(1.5, 43)	(0.8, 27)	(1.5, 36)
Inter-specific—24-h (SD, COV)	(0.4, 10)	(0.8, 17)	(0.8, 23)	(1.3, 43)	(0.7, 17)
Average—% night	6.6	5.9	9.2	3.4	6.3
Intra-specific—% night (SD, COV)	(4.1, 62)	(2.7, 44)	(4.3, 46)	(1.6, 45)	(2.4, 38)
Inter-specific—% night (SD, COV)	(1.4, 21)	(0.9, 15)	(2.4, 26)	(1.9, 56)	(1.0, 16)
<i>Meteorological conditions</i>					
Wind (U , 24-h avg, m s^{-1})	1.1	0.89	0.89	1.1	1.0
% night	23	20	28	38	27
Vapour pressure deficit (D , 24-h avg, kPa)	0.64	0.89	0.49	0.37	0.60
% night	23	21	22	19	21
$U \times D$ (24-h avg, kPa m s^{-1})	0.84	1.2	0.70	0.80	0.89
% night	14	11	13	7	11.3

Values for sap flux represent averages of three to six trees per species.

In this study, we defined ‘night’ to be characterized by $Q < 1 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Method for partitioning storage recharge vs nocturnal transpiration

Total $J_{s,n}$ was calculated as the sum of E_n and recharge of tree hydraulic capacitance (Daley and Phillips 2006). To provide estimates of recharge vs E_n , the following method was employed: two consecutive days characterized by similarly high daytime D , but contrasting nocturnal D (i.e., high D on the first night, low D on the second night) were identified during the late spring period. J_s extending into nocturnal hours of the first, high- D night was assumed to reflect a combination of E and storage recharge. J_s extending into nocturnal hours of the second, low- D night was assumed to reflect primarily storage recharge, to the extent that there was little to no nocturnal physical driving force for E_n . Because the two days were consecutive, characterized by similar daytime meteorological and

soil moisture conditions, the recharge estimate from the second, low- D night was deemed appropriately superimposable upon the recharge-plus-transpiration estimate from the first, high- D night, allowing deconvolution of nocturnal $J_{s,n}$ into recharge and E_n .

There are several limitations to the above-described method. This method likely overestimates recharge relative to E_n , since we attributed all nocturnal flux on the low- D night to recharge although we observed some measureable D (and thus driving force for E_n) on every night following a high- D day. Thus, overestimates of recharge yield conservative estimates of E_n . This method may also overestimate recharge by considering sap flux dynamics during 2 days characterized by higher than average daytime evaporative demand and E . Finally, since soil moisture was high throughout this study due to unusually high precipitation, we could not assess the degree to which recharge varies with variation in soil moisture (e.g., Phillips et al. 1996).

Results

Inter-specific and seasonal variation in nocturnal sap flow

Averaged across all eight species over the year-long study period, $J_{s,n}$ averaged ca. 6% of total J_s (Table 3). Species variation in $J_{s,n}$ was remarkably small, ranging from a minimal annual average of 4.9% (*E. argophloia*) to a maximum of 7.3% (*E. dunnii*). More pronounced was seasonal variation in $J_{s,n}$ (Table 3). Averaged across species, $J_{s,n}$ was highest in autumn (9.2% of total J_s), followed by spring (6.6%), summer (5.9%) and winter (3.4%). In contrast to moderate seasonal variation in $J_{s,n}$, nocturnal D varied little among seasons (as a percentage of 24-h D ; Table 3). Better correspondence to seasonal variation in $J_{s,n}$ was observed in ($U \times D$), especially in the low values of both $J_{s,n}$ and ($U \times D$) in winter (Table 3).

On selected nights when $J_{s,n}$ was expected to be largest (i.e., nights of elevated D and U), in most species and seasons, a decreasing 'tail' of J_s extended into night hours, indicating some level of nocturnal recharge (Figure 1). In several cases (especially the night of 6–7 January 2008; Figure 1), $J_{s,n}$ increased during the night after reaching a minimum, in concert with nocturnal increase in D and U , indicating E_n .

Partitioning nocturnal transpiration and storage recharge

Recharge of hydraulic capacitance, estimated from two consecutive days of ideal diurnal and nocturnal weather conditions (night of small D following night of large D ; 14–15 December 2007), averaged 33% of total $J_{s,n}$, ranging from a minimum of 16% (*E. occidentalis*) to a maximum of 53% (*E. argophloia*) (Figure 2). Variation in estimated recharge as a proportion of total $J_{s,n}$ was unrelated to tree size (e.g., tree height; $P = 0.38$) or ecological attributes of the species (e.g., habitat water availability) (Brooker and Kleinig 1999, 2001).

Nocturnal leaf physiology

Leaf surface conductance (g_s , $\text{mol m}^{-2} \text{s}^{-1}$) was 86% lower at night compared with daytime in the six species evaluated over two 24-h periods (Figure 3; first 24-h period shown). Over both nights and across species, $g_{s,n}$ averaged $18 \text{ mmol m}^{-2} \text{s}^{-1}$, while daytime g_s averaged $135 \text{ mmol m}^{-2} \text{s}^{-1}$. Over both nights and across all species, leaf E_n averaged 2.5% of the 24-h total leaf E (obtained by time integration of data in Figure 3). By species, the nocturnal sum of leaf E_n , expressed as a percentage of 24-h leaf E , was correlated with species-specific $J_{s,n}$ ($r^2 = 0.73$; $P = 0.031$), but not with estimates of the nocturnal sum of E_n estimated from sap flux ($P = 0.21$; relationship not shown). Median estimated g_{cut} from eight eucalypt species was $3.3 \text{ mmol m}^{-2} \text{s}^{-1}$, ranging from 1.5 to $5.6 \text{ mmol m}^{-2} \text{s}^{-1}$ (*E. cladocalyx*, *E. saligna*, respectively).

