Xylem sap flow and stem hydraulics of the vesselless angiosperm *Drimys granadensis* (Winteraceae) in a Costa Rican elfin forest

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ABSTRACT

For decades, botanists have considered Winteraceae as the least modified descendents of the first angiosperms primarily because this group lacks xylem vessels. Because of a presumed high resistance of a tracheid-based vascular system to water transport, Winteraceae have been viewed as disadvantaged relative to vessel-bearing angiosperms. Here we show that in a Costa Rican cloud forest, stem hydraulic properties, sapwood area- and leaf area-specific hydraulic conductivities of *Drimys granadensis* L. (Winteraceae) are similar to several co-occurring angiosperm tree species with vessels. In addition, *D. granadensis* had realized midday transpiration rates comparable to most vessel-bearing trees. Surprisingly, we found that *D. granadensis* transpired more water at night than during the day, with actual water loss being correlated with wind speed. The failure of stomata to shut at night may be related to the occlusion of stomatal pores by cutin and wax. Our measurements do not support the view that absence of xylem vessels imposes limitations on water transport above those for other vesselled plants in the same environment. This, in turn, suggests that a putative return to a tracheid-based xylem in Winteraceae may not have required a significant loss of hydraulic performance.

Key-words: *Drimys granadensis*; angiosperm evolution; cloud forest; xylem vessels.

INTRODUCTION

Interest in the patterns and processes of early angiosperm evolution has been renewed in the wake of phylogenetic analyses (Crane 1985; Doyle & Donoghue 1986; Crane, Friis & Pedersen 1995; Taylor & Hickey 1996; Doyle 1998; Soltis *et al*. 1998; Mathews & Donoghue 1999). Among the outstanding issues that have generated much discussion, the origin of xylem vessels among basal angiosperm remains especially contentious (Young 1981; Donoghue & Doyle 1989; Baas & Wheeler 1996; Carlquist 1996; Herendeen, Wheeler & Baas 1999). Vessels are water-conducting tubes in the xylem formed from files of cells (each cell referred to as a vessel element) in which the axial walls are partially or wholly removed during maturation (Zimmermann 1983; Gifford & Foster 1989). Plants with vessels generally have greater hydraulic conductance to water flow than those relying on tracheids because tracheids are generally small in size (both diameter and length) and lack specialized perforation plates (Zimmermann 1983; Tyree & Ewers 1991; 1996; Sperry 1995). The potential for increased hydraulic capacity has led some botanists to suggest that xylem vessels may have been a key innovation that allowed early angiosperms to shift from wet habitats into drier or more disturbed environments, develop larger undissected leaves, and support greater leaf gas exchange rates (Carlquist 1975; 1996; Young 1981; Doyle & Donoghue 1986; Bond 1989). A corollary of this is that angiosperms which lack vessels, such as the Winteraceae, will be ecologically restricted and/or hydraulically challenged, a view that has shaped much of the discussion on the early evolution of the angiosperms (Bailey 1944; Carlquist 1975; Dickison 1975; Cronquist 1988; Thorne 1996).

Members of the Winteraceae (four to six genera, ≈ 65 spp.) are the taxa most often described as the least modified descendents of the first angiosperms (Bailey & Thompson 1918; Bailey 1944; Smith 1945; Takhtajan 1969; Cronquist 1981; 1988; Thorne 1996). This view arises from the general occurrence of Winteraceae in cool, wet habitats such as cloud forests and temperate rainforests (Carlquist 1975; Feild, Zwieniecki, Holbrook 2000), as well as the existence of other putatively primitive morphological characters, an Early Cretaceous fossil pollen record, and post-Gondwanan distribution (Smith 1945; Raven & Axelrod 1974; Gifford & Foster 1989; Thorne 1996; Doyle 2000). A major factor influencing all discussions on both the ecological and evolutionary status of the Winteraceae is the assumption that the absence of xylem vessels places severe restrictions on their water transport system (Bailey & Nast 1944; Carlquist 1975; 1996; Thorne 1996). However, the view of Winteraceae as intrinsically subject to hydraulic limitations imposed by vesselless wood has never been examined from a functional perspective. An example of the need for empirical studies is recent work that demonstrates that waxy stomatal occlusions or 'plugs' in Winteraceae, long believed to restrict water loss, play a role in shedding excess water from leaves (Feild *et al*. 1998). Furthermore, closer

examination of the paleobiogeography and ecology of Winteraceae emphasizes their long association with cold climates, suggesting that the evolution of a vesselless vascular pattern may be more closely related to temperature extremes rather than avoidance of dry conditions (Doyle, Hotton & Ward 1990; Doyle 2000; Feild *et al*. 2000).

