

## Nighttime transpiration in woody plants from contrasting ecosystems

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**Summary** It is commonly assumed that transpiration does not occur at night because leaf stomata are closed in the dark. We tested this assumption across a diversity of ecosystems and woody plant species by various methods to explore the circumstances when this assumption is false. Our primary goals were: (1) to evaluate the nature and magnitude of nighttime transpiration,  $E_n$ , or stomatal conductance,  $g_n$ ; and (2) to seek potential generalizations about where and when it occurs. Sap-flow, porometry and stable isotope tracer measurements were made on 18 tree and eight shrub species from seven ecosystem types. Coupled with environmental data, our findings revealed that most of these species transpired at night. For some species and circumstances, nighttime leaf water loss constituted a significant fraction of total daily water use. Our evidence shows that  $E_n$  or  $g_n$  can occur in all but one shrub species across the systems we investigated. However, under conditions of high nighttime evaporative demand or low soil water availability, stomata were closed and  $E_n$  or  $g_n$  approached zero in eleven tree and seven shrub species. When soil water was available,  $E_n$  or  $g_n$  was measurable in these same species demonstrating plasticity for  $E_n$  or  $g_n$ . We detected  $E_n$  or  $g_n$  in both trees and shrubs, and values were highest in plants from sites with higher soil water contents and in plants from ecosystems that were less prone to atmospheric or soil water deficits. Irrespective of plant or ecosystem type, many species showed  $E_n$  or  $g_n$  when soil water deficits were slight or non-existent, or immediately after rainfall events that followed a period of soil water deficit. The strongest relationship was between  $E_n$  or  $g_n$  and warm, low humidity and (or) windy ( $> 0.8 \text{ m s}^{-1}$ ) nights when the vapor pressure deficit remained high ( $> 0.2 \text{ kPa}$  in wet sites,  $> 0.7 \text{ kPa}$  in dry sites). Why  $E_n$  or  $g_n$  occurs likely varies with species and ecosystem type; however, our data support four plausible explanations: (1) it may facilitate carbon fixation earlier in the day because stomata are already open; (2) it may enhance nutrient supply to distal parts of the crown when these nutrients are most avail-

able (in wet soils) and transport is rapid; (3) it may allow for the delivery of dissolved  $\text{O}_2$  via the parenchyma to woody tissue sinks; or (4) it may occur simply because of leaky cuticles in older leaves or when stomata cannot close fully because of obstructions from stomatal (waxy) plugs, leaf endophytes or asymmetrical guard cells (all non-adaptive reasons). We discuss the methodological, ecophysiological, and theoretical implications of the occurrence of  $E_n$  or  $g_n$  for investigations at a variety of scales.

*Keywords:* nighttime stomatal conductance, porometry, sap flow, water balance, water relations, woody plants.

### Introduction

“... there is much contradictory evidence as to whether or not the stomata of the majority of plants close at night.”

*Francis Darwin (1898)*

A longstanding notion among plant physiologists, which is captured by Francis Darwin's comment, is that C3 plants close their stomata in the dark (see also Meidner and Mansfield 1965, and citations in Kramer and Boyer 1995 and Nobel 1999). Despite evidence to the contrary, this concept has persisted since the earliest observations of stomatal behavior (Leitgeb 1886, Darwin 1898). Over 100 years ago Darwin (1898) estimated that 87% of terrestrial plants open their stomata at night, with values for wetland and aquatic species approaching 100%. Nevertheless, the assumption that stomata close in the dark (at night) is consistent with the nearly universal observation that stomatal conductance in daylight is at its highest over a diel cycle (Kramer and Boyer 1995, Richie 1974). Furthermore, a range of studies have shown that the

ionic balance (mostly  $K^+$  and  $Cl^-$  flux) and the abscisic acid concentration in the guard cell complex can change over the day–night period and that these changes are associated with changes in guard cell turgor pressure that are known to elicit closure when light is largely absent (Buckley 2005). Important exceptions to this pattern were reported in trees over 30 years ago (Hinckley and Scott 1971, Hinckley and Ritchie 1973), as well as more recently in other woody plants (Donovan et al. 2000, 2003) and a variety of herbaceous species (Muchow et al. 1980, Snyder et al. 2003). Yet, despite this evidence, the predominant view advocated in most biology and even plant physiology textbooks describing stomatal function is that the stomata of leaves of non-CAM plants are closed in the dark. It follows therefore that most studies of water balance at the whole-plant-, ecosystem- and catchment-scales have assumed nocturnal transpiration to be zero.

Recent advances in sap flow methods (see Burgess et al. 2001a) have facilitated precise and continuous measurement of plant water use over day–night cycles. Using such methods (and others), a growing number of studies, including those presented here, have revealed previously unknown patterns of nighttime water flux and loss from plant crowns across a variety of woody taxa (Burgess et al. 1998, Hultine et al. 2004, Burgess and Dawson 2004, Bucci et al. 2004, Oliveira et al. 2005a). As we prepared this publication at least 20 studies, not including papers in this issue of *Tree Physiology*, have reported a continued flux of water into plants at night, well after stomata were assumed to be closed (e.g., Becker 1998, Benyon 1999, Iritz and Lindroth 1994, Hogg and Hurdle 1997, Gutiérrez and Santiago 2006, Green et al. 1989, Oren et al. 1999, Donovan et al. 1999, 2001, 2003, Snyder et al. 2003, Grulke et al. 2004). Many of these same studies also show water loss from leaves at night. Nighttime water flux in stems and leaves occurs because leaf water potential declines over the course of daytime transpiration to the point that, when transpiration ceases, a water potential gradient between leaf and soil remains (Hinckley 1971). This water potential gradient drives water movement that can be detected by sap flow sensors (see Burgess and Dawson 2004). Until recently, much of this flux was attributed to refilling of partially depleted xylem water stores that were exhausted during daylight hours by plant transpiration (Oren et al. 1999). However, further analysis of sap-flow behavior based on improved methods, which allow detection of very low sap-flow rates in the xylem (e.g., Burgess and Dawson 2004, Burgess et al. 1998, 2001b, Hultine et al. 2004, Brooks et al. 2002, Oliveira et al. 2005a), have shown that a significant fraction of the sap flow that occurs at night is not limited to the refilling of depleted xylem water stores. These and many previous results based on other methods (e.g., Hinckley and Ritchie 1973) suggest that many plants transpire at night.

Incomplete closure of stomata at night with continued transpirational water loss has important implications for some of the commonly used methods in plant water relations research as well as some long-standing theories in plant science (Donovan et al. 1999, 2001, 2003, Bucci et al. 2004). In addition, many plant-, stand- and catchment-based models of water flux

assume no water flux occurs through plants at night (Iritz and Lindroth 1994, Green et al. 1989, Vertessy et al. 1997, but see Fisher et al. 2007). If this assumption is incorrect then models assuming that evapotranspiration at night is zero (Iritz and Lindroth 1994, Benyon 1999) will underestimate fluxes and incorrectly assess site water balance (see Fisher et al. 2007).

Here, we present data demonstrating that nighttime transpiration ( $E_n$ ) and nighttime stomatal conductance to water vapor ( $g_n$ ) are widespread among a range of tree and shrub species inhabiting a broad range of environments. For some species that show  $E_n$  or  $g_n$ , nocturnal water loss can represent a significant fraction of the total daily water used and therefore of total ecosystem water loss. We present selected examples and, based on these and a broader review of the literature, discuss the patterns emerging from these observations and the implications for ongoing and future water relations investigations at scales ranging from the individual plant to whole catchments. We conclude that our findings have important implications for the selection of appropriate sap-flow methods (e.g., the compensation heat pulse method is a poor choice if quantitative assessments of  $E_n$  are desired) and for an established body of water relations theory that assumes water loss from leaves at night does not occur.

## Materials and methods

### Study sites and species

Our investigations were carried out at 10 study sites (Table 1) representing six biomes on three continents, plus the Hawaiian Islands (see Table 2). Our goal in choosing sites and species was to measure sufficient diversity to assess the occurrence of  $E_n$  or  $g_n$ , or both, in phylogenetically divergent woody plants exposed to different site conditions.

Our studies of  $E_n$  based on the heat ratio sap-flow method (HRM; see the methods section that follows) in the coniferous coastal redwood ecosystem were carried out at two locations in coastal California as previously described by Burgess and Dawson (2004). The following tree and shrub species were studied: *Sequoia sempervirens* Lamb. ex D. Don (coast redwood), *Pseudotsuga menziesii* (Mirb.) Franco. (Douglas-fir), *Lithocarpus densiflora* (Hook. and Arn.) Rehd. (tanbark oak) and *Umbellularia californica* Nutt. (California bay-laurel). The redwood trees examined were canopy dominants, whereas the other species were mature sub-dominants growing in open forest gaps and were not shaded by larger neighbors. One understory shrub species, *Gaultheria shallon* Pursh (salal) at two of these sites was also studied by a porometric method.