Pre-dawn Ψ_1 showed small but significant evidence of nocturnal disequilibrium with twig water potential. For example, on average, leaves open to the night air exhibited lower pre-

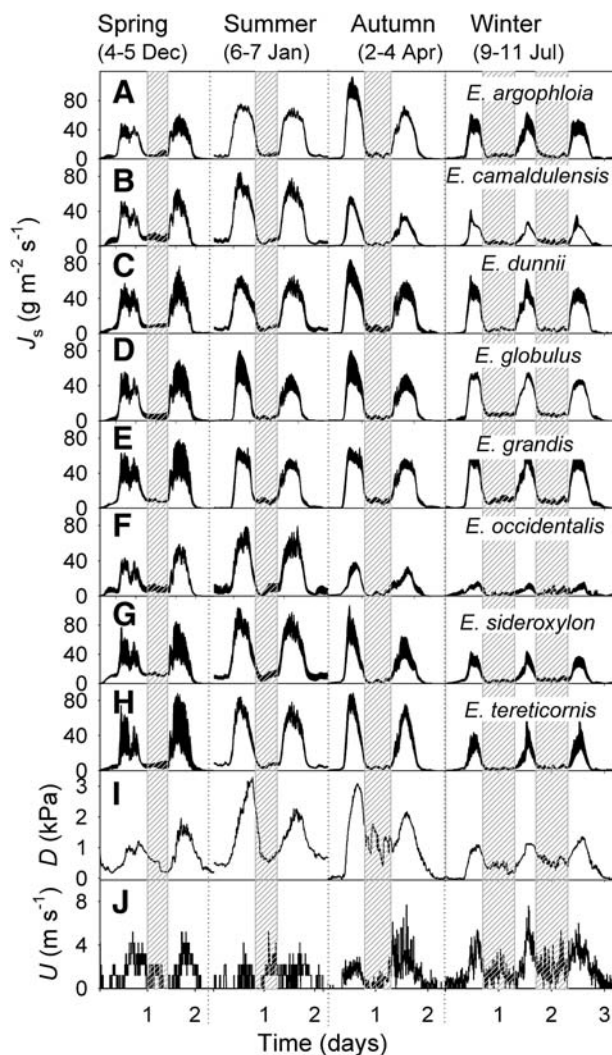


Figure 1. (A)–(H) Sap flux density (J_s , $\text{g m}^{-2} \text{s}^{-1}$) during seasonal periods (2007–08) of enhanced (I) nocturnal vapour pressure deficit (D , kPa) and (J) wind (U , m s^{-1}). Hatched periods indicate night ($Q < 1 \text{ mmol m}^{-2} \text{s}^{-1}$). In (A)–(H), line thickness represents standard errors of means of two to six trees for each species. Dashed vertical lines denote separation between seasonal periods.

dawn leaf water potential (-0.55 MPa) than bagged leaves (-0.51 MPa ; paired t -test: $P = 0.012$; $n = 21$ leaf pairs from 21 trees). Variation of mid-day and pre-dawn Ψ_1 was substantial among species (Figure 4; first 24-h period shown). There was broad correspondence of pre-dawn with mid-day Ψ_1 ; species with lower pre-dawn Ψ_1 tended to exhibit lower mid-day Ψ_1 (Figure 4). Pre-dawn Ψ_1 by species was correlated with recharge estimates (Figure 2). For example, *E. camaldulensis* and *E. tereticornis* exhibited the highest pre-dawn Ψ_1 and the smallest estimated recharge of capacitance (Figure 2). Similarly, *E. argophloia* exhibited the lowest pre-dawn Ψ_1 and one of the highest estimates of recharge (Figure 2). In contrast, pre-dawn Ψ_1 was not generally correlated with seasonally averaged estimates of $J_{s,n}$ (Table 3).

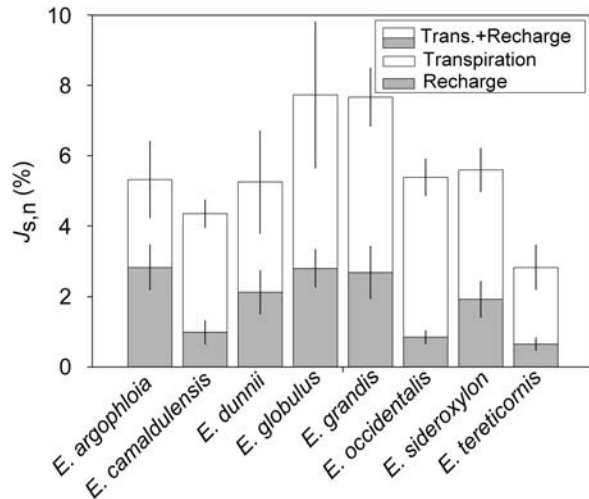


Figure 2. Estimate of nocturnal transpiration and recharge of tree water storage from nocturnal sap flow ($J_{s,n}$, % of 24-h sap flow). The sum of transpiration and recharge in a species is equal to the value of $J_{s,n}$, corresponding to the top of the open bar. Grey bars represent recharge. The distances between the tops of the grey and open bars represent transpiration. Standard errors for all bars are derived from three to six trees sampled for each species.

Linking sap flow to nocturnal physiology

During the two 24-h measurement campaigns, bole J_s and leaf level E were similarly low in the night compared with the day (Figure 5; first 24-h period shown). $J_{s,n}$, averaged over all species and the two 24-h periods, averaged 4.9% of the 24-h J_s , which was approximately double the 2.5% leaf level estimate of E_n . However, $J_{s,n}$ included both E_n and recharge, whereas the leaf measurements only included E_n . There was no significant correlation between the integrated $J_{s,n}$ over the two nights of this campaign and either pre-dawn Ψ_l , mid-day Ψ_l or mid-day-minus-pre-dawn Ψ_l ($P > 0.2$ for all).