The view of Winteraceae as ecologically and physiologically limited by the absence of xylem vessels predicts lower transpiration rates (strong stomatal control of leaf water loss) and/or less allocation to leaf area (relative to stem cross-sectional area) compared with co-occurring species with vessels. Here we present experimental data on stem hydraulic properties and realized rates of water loss by *Drimys granadensis* L. (Winteraceae), a common montane cloud forest tree of Costa Rica, and several co-occurring tree species with vessels. Our main objective was to explore the functional consequences of vesselless angiosperm wood to determine whether Winteraceae are hydraulically disadvantaged relative to vessel-bearing angiosperms. Unexpectedly, we found that *D. granadensis* transpired more at night than during the day. Experimental data detailing the possible leaf-level mechanism(s) contributing to water loss at night are provided using a closely related species, *Drimys winteri*, under controlled conditions.

MATERIALS AND METHODS
Study site and species studied

Drimys granadensis is an abundant understory to canopy tree (~ 20 m tall) that is widely distributed in the wet cloud forest highlands of Venezuela and Colombia, Central America, and southern Mexico (Smith 1943). Plants from a population of *D. granadensis* were examined in La Reserva Bosque Nuboso de Monteverde (10°12′N, 84°42′W) along the ridgecrest of the Cordillera de Tilarán at 1500 m in a typical wind-exposed 'elfin' forest stand (Lawton & Dryer 1980). Measurements were conducted from June to early August 1998. Moisture-laden trade winds and steep local topography results in high rainfall (*c.* 3000 mm y^{-1}) and humidity (saturated conditions reported more than 75% of the time) and low insolation throughout the year (Lawton 1980). In addition to direct precipitation, fog interception by the vegetation contributes a large but unknown amount to the water balance of this forest (Clark *et al*. 1998; Feild & Dawson 1998). Mean annual temperature is 17·2 °C and varies little throughout the year (Lawton 1980). The Cordillera experiences strong tradewinds throughout the year with average monthly wind speeds over a 7 year period ranging between 5 km h^{-1} and 30 km h^{-1}, with speeds of 5–10 km h^{-1} being typical of the months when this study was conducted (Lawton 1980; 1982). A complete description of the vegetation at this site can be found in Lawton & Dryer (1980).

Field measurements and collections were made in a representative patch of elfin forest that had been knocked down by cyclonic winds approximately 10 years prior to this study (R. O. Lawton, personal communication). The vegetation consisted of a short (~ 4 m tall) regenerating thicket of vigorous gap-colonizing species intermixed with some late successional species (Lawton & Putz 1988; Lawton 1990). Because of this recent disturbance, all individuals had the majority of their leaf area exposed to the sky. Five *D. granadensis* individuals of similar size were selected for physiological studies. In addition, five individuals of seven abundant co-occurring tree species with vessels and different ecologies were examined for comparison: early successional species *Cosmibuena valerii* (Standl.) C. M. Taylor (Rubiaceae); *Didymopanax pittieri* Marchal. (Araliaceae), *Oreopanax nubigenus* Standl. (Araliaceae), *Miconia* spp. (Melastomataceae) and late-successional species *Ardisia palmana* Donn. Sm (Myrsinaceae), *Clusia stenophylla* Standl. (Clusiaceae), *Dendropanax latilobus* M. J. Cannon & Cannon (Araliaceae).

Stem hydraulic properties

Stem hydraulic conductivity (K_H) was defined as the mass flow rate of water through an excised stem segment per unit pressure gradient (kg MPa^{-1} m s^{-1}). Because of the remoteness of the field site, it was not possible to use an analytic balance to determine flow rates (Sperry, Donnelly & Tyree 1988) and a method that determines the drop in pressure across a tube of known conductance (Tyree *et al*. 1993; Zwieniecki *et al*. 2000) was substituted. An elevated reservoir (50–80 cm) was used to drive water through a 1·25 m long piece of capillary tubing (PEEK, Upchurch Scientific, WA, USA) and the stem segment, arranged in series. The reservoir water was filtered through a 0·1 μm filter before passage through the stem. Two pressure transducers (PX236, Omega Engineering Inc., Stamford, CT, USA) were used to measure the pressure at each end of the capillary tube, allowing both the pressure drop across the capillary tube and the delivery pressure to the stem segment to be determined. The capillary tube could be easily interchanged with tubes of different inner diameter (and/or length) such that the conductance of the tube was approximately equal to that of the stem. The conductance of each tube was determined by comparing the pressure drop across the tube at a known temperature to the outflow rate measured by an analytic balance (± 0·01 mg). Three delivery pressures (0·001, 0·005, 0·010 MPa) were used and a linear calibration equation determined for each individual capillary tube. Capillary tubes were calibrated before travel to Costa Rica and then immediately upon return to check for drift. After correcting for temperature, a 2% change was observed in the calibration value over a 2 month period. During all measurements, temperatures of the water reservoir and measured stems were monitored with copper-constantan thermocouples (30 American wire gauge, Omega) so that changes in flow rate resulting from changes in the viscosity of water could be removed (Sperry *et al*. 1988).