Measurements of nighttime stomatal conductance ( $g_n$ ) and water flux in *Acer saccharum* Marsh. (sugar maple) trees in a Northern Hemisphere temperate deciduous forest were measured by porometric and deuterium isotope-tracer methods as described previously (Dawson 1996, Pausch et al. 2000). *Acer rubrum* L. (red maple) trees were measured about 2 km west of this site on an exposed ridgeline on rocky, well-drained soils. This site was windier and experienced 10–15% lower relative humidity than the *A. saccharum* site. At the *A. rubrum* site, we

Table 1. Summary of study site characteristics and the species examined. Abbreviations: MAP, mean annual precipitation; and MAT, mean annual temperature.

Site	Country	Ecosystem type	Latitude, Longitude	MAP (mm)	MAT (°C)	Species examined
Sonoma	USA	Coastal temperate coniferous forest	38°24' N, 122°59' W	1037	15	<i>Sequoia sempervirens</i> <i>Pseudotsuga menziesii</i> <i>Lithocarpus densiflora</i> <i>Umbellularia californica</i> <i>Gaultheria shallon</i>
Big Basin	USA	Coastal temperate coniferous forest	37°10' N, 122°14' W	995	17	<i>Sequoia sempervirens</i> <i>Pseudotsuga menziesii</i> <i>Lithocarpus densiflora</i> <i>Umbellularia californica</i> <i>Gaultheria shallon</i>
Upstate New York	USA	Temperate deciduous forest	42°21' N, 76°24' W	1470	19	<i>Acer saccharum</i> <i>Acer rubrum</i> <i>Pinus strobus</i> <i>Vaccinium</i> spp.
Blodgett Forest	USA	Montane temperate coniferous forest	38°53' N, 120°37' W	1290	12	<i>Pinus ponderosa</i> <i>Arctostaphylos manzanita</i> <i>Ceanothus cordulatus</i>
Tonzi Ranch	USA	Mediterranean oak savanna-chaparral	38°25' N, 120°57' W	560	16	<i>Quercus douglasii</i>
Bolinas Ridge	USA	Mediterranean oak savanna-chaparral	37°54' N, 122°37' W	1250	12	<i>Quercus agrifolia</i> <i>Lithocarpus densiflora</i> <i>Arctostaphylos glandulosa</i> <i>Ceanothus cuneatus</i> <i>Rhamnus californica</i> <i>Heteromeles arbutifolia</i>
Waikamoi	USA	Tropical montane forest	20°48' N, 156°13' W	5000	16	<i>Metrosideros polymorpha</i>
IBGE-RECOR	Brazil	Neotropical savanna	15°56' S, 47°53' W	1450	21	<i>Byrsonima verbascifolia</i> <i>Roupala montana</i> <i>Vellozia flavicans</i>
Floresta Nacional do Tapajós	Brazil	Tropical evergreen forest	2°26' S, 54°42' W	2000	25	<i>Coussarea racemosa</i> <i>Protium robustum</i> <i>Manilkara huberi</i>
Corrigin Water Reserve	Australia	Mediterranean woodlands and shrublands	32°19' S, 117°52' E	376	17	<i>Allocasuarina campestris</i> <i>Dryandra cirsioides</i> <i>Isopogon gardneri</i> <i>Hakea subsulcata</i> <i>Banksia sphaerocarpa</i> <i>Actinostrobos arenarius</i> <i>Eucalyptus salmonophloia</i> <i>Eucalyptus albida</i> <i>Dryandra sessilis</i> <i>Nuytsia floribunda</i> <i>Eucalyptus wandoo</i>

measured  $g_n$  on open-grown *Pinus strobus* L. (eastern white pine) saplings ranging in height from 8.2 to 11.6 m as well as on two 1–2 m tall shrubs of *Vaccinium* spp. (blueberry). Mean precipitation for these sites was 1470 mm annually, 57% of which falls as snow, with a mean growing season temperature of 19 °C (Table 1).

We used the HRM to measure sap flow at two other inland sites: a mid-elevation coniferous forest in the Sierra Nevada Mountains (California) dominated by *Pinus ponderosa* Dougl ex P. Laws. (ponderosa pine) and two woody shrubs, *Arctostaphylos manzanita* Parry (manzanita) and *Ceanothus cordulatus* Kellogg (buckbrush), and an oak–savanna site on the east

side of the great central valley of California dominated by the hardwood tree species *Quercus douglasii* Hook and Arn. (blue oak). Both sites were studied during the 2005 growing season (cf. Fisher et al. 2007).

Species that inhabit the widespread chaparral vegetation of California were studied at Bolinas Ridge, a near-coastal site just north of San Francisco (as described by Kennedy and Sousa 2006) that receives about 1250 mm of precipitation annually and has a mean annual temperature of 12 °C (Table 1). This site also receives an additional 200–340 mm of “occult” precipitation from coastal fog during the summer months. Two tree species, *Q. agrifolia* Nee. (coast live oak) and *L. densiflora* (Hook. and Arn.) Rehd., and four shrub species, *A. glandulosa* ssp. *glandulosa* Eastw. (manzanita), *C. cuneatus* (Hook.) Nutt. (buckbrush), *Rhamnus californica* Esch. (coffeeberry) and *Heteromeles arbutifolia* M. Roem. (toyon), were measured by porometry during two one-week periods in June 2000 and 2001.

In Hawaii, we investigated  $E_n$  in *Metrosideros polymorpha* Gaud. (Ohia) at Waikamoi, Maui, a montane forest, by the constant heating method, as described by Santiago et al. (2000). The site has a mean annual precipitation of about 5000 mm, and a mean temperature of 16 °C (Table 1). *Metrosideros polymorpha* grows from sea level to treeline in Hawaii, is the canopy dominant in most Hawaiian forests, and comprises 50–80% of basal area at the site. Soil waterlogging is common at the site and many of the woody species exhibit aboveground rooting (see Santiago et al. 2000).

Investigations in the neotropical savanna (Cerrado) were made at the IBGE-RECOR ecological reserve in central Brazil. Sap flow was measured by HRM on three common species (Table 1) between 2001 and 2003. The 1350-ha IBGE-RECOR ecological reserve includes extensive areas of all major physiognomic forms of Cerrado vegetation. The most common structural type is Cerrado *sensu stricto*; a savanna woodland with abundant evergreen, deciduous and semi-deciduous trees and shrubs, and a herbaceous understory with a semi-closed canopy with tree cover of about 50%. Mean annual rainfall is 1450 mm with a very distinct 5-month dry period (May–September). Less than 10% of total annual precipitation falls during this period, indicating a strong seasonality of rainfall (see Oliveira et al. 2005b for additional details).

We studied three tropical evergreen forest trees species (Table 1) at the Floresta Nacional do Tapajós, located in east-central Amazônia (see Oliveira et al. 2005a). The forest is characterized as broadleaf evergreen located on a broad, flat plateau. Mean annual rainfall is about 2000 mm with a distinct 3–5 month dry period (August–December). Less than 15% of total annual precipitation falls during this period indicating strong seasonality in rainfall. This forest can experience severe drought during El Niño events, when annual rainfall can drop to 800 mm.

Measurements at the southern hemisphere Mediterranean woodland site were made at the Corrigin Water Reserve about 2 km west of Corrigin, Western Australia. We focused our measurements of  $E_n$  on the dominant tree, *Eucalyptus salmonophloia* F. Muell. (salmon gum), a widespread canopy

species throughout the semiarid region of southwestern Australia. Rudimentary measurements of  $E_n$  were also made on six shrub and three tree species (Table 1) at this site.

#### *Sap-flow methods and measurements*

For most investigations we used the HRM (Burgess et al. 1998, 2001a, 2001b, Bleby et al. 2004, Oliveira et al. 2005a; see also Smith and Allen 1996) to make continuous measurements of sap flow in stems of the target species. This whole-plant technique was chosen because it allows bi-directional measurements of sap flow and also measures low flow rates with high precision. Corrections for misalignment of the probes were made according to Burgess et al. (2001a), but otherwise raw heat pulse velocities were used as a proxy for  $E_n$ . At the end of all of our investigations, cuts were made into the sapwood above and below the probes to stop sap flow, following the procedure suggested by Burgess et al. (2001a); this being the most accurate way to determine the reference velocity (zero) flow value. Once this zero flow value was determined, we were able to distinguish between true zero flow and the small rates of flow that are associated with  $E_n$ . For ease of comparison, we present  $E_n$  as percent of daily growing season maximum  $E$  so as to account for differences in absolute flow rates among species and individuals.