Environmental drivers of nocturnal sap flux and sensitivity to crown phenology

Variation in $J_{s,n}$ was observed among trees within the same species (Figures 5 and 6). For example, one individual of *E. occidentalis* exhibited $J_{s,n}$, while two neighbouring individuals of *E. occidentalis* of similar size and environmental conditions did not exhibit $J_{s,n}$ (Figure 6). This phenomenon occurred only on nights when there was concurrent U and elevated D (Figure 6). In the individual tree that exhibited $J_{s,n}$ on windy, dry nights, approximately one-third of the crown consisted of newly expanded foliage, while the other two trees did not have newly expanded crown foliage. Examples of this phenomenon were also observed in *E. globulus* and *E. grandis*, reflecting asynchrony in crown phenological state among individuals within a species. Spot measurements of nocturnal g_s in *E. grandis* on 2–3 and 11–12 January 2008 supported that g_s of newly expanded leaves was more than

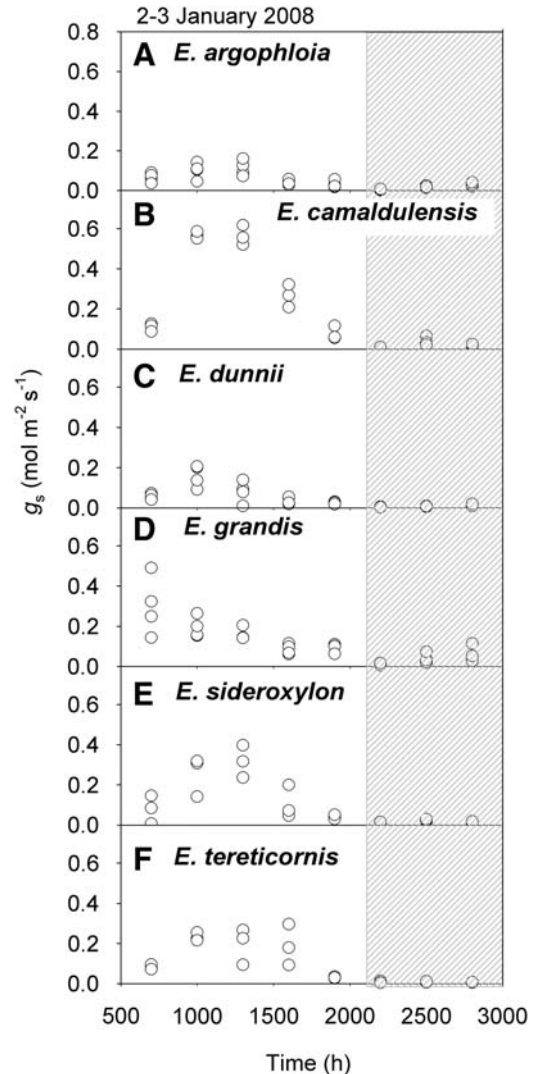


Figure 3. (A)–(F) Stomatal conductance (g_s , $\text{mol m}^{-2} \text{s}^{-1}$) during day and night (hatched zones) hours, during a 24-h sampling campaign in summer.

twice that of adjacent mature leaves: in night-time measurements (after 21:44 and 21:48 on 2 and 11 January, respectively), sample mature leaves from an *E. grandis* tree had $g_{s,n}$ of $8.4 \text{ mmol m}^{-2} \text{s}^{-1}$ (2 January) and $9.2 \text{ mmol m}^{-2} \text{s}^{-1}$ (11 January), while three young leaves on each night from the same tree had $g_{s,n}$ of $31 \text{ mmol m}^{-2} \text{s}^{-1}$ (SD = 8) (2 January) and $32 \text{ mmol m}^{-2} \text{s}^{-1}$ (SD = 11) (11 January). Supplementary measurements of nocturnal g_s performed on *E. saligna* (closely related to *E. grandis*; Eldridge et al. 1993, p. 105) saplings within 0.5 km of our principal research site showed that nocturnal g_s of newly expanded foliage was more than twice that of mature foliage (Figure 7).

Generally, $J_{s,n}$ showed greater correlation with the product of U and D (Figure 8) than with D or U alone (not shown). Specifically, averaging across seasons and species, $J_{s,n}$ was more highly correlated with $(U \times D)$ (expressed as a propor-

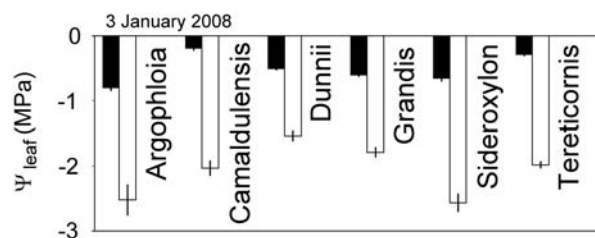


Figure 4. Pre-dawn (solid bars) and mid-day (open bars) leaf water potential (Ψ_{leaf} in MPa) measured in six *Eucalyptus* species, over a diurnal–nocturnal time course. Standard error bars are for the mean of three to four leaves/trees for each species. All values shown are for leaves that were exposed to ambient conditions (i.e., ‘unbagged’).

tion of its 24-h total; $r^2 = 0.44$, $P = 0.0002$) than with D ($r^2 = 0.30$, $P = 0.0025$) or U ($r^2 = 0.10$, $P = 0.10$) alone.