Because of logistical constraints, we were unable to provide a high-pressure flush to establish maximum stem hydraulic conductivity. Instead, stems were sampled at times when losses in hydraulic conductivity due to

embolisms were probably at a minimum (0800–0930 h on days when wind-driven rain and mist occurred). Branches were cut in the field, double-bagged in plastic, and transported immediately to the Reserve headquarters. Stem segments longer than the longest vessels or tracheids (in the case of *Drimys*) were cut from branches under water. Maximum conduit length had been previously determined by attempting to force air (~ 0·1 MPa) through stem segments as described in Zimmerman & Jeje (1981). Each end of the stem was then shaved with a fresh razor blade and attached to the flow meter. All stem segments were measured within 2 h after initial sampling in the field. Due to limitations on destructive sampling in the Forest Reserve and to minimize size-dependent variation in stem hydraulic properties across species, we restricted our observations to stem segments 3–5 mm in diameter (excluding bark). Sapwood specific conductivity (K_S; kg MPa^{-1} m^{-1} s^{-1}) was determined by dividing stem K_H by the sapwood cross-section area. Sapwood cross-section was measured with a micrometer along the major and minor axis of stem end supporting the leaf area and the surface area contributed by the pith was subtracted (Sperry *et al*. 1988). Leaf specific conductivity (K_L; kg MPa^{-1} m^{-1} s^{-1}) was determined by dividing K_H by the total leaf area supported (i.e. distal) by that branch.

Xylem sap flow, leaf water potential and micrometeorological measurements

The rate of water movement through the xylem of canopy-exposed branches was determined using heat dissipation probes (Granier 1987). Sap flow probes, consisting of two, 25-mm-long, 2-mm-diameter probes, each containing a temperature thermocouple junction and one a heating wire element, were constructed using an in-house assembly procedure similar to that outlined in Granier (1987). Two small (1·5 mm diameter) holes, spaced 15 cm apart, were drilled into the sapwood and the probes carefully inserted. The upper (downstream) probe was heated by passing a constant current through a tightly coiled length of high resistance wire that served as the heating element, while the upstream thermocouple (15 cm from the heated element) functioned as a temperature reference. The portion of the stem containing the probe was insulated with 4 cm of dry, dense foam rubber and covered with a sheet of aluminum-covered bubble-wrap to avoid temperature gradients produced by radiant heating of the stem. This housing was then double bagged with a layer of 2-mm-thick plastic which was sealed at both ends with 10-cm-diameter hose clamps tightened around the measured branch. Finally, the upper and lower ends were covered with silicon paste to prevent water infiltration into the sap flow probes by wind-driven rain or surface flow. This design was impervious to moisture infiltration for approximately 2 weeks.

Six trees were monitored for sap flow (two individuals of *D. granadensis*, and one each of *C. stenophylla*, *C. valerii*, *D. pittieri*, *O. nubigenus*) during a 1 month period. Constraints on battery power availability precluded continuous sap

flow measurements. The temperature differential (ΔT) across the two probes was recorded every 60 s and averaged over 10 min intervals with a datalogger (CR-10X, Campbell Scientific Corp., Logan, UT, USA) interfaced to a 16-differential channel multiplexer (AM416, Campbell Scientific). The contribution of stored stem water to transpiration above the sap flow gauge was assumed to be small because the branches sampled had small sapwood areas. Sap flow velocities (m s^{-1}) were calculated from ΔT using Granier's (1987) empirically determined relationship. This relationship requires that ΔT at zero sap flow be known. During the course of our study, we observed a significant flux of sap at night in *D. granadensis*. Consequently, the assumption of the constant heating method that the maximum temperature difference (ΔT_{max}) at night represents zero flow could not be used for *D. granadensis*. To calculate sap flow and transpiration for this species, we assumed that the maximum measured ΔT, which was observed during calm periods (wind speeds < 0·5 m s^{-1}), irrespective of day or night, represented the zero flow state. We recognize, however, that failure to achieve a true zero flow measurement means that actual sap flow rates will be underestimated.

Mass flow of sap was calculated by multiplying sap flow velocity by the sapwood cross-sectional area (Granier 1987). Because destructive sampling of the measured stems was not permitted in the reserve, stem sapwood areas were determined from a minimum of three small (5 mm diameter) cores extracted from the measured branches after the sap flow measurements had been terminated. Discolouration due to heartwood formation and changes in wood density indicating the pith were used to determine the non-conducting portion of the branch cross-section. Leaf area distal to the sap flow probes was determined by multiplying the total number of leaves supported by that branch by the average leaf area obtained from a subsample of 50 leaves removed randomly from each branch measured. For *D. pittieri,* which has large compound leaves, 15 leaves per branch were removed and averaged. Sap flow budgets of all species measured were calculated by summing the 10 cumulative totals of xylem sap flow from 0600 h to 1750 h (day) and from 1800 h to 0550 h (night) and expressed as a percentage of the daily total.