In California, at the montane temperate coniferous forest site (Blodgett Forest, Table 1), HRM probes were placed in five *P. ponderosa* trees, three *A. manzanita* and two *C. cordulatus* shrubs. Diameter at breast height (DBH) values for the *P. ponderosa* trees were 18, 20, 21, 24 and 24 cm. At the Mediterranean oak savanna site (Tonzi Ranch; Table 1), we measured sap flow by HRM in five *Q. douglasii* trees. Values of DBH for the *Q. douglasii* trees were 16, 18, 22, 23 and 37 cm (see also Fisher et al. 2007). Three individuals of each of the other species were investigated in the coastal temperate coniferous forest of sites (Sonoma and Big Basin, Table 1) as described in Burgess and Dawson (2004). Tanbark oak, a widespread species in the California flora, was also measured by both sap flow and porometry at our Mediterranean site (Bolinas Ridge, Table 1).

At our tropical montane forest site in Waikamoi, Hawaii (Table 1), transpiration was estimated as sap flow by the constant heating method (Granier 1985) during the months of September 1996 to February 1997. Over this period, a total of 24 trees were measured in well-drained and waterlogged soils. Because the constant heating method defines zero flow as the lowest point measured during the night, nighttime transpiration was defined conservatively as visible peaks in nighttime basal sap flow that coincided with VPD values that clearly rose above zero. Although we cannot eliminate the possibility that what we defined as zero flow for these measurements was not a true zero (i.e., no flow), visible peaks in the nocturnal sap-flow trace that occurred simultaneously with extremely rare nighttime VPD values above zero were considered indicative of  $E_n$ , at least in this species, and thus represent a conservative qualitative estimate of  $E_n$ . In instances where Granier-type probes are used and suggest the possible existence of  $E_n$ , we recommend empirical verification of  $\Delta T_{max}$  values by cutting

the xylem and excluding thermal gradients as a possible contribution (e.g., see Köstner et al. 1998).

At our southern hemisphere tropical forest site (Floresta Nacional do Tapajós; Table 1), HRM probe sets were installed in eight individuals of three species representing different functional types (see Oliveira et al. 2005a): *Coussarea racemosa* A. Rich (Rubiaceae), the most common tree species in the Tapajós forest, represented an understory type; *Protium robustum* (Swart) Porter (Burseraceae), a mid-canopy species and *Manilkara huberi* (Ducke) Stand. (Sapotaceae), representing a canopy species, also very common in this forest (I. Tohver, University of Brazilia, personal communication).

At our neotropical savanna site (IBGE-RECOR; Table 1), we selected nine individuals of three species that had contrasting leaf phenologies and were also common at the site (among the 15 most frequent and abundant species; Lenza 2005). *Byrsonima verbascifolia* L. (Malpighiaceae) is a brevideciduous tree, i.e., it is leafless for a short period of the end of the dry season. It is common in the study area and ubiquitous throughout the Cerrado (Ratter et al. 2003). *Roupala montana* Aubl. (Proteaceae) is a tree of low stature (1–3 m) that remains evergreen during both seasons but reduces its leaf area during the dry season (Franco 1998). It is the most common species in the study site (Lenza 2005) and also widespread throughout the Cerrado, occurring in at least 50% of the 376 areas surveyed by Ratter et al. (2003). *Vellozia flavicans* is a desiccation-tolerant monocotyledonous shrub (Oliveira et al. 2005b). It is common in the study site and occurs interspersed with the other species. It can grow to 1.5 m and is a true evergreen, maintaining constant leaf area throughout the year despite changing conditions. We chose only mature individuals (of similar sizes for each species) occurring within a randomly selected plot of 200 m<sup>2</sup>.

In Australia, at the Mediterranean woodlands and shrublands site (Corrigin Water Reserve; Table 1), sap flow was measured in stems of 2–3 individuals of each species, with two or three probes per tree, depending on tree size. *Eucalyptus wandoo* Blakely and *E. salmonophloea* F. Muell. are known to exhibit nighttime sap flows up and down stem tissues owing to hydraulic redistribution processes following rain (Burgess and Bleby 2006). Consequently, data were selected from a rainless period of sufficient duration that hydraulic redistribution processes could be assumed to be absent. Nighttime values were gathered between 2230 and 0430 h on numerous summer nights. Because of ongoing experiments at this site, a zero estimate by cutting xylem was made for only one probe set in a single *E. salmonophloea* tree and only these data are presented (see Figure 1). Values of  $E_n$  were normalized against maximum daytime summer transpiration, which was calculated as the mean of three half-hourly measurements made between 1130 and 1230 h for five consecutive sunny midsummer days ( $n = 15$ ). For other species, zero estimates could not be made because of ongoing measurements and so data were analyzed simply for correlations between nighttime sap flow (between 2230 and 0430 h) and VPD to provide an index of nighttime  $E_n$ . Data for *E. salmonophloea* were collected on 39 nights.

#### Nighttime stomatal conductance

Measurements of stomatal conductance in daylight ( $g$ ) and at night ( $g_n$ ) were made with a steady state porometer (Model LI-1600, Li-Cor, Lincoln, NE) on six trees each of *Acer saccharum* and *A. rubrum* and five trees of *Pinus strobus*. We measured outer-crown sun leaves in the mid-crown (~10 m high) and lower crown (~2 m high). Because it is likely that there would be interspecific variation in both the closure of stomata in response to darkness or in the magnitude of  $g_n$ , all of our gas exchange measurements were made 3 h after the light at the site decreased to below 2  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . We measured the conditions inside an empty and a closed chamber just before each leaf measurement to be sure that the baseline atmospheric humidity was well matched to the conditions surrounding each leaf just before it was measured. When using the LI-1600 to make an empty or a closed chamber measurement, the chamber was held beside the measurement leaf but was either open to air, or it was closed with a plastic or Al-foil “leaf” in the chamber, respectively. In most cases, these measurements were identical. In cases where condensation might have formed, the plastic or Al-foil “leaf” value was higher, with the difference representing freely evaporating water from the surface and not  $g_n$ . Under these circumstances the leaves were not measured. Measured values obtained on leaves that fell below ~10  $\text{mmol m}^{-2} \text{s}^{-1}$  were considered to be below the accuracy of the instrument (M. Barbour and T. Buckley, Landcare, New Zealand, personal communication) and were therefore excluded from the analyses.

#### Deuterium isotope tracers

During one dry period (July) in the 1994 growing season, six *A. saccharum* trees at the temperate deciduous forest site (Upstate New York; Table 1) were selected to receive isotopically enriched water; three trees were randomly assigned to one of the two treatments below. All trees ranged between 21 to 24 m in height. The hydrogen isotope composition is expressed in delta notation ( $\delta\text{D}$ , see Dawson and Brooks 2001). Deep soil water at the site (before irrigation) had a  $\delta\text{D}$  value of  $-57 \pm 4.9\text{‰}$  ( $n = 86$  over 4 years). The isotopically enriched water had a  $\delta\text{D}$  value of  $250 \pm 3.2\text{‰}$  ( $n = 16$  over the experimental and analysis period). This enriched water source was applied in two ways: either injected directly into the stem 50 cm above the soil surface using a modified intravenous-bag fitted with a hypodermic needle inserted into a small hole drilled into the sapwood of each tree that was elevated above the entry point to provide a slightly positive pressure (hydraulic head) to minimize embolism (modified from Calder et al. 1986); or applied uniformly to the soil at a rate equivalent to a 3 mm rainfall event from the crown drip-line inward to the base of the tree at 1800 h. Stems were sampled 2 h before the tracer application and then, every 3 h after, it was applied from 2100 h until 0900 h the following day. Stems and leaves were also sampled 30 h after labeling. For the values presented (see Table 3), daylight rates were determined in a separate experiment. At each sampling period, terminal shoots ( $n = 3$  to 5) and leaves at two crown heights were sam-

pled. The isotope data are shown as: (1) the percent difference from the pretreatment (see Figure 8); and (2) rates of water loss (for our case,  $E_n$ ) calculated based on the tracer counts/convective–dispersive equations outlined by Calder et al. (1986), where changes in isotope concentration per unit time and distance traveled are used to obtain a rate of transpiration. For accurate estimates, the method requires knowledge of the cross-sectional area of conducting xylem, estimated as described by Pausch et al. (2000), and assumes a given porosity. A concentration–time curve is obtained and from this curve a flow velocity and rate are calculated. A full description of the methods and the calculations for determining rates of transpiration based on this approach is given by Calder et al. (1986) and Calder (1992). All samples were collected in airtight vials and stored frozen until they were cryogenically extracted (see Ehleringer et al. 2000), and isotope analyses were performed as outlined by Dawson (1996). Where noted, the leaves directly adjacent to the sample stems were also measured at the same time with an LI-1600 porometer.