Discussion

Eucalyptus exhibits conservative nocturnal water use

Nocturnal J_s ($J_{s,n}$) in *Eucalyptus* was relatively small (ca. 6% of total J_s) and remarkably uniform among the eight ecologically diverse species growing in a common environment. These results were similar to those of Benyon (1999) who reported $J_{s,n}$ to be 5% of total J_s in *E. grandis*. Although small as a percentage of 24-h flux, our results must be placed within the context of relatively large absolute g_s and J_s observed in all eight species during daylight hours. Maximum daytime g_s of the *Eucalyptus* studied here (ca. 0.2 – 0.6 mol $\text{m}^{-2} \text{s}^{-1}$) was large compared with values reported for comparable *Eucalyptus* species (ca. 0.1 – 0.4 mol $\text{m}^{-2} \text{s}^{-1}$, summarized in Bell and Williams 1997). Most likely, high g_s in our study reflected relatively high soil moisture observed during the entire experimental period. Moreover, in general, our estimates of maximum daytime g_s were high relative to those reported for evergreen woody species (Körner et al. 1979). The average $g_{s,n}$ estimated here (18 mmol $\text{m}^{-2} \text{s}^{-1}$) was small relative to daytime g_s , but within the range of night-time g_s found in other woody species (e.g., xeric species listed in Dawson et al. 2007, their Table 2, which ranged from 0 to 40 mmol $\text{m}^{-2} \text{s}^{-1}$).

The curvilinear responses of g_s and $g_{s,n}$ to $U \times D$ indicate strong stomatal responses to increasing $U \times D$. Similarly, Bucci et al. (2004) found curvilinear responses of $g_{s,n}$ to nocturnal D . Moreover, estimates of g_{cut} suggest that stomata may play a larger role than cuticular water loss in $g_{s,n}$ in our study. Indeed, the estimates of g_{cut} here (range = 1.5 – 5.6 mmol $\text{m}^{-2} \text{s}^{-1}$) are likely overestimates of g_{cut} , since residual stomatal conductance could not be ruled out in the method we used. From the literature, the only value of g_{cut} for eucalypts we could find (*E. pauciflora* reported by Cochrane and Slayter 1988) was 6 mmol $\text{m}^{-2} \text{s}^{-1}$ —about a third of our average $g_{s,n}$. More broadly, in a survey of 57 non-crop species, when contributions of stomata could be physically

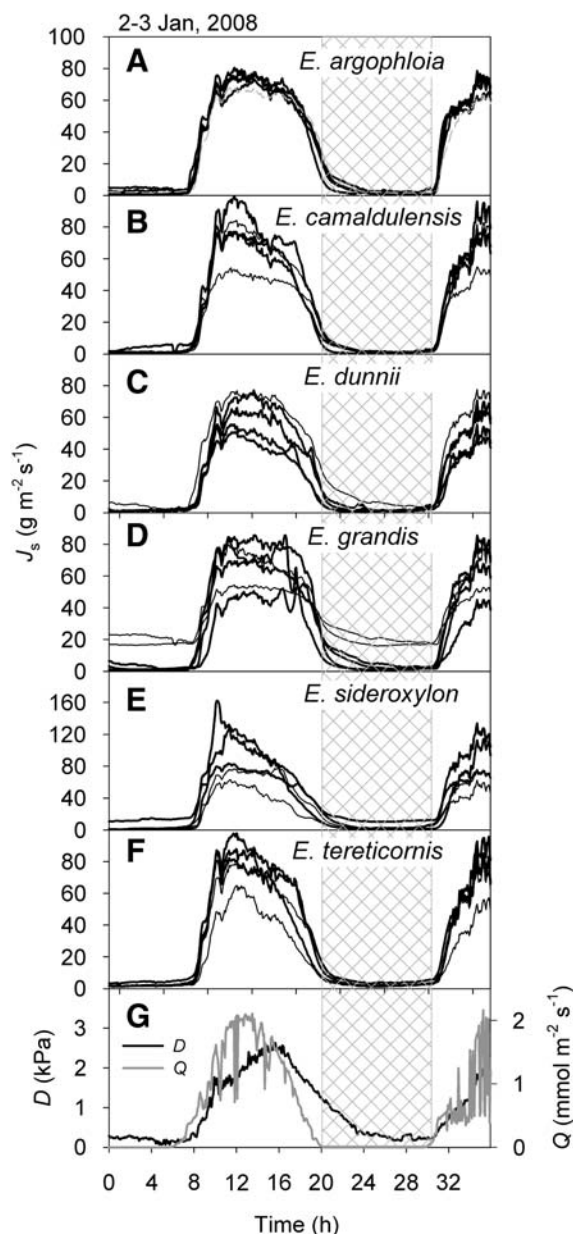


Figure 5. (A)–(F) Sap flux density (J_s , $\text{g m}^{-2} \text{s}^{-1}$) and (G) vapour pressure deficit (D , kPa) and photosynthetically active radiation (Q , mmol $\text{m}^{-2} \text{s}^{-1}$), during day and night (hatched zones) hours.

excluded, median cuticular conductance was ca. 0.7 mmol $\text{m}^{-2} \text{s}^{-1}$ (25th and 75th percentiles = 0.12 and 2.0 mmol $\text{m}^{-2} \text{s}^{-1}$, respectively) (Kerstiens 1996). In several species, Meidner (1986) found average and maximum cuticular conductances of 2 and 8 mmol $\text{m}^{-2} \text{s}^{-1}$, respectively. Caird et al. (2007) summarize the literature on $g_{s,n}$ and find that most values of $g_{s,n}$ below 20 mmol $\text{m}^{-2} \text{s}^{-1}$ may be considered functionally g_{cut} in that residual stomatal conductance may no longer be under guard cell control.