Leaf water potentials were determined with a stainless steel pressure chamber and a cylinder of compressed nitrogen gas, operated as outlined in Boyer (1995). Leaves for water potential measurements were cut from stems and quickly sealed in moist plastic bags to reduce additional water loss. At each measurement time, three leaves from five individuals per species were sampled. Water potentials were determined at midday five times during the study period and predawn water potentials at 0230 h were measured twice from the same trees measured for midday values.

Photosynthetic photon flux density (PPFD), air temperature, and wind speed were monitored every 60 s and averaged over 10 min intervals. Sensors were mounted on a steel tower near the forest canopy layer (~ 3 m above the ground) in a nearby forest gap. Light measurements were

made with a quantum sensor (Li-190, Li-Cor Inc., Lincoln, NE USA), air temperature using a dual relative humidity and temperature probe (HMP35C, Campbell Scientific), and wind speed with a cup anemometer (Campbell Scientific). Because of the prevailing wind-driven mist and rain at this site, the humidity sensor chip became over-hydrated and relative humidity measurements were not reliable.

Stomatal responses of *Drimys winteri* to light and carbon dioxide

For gas-exchange studies, five plants of *D. winteri* were grown from cuttings under greenhouse conditions (day/night temperatures of 27/22 °C, 400–450 μmol photons m^{-2} s^{-1} PPFD for 10 h, relative humidity 50–80%) at Harvard University. Stomatal conductance to water vapoUr (g_s, mmol H$_2$O m^{-2} s^{-1}) was measured with a portable photosynthesis system (LI 6400, Li-Cor Inc., Lincoln, NE, USA). Leaves were illuminated for measurement using an array of red light-emitting diodes (Li-Cor Inc.). Previous measurements (T. S. Feild, unpublished results) indicated that *D. winteri* does not require blue light to achieve maximal stomatal opening. Prior to beginning each experiment, leaves were pre-illuminated under saturating light (700 μmol photons m^{-2} s^{-1}), low vapour pressure deficit (VPD, 0·6 kPa), and constant CO$_2$ (400 μmol mol^{-1}) atmosphere for 3 h to ensure maximal stomatal opening. The carbon dioxide sensitivity of *D. winteri* stomata was observed by exposing leaves to step changes in CO$_2$ concentration going from high to low CO$_2$ (600, 500, 400, 300, 200, 150, 100, 50, 0 μmol mol^{-1}) at constant light (700 ± 5 μmol photons m^{-2} s^{-1}), temperature (25 ± 0·1 °C), and VPD (0·6 ± 0·2 kPa). CO$_2$ concentration was changed only after g_s reached a steady-state value, typically 20–30 min after the previous change in CO$_2$ level. Stomatal responses to light were inferred from changes in stomatal conductance as light levels were varied from high to low PPFD (1500, 1000, 700, 500, 300, 200, 100, 50, 20, 0 μ photons m^{-2} s^{-1}) at constant temperature, carbon

dioxide (400 ± 5 μmol mol^{-1}) and VPD as above. The youngest, fully expanded leaf from each of five well-watered plants was measured.

RESULTS

Stem hydraulic properties

We found a significant (four- to fivefold) variation in stem hydraulic properties across the eight co-occurring tree species sampled (Table 1). This amount of variability is comparable to range of stem hydraulics reported in other studies which have surveyed a variety of co-occurring tree species (Zotz *et al*. 1998; Becker, Tyree & Tsuda 1999). The intrinsic capacity of the xylem system to conduct water (sapwood specific conductivity, K_S) varied from 0·12 kg MPa^{-1} m^{-1} s^{-1} in *C. stenophylla* to 0·65 kg MPa^{-1} m^{-1} s^{-1} in *D. pittieri* (Table 1). Within this range of variation, K_S of vesselless *D. granadensis* stems was approximately two-thirds of the maximum K_S measured (Table 1). The hydraulic conductivity of *D. granadensis* wood was greater than *C. stenophylla* and *A. palmana,* while similar to that of *C. valerii* (Table 1). The ability of *D. granadensis* stems to supply a supported leaf area with water (leaf specific conductivity, K_L) was approximately 50% of the greatest K_L measured (Table 1). *D. granadensis* K_L was 60% and 20% greater than *C. stenophylla* and *O. nubigenus*, respectively (Table 1). K_L is determined by the hydraulic conductivity of the sapwood (K_S) and the ratio of sapwood to leaf area (i.e. Huber value, Zimmermann 1983; Tyree & Ewers 1991; 1996). Consistent with other studies that have surveyed a range of co-occurring species, we observed a relatively large variation in the ratio of sapwood to leaf area allocation among co-occurring species in this elfin montane cloud forest ranging from 5·3 for *O. nubigenus* to 20·8 for *A. palmana* (Table 1, Tyree & Ewers 1991; 1996; Zotz *et al*. 1998; Becker *et al*. 1999). Huber values of *Drimys* were well within the range of plants with vessels sampled in this cloud forest indicating a similar pattern of allocation to sapwood