## Results

From the large amount of data we collected, only a few examples from a small number of the species are presented here to illustrate particular patterns. These examples are representative of the responses that we observed in the wide variety of tree and shrub species across the environments we studied. A summary of the range of daylight and nighttime values we obtained from all of our studies is presented in Table 2.

### Nighttime sap flow

Our California measurements spanned a longitudinal transect across four ecosystems types: the coastal chaparral, the moist coastal redwood forest, the dry oak and grass savanna of the Central Valley, and the montane conifer-dominated forests of the Sierra Nevada Mountains. Although these ecosystems experience contrasting microclimatic influences, they have similar timing of major wintertime storm events and seasonal patterns of drought.

Burgess and Dawson (2004) found a strong positive correlation ( $r^2 = 0.84$ ) between nighttime (0030 to 0530 h) sap flow and VPD in *Sequoia sempervirens* growing in Sonoma County, California. Nighttime rates of water loss exceeded 20% of maximum transpiration rate measured at noon under warm and dry summer conditions. Nighttime transpiration rates of 10 to 12% of summer maximum were common on dry, often windy nights, but on some occasions, rates exceeding 40% of maximum were observed (when nighttime VPD exceeded 3.0 kPa and was associated with wind velocities of  $0.8 \text{ m}^2 \text{ s}^{-1}$ ; data not shown). Several other species from this temperate coniferous forest showed modest rates (6–11% of daily maximum transpiration rates; Table 1) of  $E_n$  that were associated with VPD values in excess of 0.2 kPa (when relative humidity dipped below 90%); in these cases there was also a strong positive correlation between  $E_n$  and VPD for *P. menziesii* ( $r^2 = 0.71$ ), *L. densiflora* ( $r^2 = 0.66$ ) and *U. californica*

( $r^2 = 0.59$ ) and between  $g_n$  and VPD for the understory shrub species, *G. shallon* ( $r^2 = 0.64$ ). Our diurnal sap-flow traces indicate that little of the  $E_n$  we quantified was attributable to xylem refilling at the end of the day. For example, continuous sap-flow traces obtained from three crown positions in *S. sempervirens* trees declined to near zero within 1 to 1.5 h after dusk on high humidity nights, but declined quickly and then remained at elevated flow rates on low humidity nights (see Figure 2 of Burgess and Dawson 2004). This 1 to 1.5 h decay in sap flow rate to a stable, non-zero value on low humidity nights was observed in all of the tree species we investigated and, we believe, represents xylem storage tissue recharge (data not shown).

Fisher et al. (2007) reported similar coefficients of determination between VPD and nocturnal sap flow for *Q. douglasii* ( $r^2 = 0.79$ ) at the oak-savanna site and *P. ponderosa* ( $r^2 = 0.71$ ) in the Sierra Nevada Mountains. Nighttime transpiration as a percentage of daily total transpiration was around 20% for both sites and species. Nighttime water loss was minimal for the Sierra Nevada understory shrubs, *A. manzanita* and *C. cordulatus*.

In the Mediterranean *Eucalyptus* woodlands of the Southern Hemisphere, we found a high correlation ( $r^2 = 0.69$ ) between VPD and  $E_n$  in *E. salmonophloia* (Figure 1). For the one probe set for which a zero reference was made, rates of  $E_n$  were up to 18% of maximum daytime rates. Sap-flow traces from other probe sets for which a zero reference was not obtained were similar to those presented and broadly confirm the patterns and flow rates we report. We also observed correlations between nighttime sap flow and VPD greater than 0.5 kPa in *E. wandoo* (tree;  $r^2 = 0.69$ ), *Allocasuarina arenarius* (shrub;  $r^2 = 0.61$ ) and *Nuytsia floribunda* (tree;  $r^2 = 0.53$ ). Coefficients of determination ( $r^2$ ) of 0.45, 0.39, 0.34 and 0.32 were found in *E. albidula* Maiden and Blakely (tree), *Dryandra sessilis* R. Br. (shrub), *Isopogon gardneri* Foreman (shrub) and *Dryandra sessilis* (Knight) Domin var. *sessilis* (shrub), respectively, whereas the remaining species (e.g., *Hakea subsulcata* Meisn. (shrub), *Banksia sphaerocarpa* R. Br. var. *sphaerocarpa* (shrub), *Actinostrobos arenarius* C.A. Gardner (tree) had  $r^2$  values ranging from 0.25 to near zero (S. Burgess et al., unpublished results). There was considerable variation among individuals and the values reported are the strongest relationships we found; we consider them an index of the potential for  $E_n$ .

The montane forest of Waikamoi, Hawaii typically experiences high humidities with nighttime VPDs near zero. In *M. polymorpha*, we commonly observed a slow decline in transpiration at the end of the day, reaching a steady rate that was assumed to be zero approximately 2–4 h after sunset. We consider the lag from sunset to this steady rate represents xylem storage tissue recharge. However, we also observed that, after such recharge events, sap flow occasionally peaked again in the dark and that these peaks coincided with non-zero VPD (Figure 2). Nighttime peaks in sap flow were observed only at times of non-zero VPD, supporting the interpretation that nighttime peaks represent  $E_n$ . This qualitative indication of  $E_n$  warrants further investigation with quantitative methods. Re-

Table 2. Daytime and nighttime transpiration rates by ecosystem type, location and tree (t) and shrub (s) species. Data type is indicated in parenthesis as follows: transpiration,  $E$ , as sap velocity in  $\text{cm h}^{-1}$ ; stomatal conductance,  $g$ , in  $\text{mmol m}^{-2} \text{s}^{-1}$ . Data daylight values, nighttime values and the nighttime value as a proportion of the mean daylight value. Values at the higher end of each range are generally associated with summertime or peak growing season maxima.

Biome	Type	Day	Night	Night/Day
Coastal temperate and temperate deciduous and coniferous forests, USA				
<i>Acer saccharum</i> (g)	t	130–355	4.5–77	0.05–0.25
<i>Acer rubrum</i> (g)	t	119–322	2.2–58	0.02–0.20
<i>Pinus strobus</i> (g)	t	52–222	10.1–40	0.018–0.17
<i>Vaccinium spp.</i> (g)	s	37–209	9.5–60	0.07–0.22
<i>Sequoia sempervirens</i> (E)	t	9–27	0.5–7.6	0.03–0.18
<i>Pseudotsuga menziesii</i> (E)	t	6–33	0.2–3.3	0.01–0.07
<i>Lithocarpus densiflora</i> (E/g)	t	11–40 / 82–195	3.4–9.0 / 3.0–44	0.07–0.11
<i>Umbellularia californica</i> (E/g)	t	23–31 / 52–178	0–1.2 / 3.0–19	0.01–0.05
<i>Gaultheria shallon</i> (g)	s	36–188	5.7–38.3	0.07–0.21
<i>Pinus ponderosa</i> (E)	t	1–19	0–2	0–0.21
Mediterranean oak-savanna, USA				
<i>Quercus douglasii</i> (E)	t	1–20	0–3	0–0.18
<i>Arctostaphylos manzanita</i> (E)	s	0.5–16	0–1	0–0.06
<i>Ceanothus cordulatus</i> (E)	s	0–15	0–1	0–0.07
Mediterranean chaparral, USA				
<i>Quercus agrifolia</i> (g)	t	15–183	0–9.5	0–0.02
<i>Lithocarpus densiflora</i> (E/g)	t	8–34 / 39–147	1.9–3.6 / 1.0–22	0.03–0.08
<i>Arctostaphylos glandulosa</i> ssp. <i>glandulosa</i> (g)	s	22–244	2.5–23.8	0.05–0.15
<i>Ceanothus cuneatus</i> (g)	s	48–326	0–0.6	0–0.01
<i>Rhamnus californica</i> (g)	s	33–190	12.4–40	0.03–0.08
<i>Heteromeles arbutifolia</i> (g)	s	21–277	0	0
Tropical montane forest, Hawaii, USA				
<i>Metrosideros polymorpha</i> (E)	t	0.3–1.8	0–0.3	0–0.18
Tropical evergreen forests, Brazil				
<i>Coussarea racemosa</i> (E)	t	3–16	0–4.0	0.01–0.25
<i>Manilkara huberi</i> (E)	t	1–11	0–1.0	0.01–0.11
<i>Protium robustum</i> (E)	t	1–11	0–1.5	0.01–0.13
Neotropical savanna, Brazil				
<i>Byrsonima verbascifolia</i> (E)	t	0–30	0–3.5	0.01–0.12
<i>Roupala montana</i> (E)	t	2–35	0–5.0	0.01–0.14
<i>Vellozia flavicans</i> (E)	s	0–18	0–2.0	0.01–0.11
Mediterranean woodlands and shrublands, SW Australia				
<i>Eucalyptus salmonophloia</i> (E)	t	2–8	0–1.5	0–0.18

regardless of whether the pattern is quantitative or not, this type of response was observed in all three species studied and corresponded to the nighttime movement of dry air masses over the islands, which is consistent with the assumption that the elevated basal sap flow was indicative of  $E_n$ . When air relative humidity returned to near 100% and VPD was essentially zero (between 0330 and 0600 h in Figure 2), sap flow continued, presumably due to xylem refilling. Although absolute nighttime sap flow rates were low because of the high humidity in this moist forest, nighttime sap flow constituted up to 17% of mean maximum daily rates.