The small differences in $J_{s,n}$ or E_n between *Eucalyptus* species did not correlate with habitat water availability (Brooker

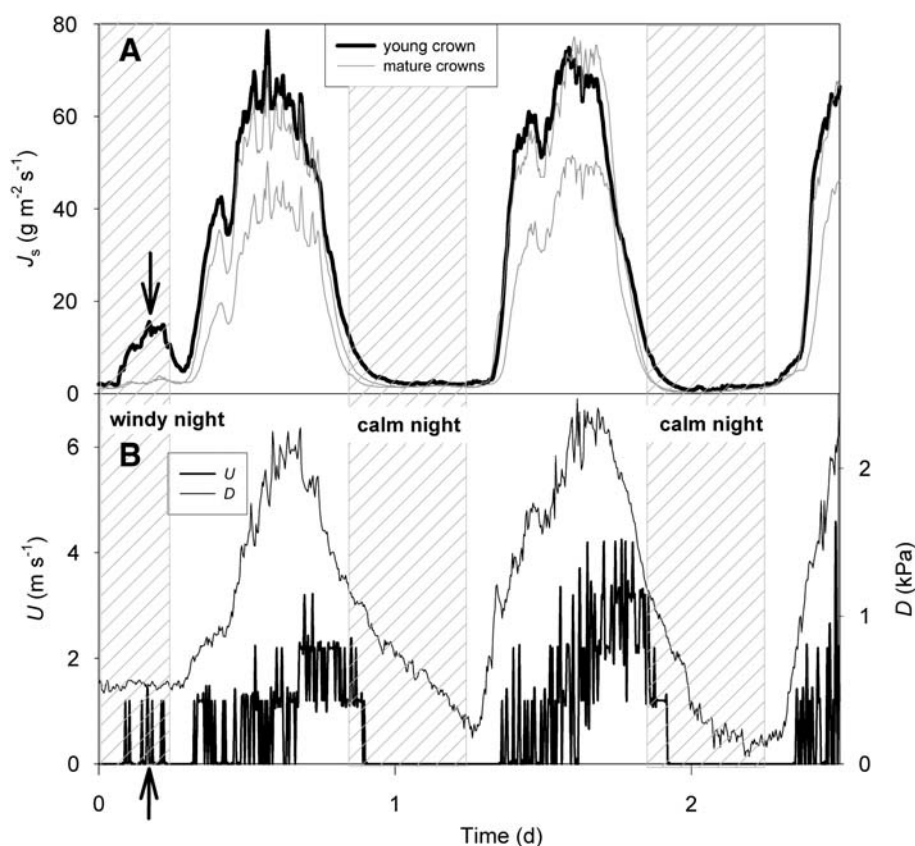


Figure 6. An example of the interaction of crown phenological state, nocturnal vapour pressure deficit and wind speed that, in combination, drive nocturnal sap flux. (A) Sap flux density (J_s , $\text{g m}^{-2} \text{s}^{-1}$) in three *E. occidentalis* trees. The black line represents a tree with ~35% young, bright green crown foliage; grey lines represent neighbouring trees with only dark green foliage. Hatched areas indicate night. (B) Vapour pressure deficit (D , kPa) and wind speed (U , m s^{-1}). On calm nights, trees did not exhibit nocturnal flux, independent of D . In contrast, when windy nights coincide with higher D (black upward arrow in B), nocturnal flux occurs in trees having a larger proportion of young crown foliage (black downward arrow in A).

and Kleinig 1999, 2001). Other studies have shown substantial inter-specific variation in nocturnal water transport among sympatric species (e.g., Lucas et al., unpublished data,

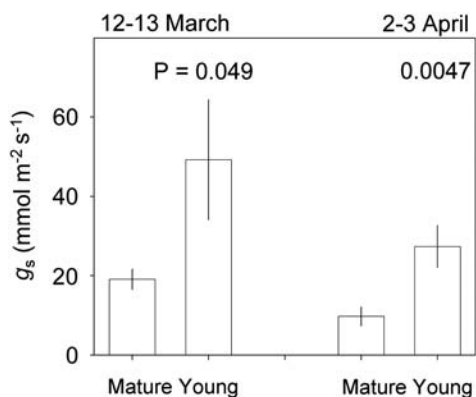


Figure 7. Nocturnal stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$) in young and mature leaves of *E. saligna* saplings on nights (22:00–01:00) in late summer (12–13 March) and autumn (2–3 April) 2008. Standard error bars were computed from 54 mature leaves and 18 young leaves collected from the same 18 trees.

Daley and Phillips 2006, Marks and Lechowicz 2007). Yet similar to the findings here, such variation was not associated with habitat water availability (Marks and Lechowicz 2007). The small inter-specific variation in $J_{s,n}$ observed in our study provided too limited a range to correlate $J_{s,n}$ with other species-specific ecological characteristics (e.g., Marks and Lechowicz 2007). Nonetheless, we have characterized *Eucalyptus* nocturnal physiology under high soil moisture conditions that presumably promote maximum nocturnal vapour exchange (sensu Christman et al. 2008).