Table 1. Averages (± SE) of leaf specific conductivity (K_L), sapwood specific conductivity (K_S), and Huber value (*HV*; ratio of sapwood area to leaf area) for *D. granadensis* compared with seven co-occurring species of elfin cloud forest trees with vessels

Species	Family	$K_L \times 10^4$ kg MPa^{-1} m^{-1} s^{-1}	K_S kg MPa^{-1} m^{-1} s^{-1}	$HV \times 10^4$
Vesselless species				
Drimys granadensis	Winteraceae	3·3 ± 0·05[a]	0·39 ± 0·02[a]	8·4 ± 0·02[a]
Vessel-bearing species				
Ardisia palmana	Myrsinaceae	6·2 ± 0·04[b]	0·23 ± 0·03[b]	20·8 ± 0·01[b]
Clusia stenophylla	Clusiaceae	1·3 ± 0·01[c]	0·12 ± 0·1[c]	11·6 ± 0·01[c]
Dendropanax latilobus	Araliaceae	6·2 ± 0·06[b]	0·64 ± 0·04[d]	6·8 ± 0·02[d]
Didymopanax pittieri	Araliaceae	6·3 ± 0·02[b]	0·65 ± 0·07[d]	7·9 ± 0·01[e]
Cosmibuena valerii	Rubiaceae	4·1 ± 0·06[a]	0·39 ± 0·06[a]	10·4 ± 0·01[c]
Miconia spp.	Melastomataceae	4·5 ± 0·04[a]	0·51 ± 0·09[d]	8·8 ± 0·02[c]
Oreopanax nubigenus	Araliaceae	2·6 ± 0·07[d]	0·48 ± 0·07[e]	5·3 ± 0·01[f]

Two stem segments were measured from five individuals of each species ($n = 10$). Species with a different letter are significantly different from each other at least $P < 0.01$ level of significance (Student's *t*-test).

area relative to leaf area to co-occurring angiosperms with vessels (Table 1).

Realized rates of transpiration and water potential gradients

Maximum transpiration rates (E_{max}) ranged from 0·65 mmol m^{-2} s^{-1} in *O. nubigenus* to 1·72 mmol H$_2$O m^{-2} s^{-1} in *D. pittieri* (Table 2). Compared with vesselled species, *D. granadensis* had greater E_{max} than *C. valerii* and *O. nubigenus*. However, *C. stenophylla* and *D. pittieri* had E_{max} values 20–30% greater that those observed in *D. granadensis* (Table 2).

D. pittieri, *C. valerii*, *C. stenophylla*, and *O. nubigenus* showed consistent midday leaf water potentials (Ψ_L) that varied from − 0·3 to − 0·45 MPa (Table 2). In contrast, midday water potentials of *D. granadensis* were highly variable compared with the other species, ranging from 0·0 to − 0·45 MPa. However, when data from windy (wind speeds greater than 0·8 m s^{-1}) versus calm (wind speeds lower than 0·8 m s^{-1}) days were considered separately, a clear pattern emerged. Midday Ψ_L was significantly more negative (average $\Psi_L = - 0·37$ MPa; SD $= 0·1$; $n = 5$) on windy days than on calm days (average $\Psi_L = - 0·15$ MPa; SD $= 0·2$; $n = 4$). In contrast to *D. pittieri* and *C. stenophylla* which had predawn Ψ_L near zero, predawn Ψ_L of *D. granadensis* varied from − 0·25 to −0·35 MPa (Table 2). In *O. nubigenus* and *C. valerii*, predawn leaf water potentials were somewhat less negative, varying from − 0·05 to − 0·12 MPa and 0·0 to − 0·1 MPa (Table 2).

Diurnal patterns of transpiration

Average photosynthetic photon flux densities (PPFD) at 1200 h on rare periods when the clouds cleared at midday were 1750 μmol photons m^{-2} s^{-1} PPFD (SD = 100; $n = 5$), while on cloudy days, average midday values were 800 μmol photons m^{-2} s^{-1} PPFD (SD = 200; $n = 25$). Air temperature varied between 5 °C (SD = 1·3; $n = 5$) and 3 °C (SD = 0·8; $n = 25$) diurnally on partly cloudy and cloudy days, respectively. Wind speeds, however, varied widely over the course of the day during the 1 month study period from the stalling speed of the anemometer ($\sim$ 0·49 m s^{-1}) to 4·6 m s^{-1}. Wind speeds were generally lowest after midday, increased dramatically at night, and lasted until late morning (Fig. 1b). Night-time observations revealed that these periods of windy weather were associated with clouds and mist.