In the Amazon, we found a strong positive correlation between  $E_n$  and VPD in the tree species *C. racemosa* ( $r^2 = 0.54$ ; Figure 3). This was also observed in a tree common to the Brazilian Cerrado (savanna), *B. verbascifolia* ( $r^2 = 0.58$ ; Fig-

ure 4). On average,  $E_n$  was about 57% of maximum daylight dry season  $E$  for *B. verbascifolia* and about 12% for *C. racemosa*. As in *S. sempervirens* in California, rates of  $E_n$  approached between 10 and 30% of maximum transpiration rate measured at midday during the hot dry season for both species. Patterns of  $E_n$  in individual *R. montana*, *V. flavicans* (in Oliveira et al. 2005b), *M. huberi* and *P. robustum* are consistent with the patterns represented in Figures 3 and 4 (see also Table 2).

#### Nighttime stomatal conductance

As with our other study species, in two common maple tree species of deciduous forest in northeastern USA, we found a strong positive correlation ( $r^2 = 0.64$ ) between nighttime sto-

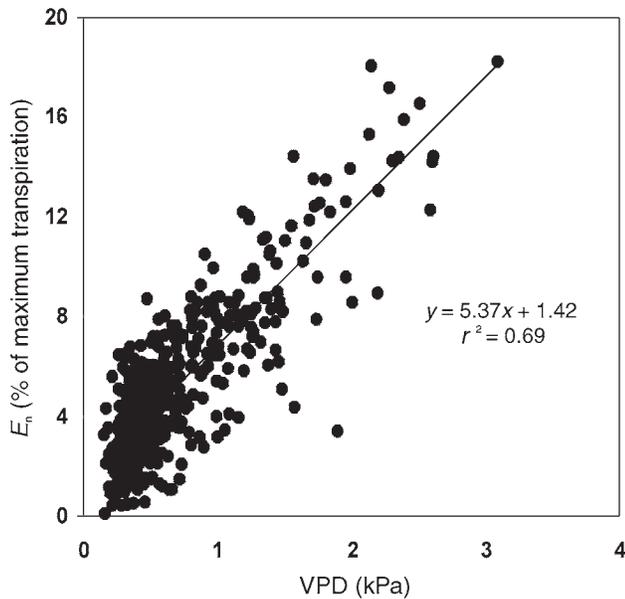


Figure 1. Relationship between vapor pressure deficit (VPD, kPa) and nighttime transpiration ( $E_n$ ) in *Eucalyptus salmonophloia* expressed as % of summertime maximum transpiration rate. Measurements were made between 2230 and 0430 h over 39 summer nights in 2004.

matal conductance,  $g_n$  and the leaf-to-air vapor pressure gradient, VPG (Figure 5). These direct measurements of water loss from leaves were made at several different times of the growing season over a period of four years. Values were generally higher in plants with low midday xylem pressure potentials ( $< -0.9$  MPa); data not shown, but see Figure 6). Overall,  $g_n$  was low, between 5 and 9% of daily maximum  $g$ , though on dry (VPG  $> 1.6$  kPa) nights it approached 25% of the mean daily maximum  $g$  (Figure 5).

For maple trees, we commonly observed  $g_n$  for up to 5 full days (~120 h) following a significant ( $> 20$  mm) summer rainfall event (Figure 6a), which also enhanced daylight  $g$  in both *A. saccharum* and *A. rubrum* (Figure 7). When  $g_n$ ,  $g$  and their ratio are plotted in relation to the number of days following rainfall, we see a strong ( $r^2 = 0.92$ ) negative correlation with the highest values immediately following rainfall (Figure 7). This relationship provides a strong and predictable index of water loss from plants at night based on daylight values and has important implications for modeling studies at the ecosystem and catchment scales.

#### Nighttime transpiration estimated by deuterium labeling experiments

The results of our deuterium labeling experiments are shown in Table 3 and Figure 8 using both the stem base injection (Figure 8A) and soil irrigation (Figure 8B) treatments. Both treatments gave similar patterns, though isotope applied by direct stem injection was detected sooner in both stem and leaf tissues (Figure 8A) compared with isotope applied in the soil irrigation treatment, reflecting the greater distance water applied to the soil must travel. Although the isotope could be de-

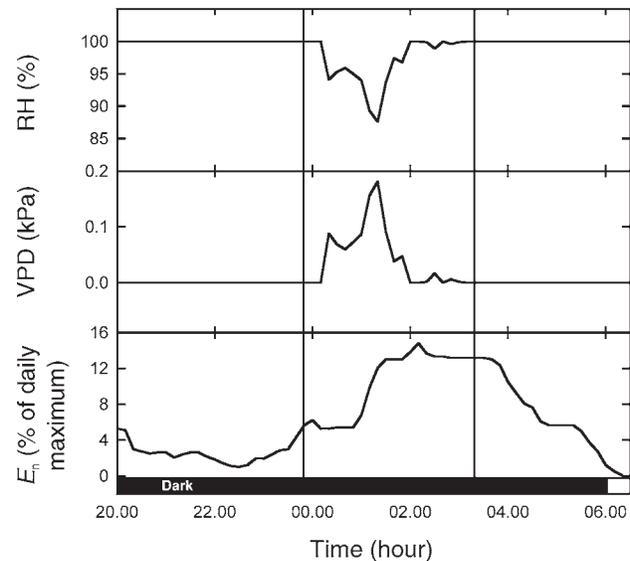


Figure 2. Nighttime transpiration expressed as percent of maximum daily sap flow for *Metrosideros polymorpha* from 2000 to 0600 h at Waikamoi, Maui, Hawaii, in November 1996. The bar along the bottom of the figure shows the dark period. Sap flow at the stem base rises in the night when relative humidity (RH; top panel) drops and the vapor pressure deficit (VPD, kPa) rises (0000 to ~0300 h). The slow decay of transpiration back to near zero from ~0300 to 0600 h is likely to be xylem refilling.

tected within 3 h of application, maximum stem and leaf labeling was not observed until 12–15 h after application, or between 0600 and 0900 h. Some of the highest accumulations

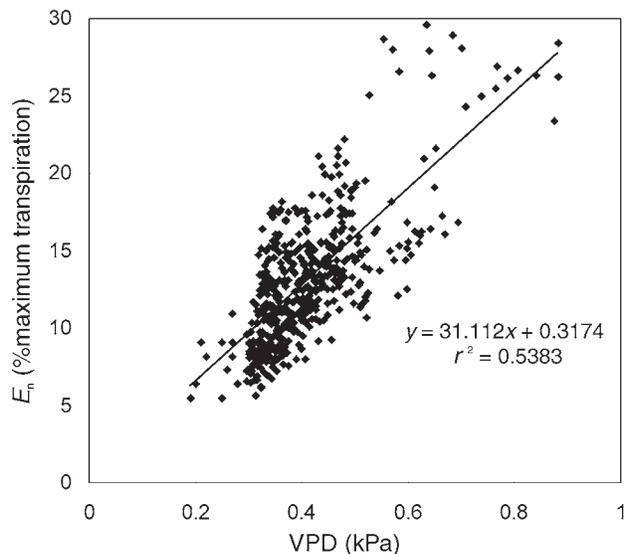


Figure 3. Relationship between vapor pressure deficit (VPD, kPa) and nighttime transpiration ( $E_n$ ) in *Cousarea racemosa* (in an Amazonian forest) expressed as % of daylight maximum transpiration rate. Measurements were made between 2330 and 0530 h over 28 nights in the dry season of 2001.