Nocturnal vapour exchange promoted by a combination of wind, vapour pressure deficit and young foliage

Our finding that concurrent U and D drives $J_{s,n}$ extends the observations of Benyon (1999) who found that U and D influenced $J_{s,n}$ in *E. grandis*, but who analysed them as additive, rather than synergistic, drivers of $J_{s,n}$. Other studies have appreciated the role of wind as a source of energy advection to support transpiration, but not at night (e.g., Taylor et al. 2001). In this study, the product ($U \times D$) was superior to individual U or D or a linear combination of U and D as a predictor of $J_{s,n}$. This was based on two metrics: (i) largest

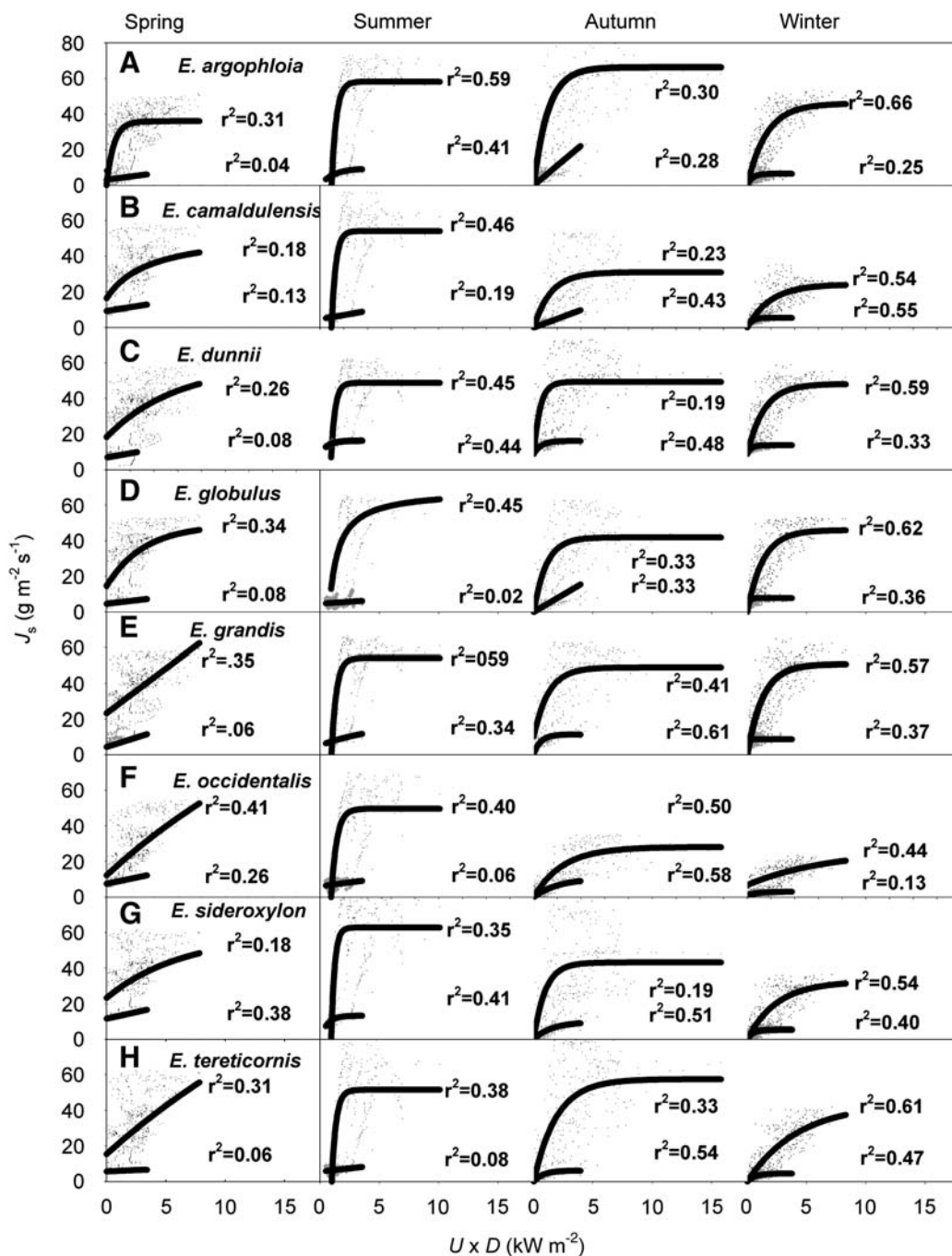


Figure 8. (A)–(H) Sap flux density (J_s , $\text{g m}^{-2} \text{s}^{-1}$) during the same periods as in Figure 1, plotted against the product of wind and vapour pressure deficit ($U \times D$, kW m^{-2}). In each set of plots, two curve fits are shown, representing daytime conditions (upper curve, black dots) and night-time conditions ($Q < 1 \mu\text{mol m}^{-2} \text{s}^{-1}$; lower curve, grey dots).

correlation coefficient (r^2) and (ii) zero intercept (intercept $P = 0.45$), compared with a significant (non-zero) intercept term between $J_{s,n}$ and D ($P = 0.033$). A zero intercept term for the relationship between $J_{s,n}$ and ($U \times D$) facilitated a simple physical interpretation, in contrast to a flux-driving force relationship that exhibits a non-zero intercept. One possible interpretation of the non-zero intercept between $J_{s,n}$ and D was that positive values of $J_{s,n}$, occurring when D is zero, repre-

sent capacitance recharge. While we were unable to rigorously evaluate this hypothesis, our observations indicate a potentially new method for partitioning E_n from capacitance recharge, wherein E_n and recharge both occur on windy, dry nights, while recharge alone occurs on calm nights, irrespective of atmospheric humidity.

While ($U \times D$) sets physical conditions in which $J_{s,n}$ can occur, young leaves and crowns with large proportions of

young leaves respond most strongly to this physical driver of $J_{s,n}$. Thus, $J_{s,n}$ is expected to be highest in any tree when three concurrent conditions are met: (i) young crowns are exposed to (ii) dry and (iii) windy nights.