Diurnal sap flow varied widely among the species measured, ranging from 387 g day^{-1} in *C. valerii* to 2019 g day^{-1} in *D. granadensis* (Table 2). Among the vessel-bearing species, *D. pittieri* and *C. stenophylla* had similar diurnal sap flow (861 and 830 g d^{-1}, respectively) which were twofold greater than measured in *C. valerii* and *O. nubigenus* (Table 2). Both trees of *D. granadensis* had greater daily sap flow than did any of the vessel-bearing cloud forest trees (Table 2). When diurnal sapflow was normalized either by sapwood area or leaf area, water loss rates of *D. granadensis* equalled or exceeded those of any of the vessel-bearing trees. The larger water loss from *D. granadensis* was, in part, due to the substantial sap flow at night. *D. granadensis* trees lost more water at night than during the day and the highest transpiration rates observed in *D. granadensis* occurred at night (Fig. 2). Interestingly, *O. nubigenus* and *C. valerii* showed a similar water-use budget, with nearly half of the estimated diurnal water loss occurring during the night (Table 2). In contrast, *D. pittieri* exhibited the more typical pattern of maximum water loss rates near midday. *C. stenophylla* also had high transpiration rates at midday, although on cloudy days maximum rates of

Table 2. Morphological and water-use characteristics of *D. granadensis* and four co-occurring species with vessels

Species	Height (m)	Sapwood area (cm^2)	Leaf area (m^2)	Leaf area Sapwood area (m^2/cm^2)	Diurnal total sap flow (g d^{-1})	% day versus % night	Maximum transpiration rate (mmol m^{-2} s^{-1})	Pre-dawn Ψ_{leaf} (MPa)	Midday Ψ_{leaf} (MPa)
Vesselless species									
Drimys granadensis 1	2·3	12·6	0·65	0·051	1346 ($n = 3$)	40/60 ($n = 3$)	1·44 ± 0·24 ($n = 5$)	− 0·22 ± 0·008 ($n = 2$)	− 0·32 ± 0·39 ($n = 9$)
D. granadensis 2	3·5	25·8	2·66	0·103	2019 ($n = 3$)	31/69 ($n = 3$)	1·32 ± 0·12 ($n = 5$)	− 0·25 ± 0·012 ($n = 2$)	− 0·36 ± 0·41 ($n = 9$)
Vessel-bearing species									
Clusia stenophylla	3·8	22·9	1·25	0·055	830 ($n = 3$)	64/36 ($n = 3$)	1·67 ± 0·23 ($n = 5$)	0·0 ($n = 2$)	− 0·33 ± 0·013 ($n = 9$)
Cosmibuena valerii	3·3	23·5	2·07	0·088	387 ($n = 3$)	54/46 ($n = 3$)	0·66 ± 0·08 ($n = 5$)	0·0 ($n = 2$)	− 0·40 ± 0·008 ($n = 9$)
Didymopanax pittieri	4·0	11·0	1·71	0·155	861 ($n = 3$)	69/31 ($n = 3$)	1·72 ± 0·18 ($n = 5$)	0·0 ($n = 2$)	− 0·26 ± 0·009 ($n = 9$)
Oreopanax nubigenus	2·2	20·0	3·43	0·172	702 ($n = 3$)	33/67 ($n = 3$)	0·65 ± 0·11 ($n = 5$)	− 0·1 ± 0·001 ($n = 2$)	− 0·21 ± 0·008 ($n = 9$)

Values for maximum sap flow Height rates and diurnal water use budgets (e.g. percentage diurnal water lost in the day versus night) were determined for a minimum of three days of continuous measurement. Leaf potentials are expressed as means ± SE over nine mostly sunny days (averages per day are from three leaves).
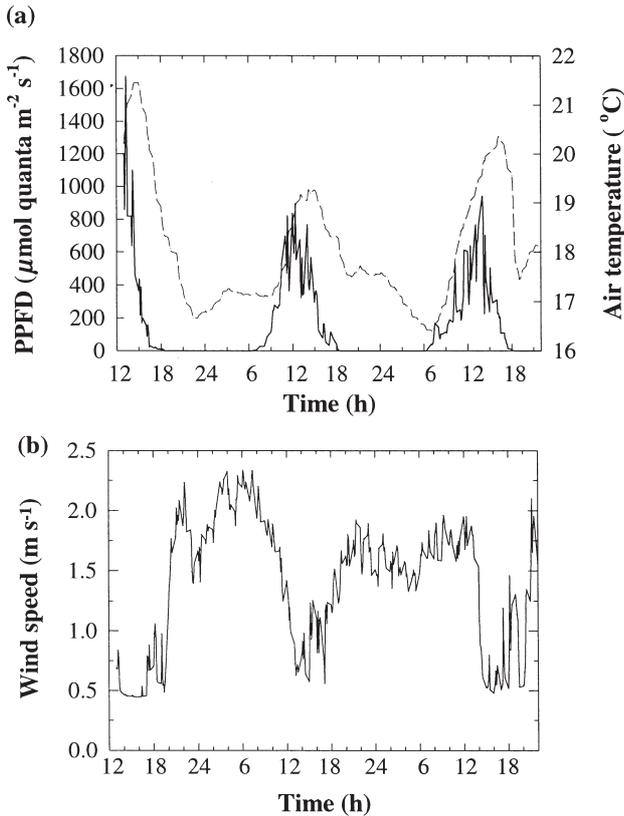
(a)