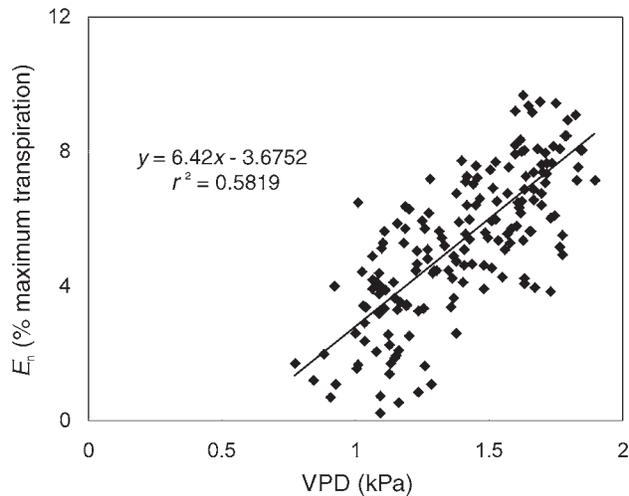


Figure 4. Relationship between vapor pressure deficit (VPD, kPa) and nighttime transpiration ( $E_n$ ) in *Byrsonima verbascifolia* expressed as % of daylight maximum transpiration rate. Measurements were made between 2330 and 0530 h over 14 nights in the dry season of 2001.

of label occurred at 0900 h and were likely enhanced as a result of stomatal conductance increasing in the early morning (after 0650 h on the day of observation) with increasing daylight. The accumulation of isotope label with time in the dark suggests that water was being lost from leaves (cf. Calder 1992) and this is corroborated by our porometric data (Table 2, Figures 5–7). Calculations based on the data shown in Figure 8 suggest that rates of water loss from leaves over an 8-h night were between 0.0032 and 0.0014  $\text{m}^3 \text{night}^{-1}$  (or 3.2 to 1.4l) or

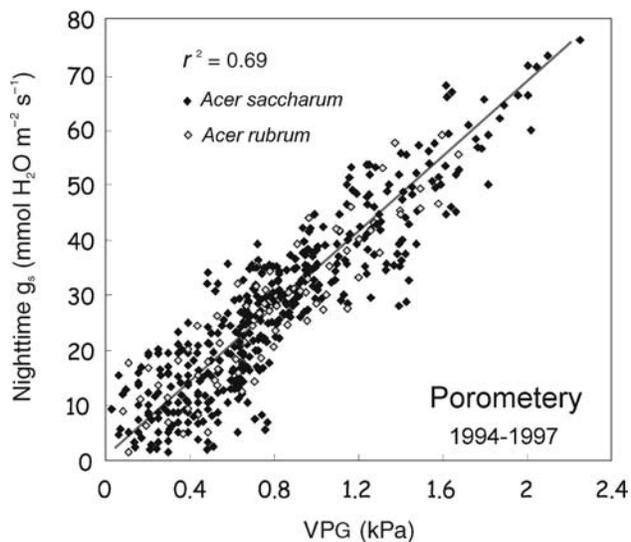


Figure 5. Relationship between the leaf-to-air vapor pressure gradient (VPG, kPa) and nighttime stomatal conductance ( $g_n$ ) in sugar (*Acer saccharum*,  $\blacklozenge$ ) and red (*A. rubrum*,  $\diamond$ ) maple. Measurements were made between 2300 and 0400 h over 77 nights during the 1994 to 1997 growing seasons.

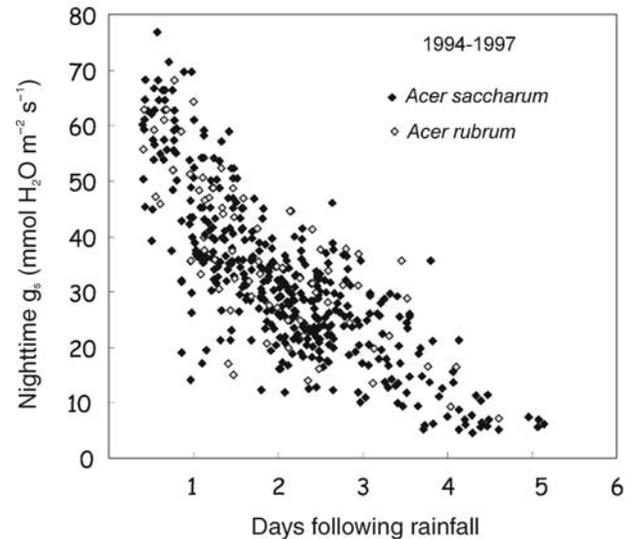


Figure 6. Maximum rates of nighttime stomatal conductance in sugar (*Acer saccharum*,  $\blacklozenge$ ) and red (*A. rubrum*,  $\diamond$ ) maple species in response to the number of days since the last rainfall event. Measurements were made between 2300 and 0400 h on 30 different nights during the 1994 to 1997 growing seasons.

between 15.1 and 6.6% of daily maximum values estimated for the same trees (Table 3).

## Discussion

Before discussing our findings, it is important to acknowledge that a large fraction of the data that we and others have presented on  $E_n$  or  $g_n$  were not originally collected with the primary goal of studying nighttime water loss. This highlights the importance of analyzing data, particularly sap flow data, in relation to the solar period (daylight, night and total) or the occurrence of  $E_n$  could be overlooked. In the studies reported here,  $E_n$  or  $g_n$  was measured in every ecosystem type investigated and in both trees and shrubs, although the degree to which  $E_n$  occurred, as indicated by the strength of the relationship between water flux and VPD or VPG, varied. For the species and ecosystems we investigated,  $E_n$  or  $g_n$  generally occurred when nighttime VPD or VPG exceeded  $\sim 0.2$  kPa for plants inhabiting ecosystem types with high water availability (e.g., at higher soil water contents or following rainfall events; Figures 2–4) or, in the drought-prone ecosystems, when it was slightly higher ( $\sim 0.7$  kPa) (Figures 1 and 5–7). In two cases,  $E_n$  occurred when nighttime wind velocities exceeded  $0.8 \text{ m s}^{-1}$  (unpublished data). Taken together, the examples presented suggest that there are two drivers of  $E_n$  in the woody plants we investigated: nighttime evaporative demand and soil water availability. Furthermore, our data, as well as those presented previously (e.g., Darwin 1898, Hinckley and Scott 1971, Hinckley and Ritchie 1973, Rawson and Clarke 1988, Matussek et al. 1995, Assaf and Zieslin 1996, Donovan et al. 1999, 2000, Synder et al. 2003, Burgess and Dawson 2004 and references therein) and in this collection of papers (Cavender-Bares et al. 2007, Dawson et al. 2007, Fisher et al.

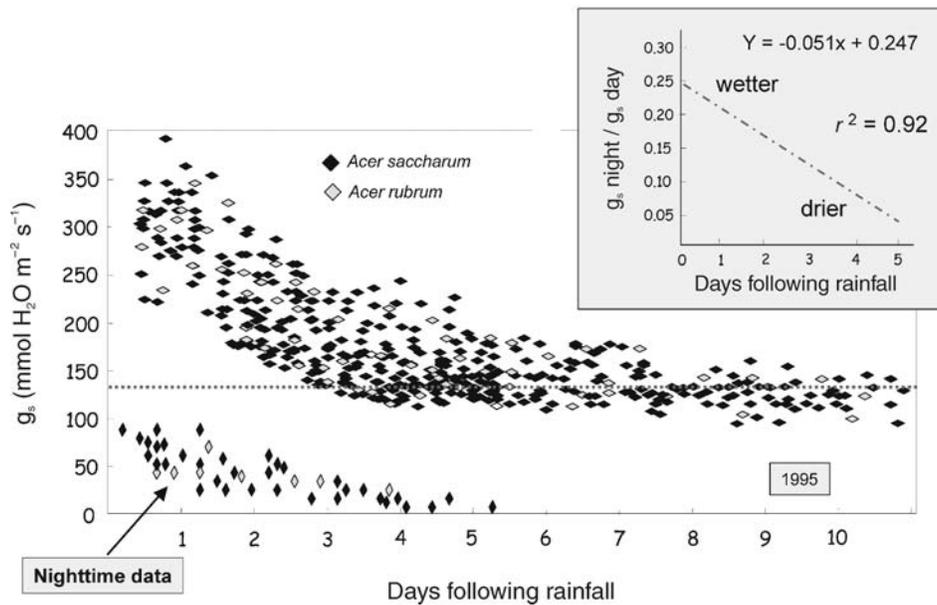


Figure 7. Daylight and nighttime stomatal conductance in the two maple tree species shown in Figures 5 and 6 in response to the number of days since the last rainfall event. Measurements were made between 2300 and 0400 h during two 10-day/night periods in the 1995 growing season. In the upper right-hand corner the ratio of these two measurements for each day following rainfall is plotted. The regression shows that there is a greater fraction of nighttime  $g_n$  during the wettest periods just following rainfall versus later.

2007, Hubbart et al. 2007, Kavanagh et al. 2007, Marks and Lechowicz 2007, Scholz et al. 2007, Seibt et al. 2007) show strong evidence that plants of all types inhabiting a wide range of ecosystems can transpire at night. This evidence suggests that the notion that stomata always close in the dark is wrong. The common occurrence of  $E_n$  and  $g_n$  appears, thus, to be much more widespread than has generally been acknowledged, and in some instances, appears to be highly predictable (Figure 7).