Given the sensitivity of $J_{s,n}$ to the covariance of meteorological and phenological variables, it is of interest to consider how strongly these variables co-vary within and across seasons. We have observed moderate inter-seasonal variation in $J_{s,n}$ (Table 3), which appears partly to be explained by seasonal variation in $U \times D$ (Table 3) and partly to be explained by seasonal variation in crown phenological state. For example, the lowest $J_{s,n}$ occurred in winter (Table 3), which was characterized by the lowest ($U \times D$), but also little or no newly expanding foliage visible among trees of the eight species. While there can be large variation in ($U \times D$) from night to night within a season and crown phenology can be highly asynchronous among individual trees within a season, this asynchrony was primarily observed within the active growing season. Therefore, the complex, asynchronous nature of foliar phenology of *Eucalyptus* (Jacobs 1955) will be an important component to consider when predicting nocturnal water flux in the active growing seasons (primarily summer, but also autumn and spring), but generally not in winter.

Conclusions

The *Eucalyptus* genus dominates Australia's woodlands. Thus, its nocturnal physiology has continental-scale importance for nocturnal vapour and energy exchange between the vegetation and the atmosphere. However, with hundreds of species in this genus (Brooker and Kleinig 1999, 2001), generalizing *Eucalyptus*'s role in large-scale nocturnal vapour exchange requires assessment of inter-specific variation. In this study, we characterized the nocturnal physiology of eight *Eucalyptus* species representing a wide range of functional diversity.

We observed three significant findings. First, we found that, even under conditions of high soil moisture in the field, *Eucalyptus* was conservative in nocturnal water loss. Second, nocturnal water loss in *Eucalyptus* was sensitive to the *product* of nocturnal vapour pressure deficit and wind, but less so to either variable individually, which represents a potentially useful meteorological predictor of nocturnal vapour exchange. This product is proportional to the aerodynamic term of the surface energy balance equation (Monteith and Unsworth 1990). Third, intra-specific variation in nocturnal water transport was greater than inter-specific variation: crowns possessing clearly larger proportions of immature leaves exhibited substantially greater potential for nocturnal water transport. To the extent that our limited observations of these phenomena in *E. occidentalis* and *E. saligna* are representative of nocturnal behaviour in other species in this genus, predicting nocturnal water flux in *Eucalyptus* may depend on

simultaneous knowledge of individual characteristics of trees and the meteorological status of the environment.

Acknowledgments

This work was supported through Discovery Project Number DP0879531 of the Australian Research Council and the University of Western Sydney International Research Initiatives Scheme (IRIS) Grant Number 71827. Further support was provided by Boston University, Fordham University and the Roberts Fund at Bowdoin College. The authors thank Renee Attard for measurements of cuticular conductance; Carolyn Hricko, Kristine Crous, David Ellsworth, Marion Liberloo, Markus Löew, Renee Attard, Renee Smith and Roslyn Woodfield for field and laboratory assistance and Jann Conroy, Remko Duursma, Derek Eamus, Sigfredo Fuentes, Oula Ghannoum, Ross McMurtrie, Belinda Medlyn and Melanie Zeppel for helpful discussions. We thank Craig Barton, Burhan Amiji, David Harland and Linda Westmoreland for logistical support and provision of meteorological data. We thank Jann Conroy and Christine Sefton for establishing the long-term research site we used for this study.

References

- Bamber, R.K. 1985. The wood anatomy of eucalypts and papermaking. *Appita J.* 38:210–216.
- Barbour, M.M., L.A. Cernusak, D. Whitehead, K.L. Griffin, M.H. Turnbull, D.T. Tissue and G.D. Farquhar. 2005. Nocturnal stomatal conductance and implications for modelling $\delta^{18}\text{O}$ of leaf-respired CO_2 in temperate tree species. *Funct. Plant Biol.* 32:1107–1121.
- Bell, D.T. and J.E. Williams. 1997. *Eucalypt ecophysiology*. In *Eucalypt Ecology*. Eds. J.E. Williams and J. Woinarski. Cambridge University Press, Cambridge, UK, p 168–196.
- Benyon, R.G. 1999. Night time water use in an irrigated *Eucalyptus grandis* plantation. *Tree Physiol.* 19:853–859.
- Brooker, M.I.H. and D.A. Kleinig. 1999. Field guide to eucalypts, volume 1: south-eastern & southern Australia. Bloomings Books, Melbourne, Australia.
- Brooker, M.I.H. and D.A. Kleinig. 2001. Field guide to eucalypts, volume 2: south western and southern Australia. Bloomings Books, Melbourne, Australia.
- Bucci, S.J., F.G. Scholz, G. Goldstein, F.C. Meinzer, J.A. Hinojosa, W.A. Hoffman and A.C. Franco. 2004. Processes preventing nocturnal equilibration between leaf and soil water potential in tropical savanna woody species. *Tree Physiol.* 24:1119–1127.
- Caird, M.A., J.H. Richards and L.A. Donovan. 2007. Nighttime stomatal conductance and transpiration in C3 and C4 plants. *Plant Physiol.* 143:4–10.
- Christman, M.A., J.H. Richards, J.K. McKay, E.A. Stahl, T.E. Jueniger and L.A. Donovan. 2008. Genetic variation in *Arabidopsis thaliana* for night-time leaf conductance. *Plant Cell Environ.* 31:1170–1178.
- Clearwater, M., F.C. Meinzer, J.L. Andrade, G. Goldstein and N.M. Holbrook. 1999. Potential errors in measurement of nonuniform sap flow using heat dissipation probes. *Tree Physiol.* 19:681–687.
- Cochrane, P.M. and R.O. Slayter. 1988. Water relations of *Eucalyptus pauciflora* near the alpine tree line in winter. *Tree Physiol.* 4:45–52.
- Cuntz, M., J. Ogee, G.D. Farquhar, P. Peylin and L.A. Cernusak. 2007. Modelling advection and diffusion of water isotopologues in leaves. *Plant Cell Environ.* 30:892–909.
- Daley, M. and N. Phillips. 2006. Interspecific variation in night time transpiration and stomatal conductance in a mixed New England deciduous forest. *Tree Physiol.* 26:411–419.