(b)

Figure 1. Representative diurnal course of (a) photosynthetic photon flux (PPFD, μmol photons m^{-2} s^{-1}) and air temperature (°C), and (b) canopy wind speed (m s^{-1}) for one partly cloudy day and two consecutive cloudy days. Climate data are for July 29 to July 31, 1998.

water loss occurred for a brief period after sunset (between 1800 h and 2000 h, Fig. 2d).

Because of the large night-time sap flow in *D. granadensis*, there was no relation between transpiration and PPFD (Fig. 3a). In contrast, *D. pittieri* showed a positive relation between transpiration and PPFD that most probably was due to stomatal opening in response to light levels (Jones 1998; Fig. 3b). Transpiration from *D. granadensis* did, however, exhibit a positive relationship with wind speed (Fig. 3c). Unlike *D. pittieri,* where the lowest transpiration rates were associated with the highest wind speeds, the highest transpiration rates from *D. granadensis,* whether at night or during the day, were associated with high wind speeds (Figs 1, 2a & b, 3a).

Stomatal responses of *Drimys winteri* to carbon dioxide and light

Measurements of stomatal conductance (g_s) of *D. winteri,* a closely related species with similar epidermal anatomy and ecology as *D. granadensis* (Bongers 1973; Suh *et al.* 1993; Feild *et al.* 1998; 2000; Karol *et al.* 2000), as a function of PPFD and external CO_2 concentration indicated low responsivity to external stimuli. In response to changes

in PPFD from high light (1000 μmol photons m^{-2} s^{-1}) to darkness, g_s of *D. winteri* decreased 8% from 109 to 101 mmol H$_2$O m^{-2} s^{-1} (Fig. 4a). As external CO_2 concentration was decreased from 600 to 0 μmol mol^{-1} under constant PPFD, g_s increased approximately 7% from 102 to 110 mmol H$_2$O m^{-2} s^{-1} (Fig. 4b). This degree of stomatal closure in response to decreasing light availability and stomatal opening in response to decreasing CO_2 concentration are substantially less than reported for a variety of plants (Jones 1998).

DISCUSSION

Our measurements on the water transport properties of *D. granadensis* in an elfin tropical montane cloud forest do not support the view that the absence of xylem vessels constrains water transport capacity (Table 1 & Table 2). This conclusion is based on the similar leaf area- and sapwood area-specific hydraulic conductivities of *D. granadensis* stems compared with several co-occurring tree species with vessels (Table 1). K_L of *D. granadensis* was in the middle range of capacities reported for other vessel-bearing angiosperm cloud forest trees and in the lower range of lowland tropical rainforest species (Zotz *et al.* 1998; Tyree & Ewers 1991; 1996). More importantly, xylem sap flow measurements show that *D. granadensis* transpired more water than all co-occurring species with xylem vessels examined in this study (Table 2). In fact, diurnal water loss by *D. granadensis* was comparable to some lowland rainforest tree species of similar size (Andrade *et al.* 1998; Zotz *et al.* 1998). This indicates that *D. granadensis* does not experience more restrictions on whole plant water use than co-occurring tree species.