#### Methodological implications

Our results have several important implications concerning methods for investigating relationships between plants and their water resources. For example, most, but not all, sap-flow methods (e.g., HRM; after Marshall 1958) estimate canopy water loss based on the assumption that nighttime water flux through trees is zero. Some sap-flow methods not only assume zero flow at night, but apply an algorithm that resets daily sen-

sor drift to zero each night (e.g., Granier 1985). Our objective here is not to cast doubt on investigations that have used these methods, particularly when used to estimate daylight transpiration, but to urge the need for care to insure the selection of an appropriate method. If the objective is to document if, or when,  $E_n$  occurs, the use of sensitive methods such as the heat-ratio sap flow method (HRM; Burgess et al. 2001a) that can resolve zero flow seems advantageous. Our data obtained with the HRM show that, for woody plants inhabiting an array of ecosystem types, nighttime transpiration occurs and therefore sap flow is rarely zero. Under conditions where  $E_n$  occurs but zero flow is assumed, as is often the case with the Granier (1985) method, the data obtained are qualitative at best and therefore any conclusion drawn about the occurrence of  $E_n$  needs verification. Our data for *M. polymorpha* (Figure 2), for example, suggest the occurrence of  $E_n$ , but we believe a zero estimate and verification that thermal gradients are not the

Table 3. Calculated rates of transpiration of *Acer saccharum* trees following deuterium tracer applications. Rates are estimated in  $\text{m}^3 \text{day}^{-1}$  for both daylight ( $E$ ) and nighttime ( $E_n$ ) periods for trees in the direct injection and soil application treatments. The first number is the rate for the upper (~20-m high) leaves and the second number is the rate for the lower (~5 m high) leaves. Rates of  $E_n$  were calculated from the data in Figure 8 and constructing an isotope concentration–time curve (after Calder 1986). Also shown are daylight ( $g$ ) and nighttime ( $g_n$ ) stomatal conductances ( $\text{mmol m}^{-2} \text{s}^{-1}$ ), determined by porometry, for comparison with the values shown in Table 2.

Treatment	$E$	$E_n$	$g$	$g_n$
<i>Injected</i>				
Tree 1	0.0202, 0.0171	0.0022, 0.0015	244, 219	41, 32
Tree 2	0.0303, 0.0194	0.0029, 0.0017	328, 309	29, 27
Tree 3	0.0321, 0.0255	0.0014, 0.0015	277, 249	34, 30
<i>Watered</i>				
Tree 1	0.0242, 0.0228	0.0023, 0.0020	259, 244	61, 36
Tree 2	0.0309, 0.0265	0.0032, 0.0024	340, 318	70, 59
Tree 3	0.0263, 0.0240	0.0027, 0.0025	292, 277	39, 32

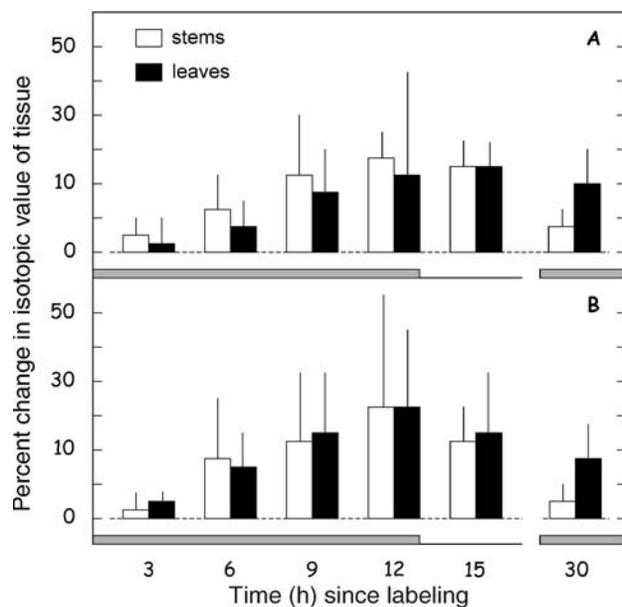


Figure 8. Percent change in the stable isotope composition of water extracted from leaves and stems every 3 h for 15 h and then again after 30 h following deuterium labeling directly to the stem base (A) or to the soil around the tree (B) (see Methods for details). Based on these isotope enrichments, the transpiration rate was calculated (see Table 3). Gray bands represent night.

cause of the observed sap flux are required before the data can be used quantitatively. We have more confidence in data obtained with the HRM because it excludes effects of thermal gradients, it is accurate at low flow rates, and it can be used in conjunction with xylem cutting to obtain an accurate zero reference.

An important question that is not fully resolved by any sap flow method currently available is how to differentiate between nighttime flows partitioned into xylem refilling (from depleted water stores) and  $E_n$ . We have suggested an approach, as have Daley and Phillips (2006) and Fisher et al. (2007), to differentiate refilling from  $E_n$ . Coupled with additional measurements such as  $g_n$ , deuterium tracer data (Figure 8, Table 3), the measurement of volumetric changes unrelated to growth using automatic high precision dendrometer bands (e.g., Irvine and Grace 1997, Peramaki et al. 2001), as well as placing HRM sap-flow sensors throughout the plant crown (see Burgess and Dawson 2004), can help to better resolve this important issue, while providing a robust way of quantifying  $E_n$  and other important parameters such as stem capacitance.

For our examples, if all nighttime sap flow were simply due to storage tissue refilling, it is unlikely there would be a strong relationship between sap flow and nighttime VPD (Figures 1–5). One difficulty of interpretation is that there is typically a decline in VPD as the night progresses and air temperatures fall, which could match the decay function in sap flow data that would indicate refilling. In this case, refilling and  $E_n$  would be difficult to partition. Hinckley and Ritchie (1973) overcame this problem with small trees by using plastic bags

that contained the plant and a wet towel. For larger trees, one approach is to search for data where VPD drops rapidly at dusk, so that refilling can be examined in the absence of a driver for  $E_n$ . In such cases, where no decay in sap flow is detected, it can be inferred that refilling is small relative to  $E_n$ . For example, in Figure 9, Night 12 shows a slight decay function that may partly be the result of a slight decrease in VPD, but also appears to include refilling. Night 14, on the other hand, has a decay function that matches that of VPD and we can surmise from night 14 that very little of the sap flow was due to refilling. Because the HRM can detect the low flow rates found at night, we believe that it may be one of the best ways to quantify and partition  $E_n$  and refilling. A second example of this approach is shown by Fisher et al. (2007) where nighttime HRM traces often displayed two distinct phases; a post-daylight decay phase and a constant phase that comes some time later; both of these phases show sap flow rates above zero and both occur at night. Fisher and colleagues suggest that the early sloped phase represents mostly xylem refilling and the later, non-zero linear phase represents nighttime transpiration (see also Figure 6 in Fisher et al. 2007). This approach or the method advocated by Phillips et al. (1997) and Daley and Phillips (2006) are useful for partitioning  $E_n$  from refilling. Our approach is empirical, whereas Phillips et al. (1997) predict transpiration rates with the Jarvis-McNaughton model and compare the predicted  $E_n$  rates versus rates obtained from sap flow measurements.

#### Ecophysiological implications

Understanding why  $E_n$  or  $g_n$  occur is challenging given the presently available data because most studies, including many of our own, were not originally designed to investigate  $E_n$  or  $g_n$ .

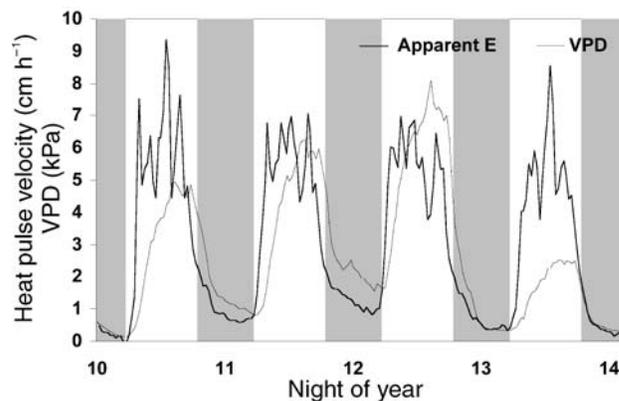


Figure 9. Heat pulse velocity ( $\text{cm h}^{-1}$ ) as an indicator of nighttime transpiration ( $E_n$ ) in *Eucalyptus salmonophloea* and its relationship to vapor pressure deficit (VPD, kPa) over five summer nights in 2004 at Corrigin, Western Australia. Gray bands represent night. The thick, black line is the sapflow trace and is closely associated with the daily course of solar radiation. The light gray line is VPD, which peaks in the afternoon when air temperature reaches its maximum and relative humidity is usually at its lowest. Transpiration (sap flow) in this species of eucalypt peaks when solar radiation is maximal and leaf energy loads are highest.

nor were the methods standardized. Therefore, a meta-analysis of  $E_n$  or  $g_n$  is not easily accomplished, precluding quick identification of the possible reasons underlying the occurrence of  $E_n$  and  $g_n$ . We offer some hypothetical reasons that, if correct, have important implications.