- Darwin, F. 1898. Observations on stomata. *Philos. Trans. R. Soc. Lond. B* 190:531–621.
- Dawson, T.E., S.S.O. Burgess, K.P. Tu, R.S. Oliveira, L.S. Santiago, J.B. Fisher, K.A. Simonin and A.R. Ambrose. 2007. Night time transpiration in woody plants from contrasting ecosystems. *Tree Physiol.* 27:561–575.
- Eldridge, K., J. Davidson, C. Harwood and G. Van Wyk. 1993. Eucalypt domestication and breeding. Oxford University Press, Oxford, UK.
- Field, C. 1985. Leaf age effect on stomatal resistance. In *Stomatal Function*. Eds. E. Zeiger, G. Farquhar and I. Cowan. Stanford University Press, Stanford, CA, USA, p 367–384.
- Gindel, I. 1970. The nocturnal behaviour of xerophytes grown under arid conditions. *New Phytol.* 69:399–404.
- Granier, A. 1985. A new method of sap flow measurement in tree stems. *Ann. Sci. For.* 42:193–200.
- Granier, A. 1987. Evaluation of transpiration in a Douglas-fir stand by means of sap flow measurements. *Tree Physiol.* 3:309–320.
- Jacobs, M.R. 1955. Growth habits of the eucalypts. Commonwealth Government Printer, Canberra, Australia.
- Kerstiens, G. 1996. Cuticular water permeability and its physiological significance. *J. Exp. Bot.* 47:1813–1832.
- Körner, Ch., J.A. Scheel and H. Bauer. 1979. Maximum leaf diffusive conductance in vascular plants. *Photosynthetica* 13:45–82.
- Levitt, J. 1967. The mechanism of stomatal action. *Planta (Berl.)* 74:101–118.
- Marks, C.O. and M.J. Lechowicz. 2007. The ecological and functional correlates of nocturnal transpiration. *Tree Physiol.* 27:577–584.
- Meidner, H. 1986. Cuticular conductance and the humidity response of stomata. *J. Exp. Bot.* 37:517–525.
- Monteith, J.L. and M.H. Unsworth. 1990. Principles of environmental physics, 2nd edn. Edward Arnold, New York.
- Noble, I.R. 1989. Ecological traits of the *Eucalyptus* L'herit subgenera *Monocalyptus* and *Symphoyomyrtus*. *Aust. J. Bot.* 37:207–224.
- Oishi, A.C., R. Oren and P.C. Stoy. 2008. Estimating components of forest evapo-transpiration: a footprint approach for scaling sap flux measurements. *Agric. For. Meteorol.* 148:1719–1732.
- Oren, R., N. Phillips, B. Ewers, D. Pataki and J. Megonigal. 1999. Sap-flux-scaled transpiration responses to light, vapour pressure deficit, and leaf area reduction in a flooded *Taxodium distichum* forest. *Tree Physiol.* 19:337–347.
- Phillips, N., R. Oren and R. Zimmerman. 1996. Radical patterns of xylem sap flow in non-, diffuse- and ring-porous tree species. *Plant Cell Environ.* 19:983–990.
- Phillips, N., M. Barbour and T.E. Dawson. 2007. Casting light on nocturnal stomatal conductance. *Tree Physiol.* 27, preface.
- Scholz, F.G., S.J. Bucci, G. Goldstein, F.C. Meinzer, A.C. Franco and F. Miralles-Wilhelm. 2007. Removal of nutrient limitations by long-term fertilization decreases nocturnal water loss in savanna trees. *Tree Physiol.* 27:551–559.
- Sefton, C.A. 2003. Genetic and environmental variation in leaf morphology of juvenile eucalypt leaves. Ph.D. Thesis. University of Western Sydney, Richmond, NSW, Australia.
- Seibt, U., L. Wingate and J.A. Berry. 2007. Nocturnal stomatal conductance effects on the ^{18}O signatures of foliage gas exchange observed in two forest ecosystems. *Tree Physiol.* 27:585–595.
- Taylor, P.J., I.K. Nuberg and T. Hatton. 2001. Enhanced transpiration in response to wind effects at the edge of a blue gum (*Eucalyptus globulus*) plantation. *Tree Physiol.* 21:403–408.
- Turnbull, M.H., D.T. Tissue, R. Murthy, X. Wang and K.L. Griffin. 2004. Nocturnal warming increases photosynthesis at elevated CO_2 partial pressure in *Populus deltoides*. *New Phytol.* 161:819–826.
- Whitehead, D., K.L. Griffin, M.H. Turnbull, D.T. Tissue, V.C. Engel, K.J. Brown, W.S. F. Schuster and A.S. Walcroft. 2004. Response of total night-time respiration to differences in total daily photosynthesis in a canopy of *Quercus rubra* L. *Global Change Biol.* 10:925–938.
- Wilkins, A.P. 1991. Sapwood, heartwood and bark thickness of silviculturally treated *Eucalyptus grandis*. *Wood Sci. Technol.* 25: 415–423.
- Zang, D., C.L. Beadle and D.A. White. 1996. Variation of sap-flow velocity in *Eucalyptus globulus* with position in sapwood and use of a correction coefficient. *Tree Physiol.* 16:697–703.
- Zeppel, M., C. Macinnis-Ng, A. Palmer, D. Taylor, R. Whitley, S. Fuentes, I. Yunusa, M. Williams and D. Eamus. 2008. An analysis of the sensitivity of sap flux to soil and plant variables assessed for an Australian woodland using a soil–plant–atmosphere model. *Funct. Plant Biol.* 35:509–520.