We are unaware of any direct evidence for why plants lose water at night, yet some testable hypotheses for the general occurrence of nighttime transpiration, in longer-lived woody plants in particular, can be stated. First,  $E_n$  may promote carbon fixation early in the daylight hours because it means that stomata are already open, and photosynthesis can begin as soon as light availability and temperature permit (see Oren et al. 1999). This corresponds with what is observed in plants from low-light environments which depend, for photosynthesis, on sunflecks (see Pearcy 1988). Second, and as suggested by Scholtz et al. (2007),  $E_n$  could enhance nutrient supply to distal parts of a woody plant crown. Third, continuous water flux at night may serve to deliver  $O_2$  to parenchyma cells in woody tissues where it is required for respiratory processes (see Daley and Phillips 2006). This may be particularly important in large trees in which the availability of oxygen to tissues is necessarily low because of the long diffusion path through water-filled woody tissues. Finally,  $E_n$  may occur simply because stomata are prevented from closing for various possible reasons, including by waxy plugs, leaf endophytes or asymmetrical guard cell complexes. It must also be the case that some small fraction of  $E_n$  reflects cuticular water loss from leaves, which likely increases as leaves age. To quantify the importance of this pathway, however, better field methods are required.

Under conditions where plants experience water deficit,  $E_n$  may come at a cost by preventing plants from repairing embolized xylem conduits as would otherwise occur at night. In addition, the lack of refilling of water storage tissues should reduce overall transpiration the following day, because plant storage reservoirs were not replenished overnight. Reduced tissue water storage due to  $E_n$  would lead to lower tissue water potential and greater risk of xylem cavitation and embolism during times of high evaporative demand (Sperry 2000; Buckley 2005). Many nights of  $E_n$  in succession may lead to a repeated cycle that prevents plants from refilling their capacitors. This would lead to further cavitation and a reduced chance of repair, thus impairing the important hydraulic re-supply cycle that plants otherwise experience with daily stomatal closure.

By using a whole-plant water balance framework, we can begin to see how  $E_n$  could reduce the efficacy of hydraulic redistribution (HR) as well as tissue capacitance,  $C$ , as:

$$RU_N - k_{HR}\Delta\Psi_{U-R} - E_n = C_R \frac{d\Psi_R}{dt} + C_S \frac{d\Psi_S}{dt} + C_L \frac{d\Psi_L}{dt} \quad (1)$$

where  $RU_N$  is nighttime root water uptake,  $C_R$ ,  $C_S$  and  $C_L$  are root, stem and leaf specific capacitances, i.e., the change in tis-

sue water content ( $\Delta W$ ) per unit change in water potential ( $d\Psi$ ; Williams et al. 2001),  $k_{HR}$  is hydraulic conductance associated with HR and  $\Delta\Psi_{U-R}$  is the water potential gradient between the uptake and release layers of the soil profile (Lee et al. 2005). Greater  $E_n$  will divert water that would otherwise be available for both HR ( $k_{HR}\Delta\Psi_{U-R}$ ) or the refilling of internal water stores (right-hand side of Equation 1). It is also apparent from Equation 1 that if pressure–volume characteristics are known, particularly the bulk elastic modulus of roots, stems and leaves, then measures of water potential and sap flow could be used to partition the proportion of nighttime sap flow that contributes to refilling internal water reservoirs versus  $E_n$ . Furthermore, for investigations of hydraulic lift or HR, it is prudent to determine if  $E_n$  exists, because if it does, it could reduce the efficacy of these processes. Williams et al. (1993) demonstrated that hydraulic lift increases when evaporative demand is reduced by cloud cover; the corollary is also true, and nighttime transpiration turns the canopy into a water sink that competes with roots in dry soil.

The model presented above suggests that  $E_n$  may complicate investigations that attempt to characterize cost–benefit relationships for comparative analyses (Westoby et al. 2002). Alternatively, if  $E_n$  is considered a plant trait with adaptive value, e.g., by enhancing nutrient status at the expense of water status, then the occurrence of  $E_n$  may produce tradeoffs that maximize partitioning of resources in plant communities with implications for species coexistence. Therefore,  $E_n$  should be most beneficial in low-nutrient systems when soil water availability and nighttime VPD are high; for example, several days of dry weather during the wet season in Mediterranean ecosystems could produce such conditions. If  $E_n$  were shown to be more prevalent under such conditions, this would provide preliminary evidence that  $E_n$  is an adaptive process for plants.

#### Theoretical implications

The finding that  $E_n$  not only occurs but is widespread in plants, and may represent a significant fraction of a plant's daily and seasonal water use, has several important implications for plant water relations theory as well as for studies that utilize plant water use data at larger scales. For example, if  $E_n$  occurs, we need to modify our assumptions about the soil–plant–atmospheric continuum and, in particular, the assumption that the drivers of water flux are important only during daylight hours. This is relevant in parameterizing climate models, where stable isotope data are used to partition plant and ecosystem carbon fluxes (see Seibt et al. 2007), as well as for accurate representation of site water balance. In cases where  $E_n$  is documented, one might use the leaf-to-air vapor pressure gradient, VPG, rather than solar radiation, as the primary driver of transpiration. This then requires estimates of nighttime leaf temperatures, relative humidity and  $g_n$  to calculate the contribution of nighttime water loss. However, if  $g_n$  varies consistently with daylight  $g$  as a function of days after rainfall, as shown in Figure 7, it may be possible to assess total plant water loss (day and night) from measurement of daylight  $g$  only. The relationship between  $g_n/g$  and days after rainfall,

shown in Figure 7, may differ among species and for plants growing under different soil water conditions, because days following rainfall serves here only as a surrogate for plant water status. Thus, we expect that plant water status should provide a better and more general predictor of  $g_n/g$  across species and site conditions.

In the context of whole-plant water balance, we can see why  $g_n$  (or  $E_n$ ) might depend on plant water status by rearranging Equation 1 to give  $E_n$  as the residual of nighttime root water uptake, the sum of the water losses through hydraulic redistribution and the change in water content of the main plant capacitors:

$$E_n = RU_N - \left( k_{HR} \Delta\Psi_{U-R} + C_R \frac{d\Psi_R}{dt} + C_S \frac{d\Psi_S}{dt} + C_L \frac{d\Psi_L}{dt} \right) \quad (2)$$

Thus,  $E_n$  should occur when nighttime root water uptake exceeds water lost either to the soil through hydraulic redistribution and or required to refill internal reservoirs. Nighttime transpiration may also occur when internal water reservoirs are depleted by the movement of water across water potential gradients to the atmosphere. Because this depletion of plant water stores will eventually result in plant water stress and stomatal closure (Tuzet et al. 2003), this latter scenario of a negative plant water balance cannot be sustained for long. In contrast, the former scenario of  $E_n$  during periods of positive plant water balance is sustainable and more likely to be exhibited. Further,  $E_n$  during periods of positive plant water balance is consistent with our observations that  $E_n$  typically occurs when humidity is low yet plant water potential is high. Such conditions are most likely to occur following periods of rain or during the transition between wet and dry seasons.

The finding that  $E_n$  is relatively common limits our ability to assess plant water status from predawn water potential measurements, because we can no longer assume that plant water potential reaches equilibrium with the soil (see Ritchie and Hinckley 1971, Donovan et al. 1999, 2000, 2001, Kavanagh 2007). This notion is based on the additional assumption that nighttime water loss via plant transpiration is zero, thereby permitting the plant–soil water potential equilibrium to be achieved. Our data show that water loss can occur at night, which therefore violates these assumptions. The implication is that, for species in which  $E_n$  occurs, or under conditions where  $E_n$  may occur, predawn measurement of plant water potential cannot be considered an accurate proxy for soil water potential. If  $E_n$  or  $g_n$  occurs, investigators will be required to measure soil water potential directly (e.g., by psychrometry) and if this is not possible, they should not calculate whole-plant hydraulic conductance from soil to leaf because it assumes predawn water potential equilibration between plant and soil.

In conclusion, experimental evidence for nighttime transpiration was found in all biomes studied. That values of  $E_n$  or  $g_n$  were commonly 5–15% of daytime maxima and could exceed 40% of maximum daylight transpiration rates demonstrates that this phenomenon can be a major determinant of plant and

site water budgets. High VPD, abundant soil water and species with relatively insensitive stomatal function produce the conditions under which  $E_n$  is likely most important; most plants experience one or more of these conditions at some point in each and every growing season. The widespread occurrence of  $E_n$  therefore requires us to: (1) rethink how to assess plant water relations; (2) choose appropriate sap flow measurement methods; and (3) retool our transpiration models to include parameters such as nighttime leaf temperature, or  $g_n$ , or both.

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