The view of the Winteraceae as hydraulically limited has led to the idea that these plants require an especially strong ability to limit rates of water loss (Bailey & Nast 1944; Cronquist 1981; Carlquist 1975; 1996). However, we found that *D. granadensis* lost as much as 60% of its diurnally transpired water at night (Table 2, Fig. 2a & b). Because night-time transpiration by C$_3$ plants does not contribute to leaf carbon gain, it is difficult to view water use by *D. granadensis* as conservative. Sap flow measurements suggest that leaf water loss by *D. granadensis* may be controlled by the leaf boundary layer conductance because stomatal aperture appears to be largely invariant over a wide range of environmental conditions (Fig. 4). We found that transpiration rates of *D. granadensis* increased dramatically with wind speed during both day and night, while water loss from other species decreased with increasing wind speed (Fig. 2). Transpiration rates from *D. granadensis* calculated from sap flow were approximately 10 times greater than measured cuticular water loss rates from a variety of plants (Boyer *et al.* 1997; Hoad, Grace & Jeffree 1997). This suggests that almost all of the water lost at night passed through open stomata of *D. granadensis.* Assuming that *D. winteri* is a suitable surrogate for *D. granadensis,* stomatal responses of *D. winteri* to light and CO_2 show that these leaves have a reduced ability to regulate stomatal
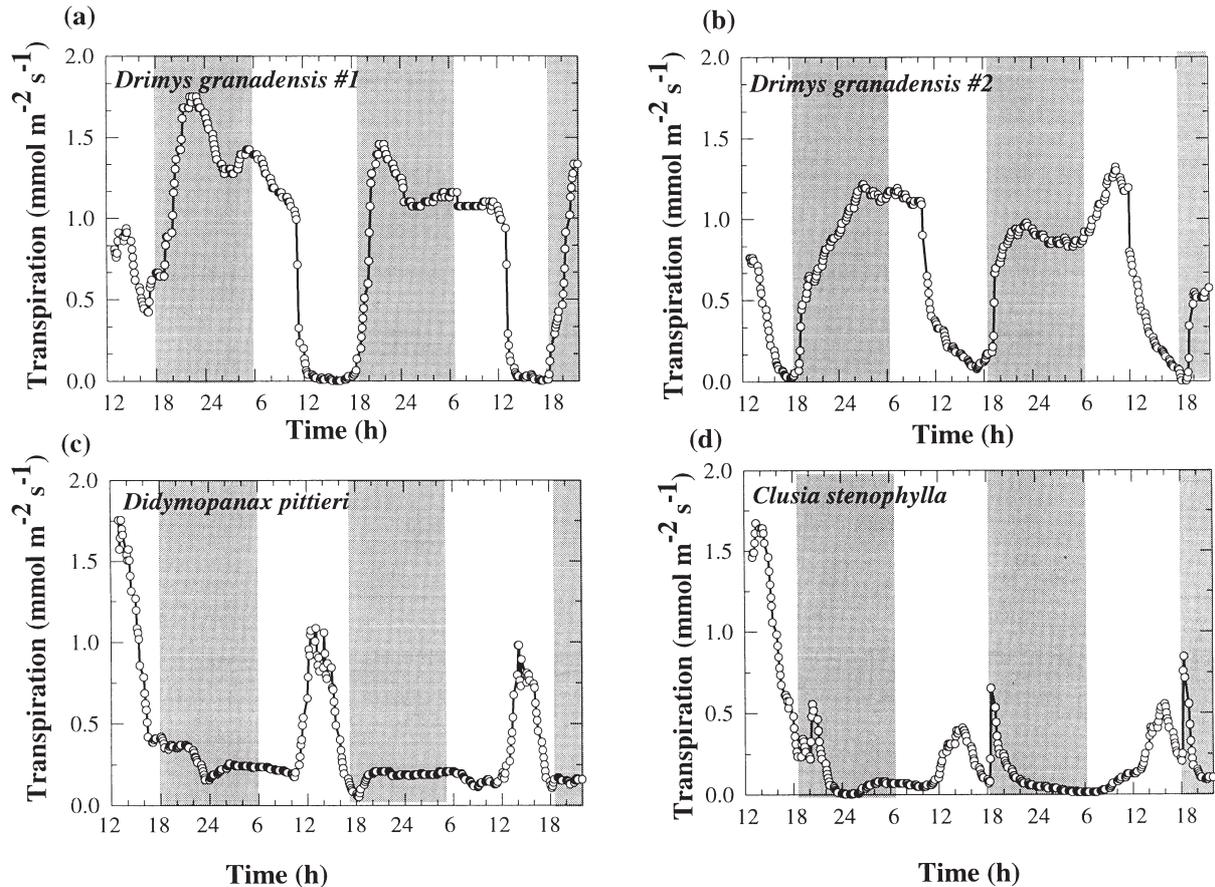
Figure 2. Diurnal course of transpiration (mmol m^{-2} s^{-1}) calculated from xylem sap flow for two trees of *D. granadensis* (a & b), and one tree of *D. pittieri* (c), and *C. stenophylla* (d) on the same days as in Figure 1.

aperture. Previous studies have also shown that *D. winteri* leaves challenged with atmospheric drought (high vapour pressure deficit, VPD) do not close their stomata (Feild *et al*. 1998). Water loss at night has been observed in a variety of temperate and tropical angiosperm trees and shrubs, although the total amounts are quite small (i.e. ~ 5% of daily whole plant water use; Green, McNaughton & Clothier 1989; Steinberg, van Bavel & McFarland 1990; Hogg & Hurdle 1997; Benyon 1999; Donovan *et al*. 1999) and conifers (Herzog *et al*. 1998; Oren *et al*. 1999). The major exceptions to this are species that exhibit Crassulacean Acid Metabolism (CAM). However, we have no evidence for nocturnal CO_2 fixation in any species of Winteraceae.

Sap flow at night is most probably a consequence of an unusual epidermal feature of *D. granadensis*. The stomata of *D. granadensis*, as in most species of Winteraceae, accumulate cutin and wax above the guard cells, forming a hydrophobic granular plug (Bailey & Nast 1944; Bongers 1973; Feild *et al*. 1998). Stomatal plugs are not solid, but riddled with air-filled cavities formed between cutin tubes and wax crystals that allow for moderate gas exchange (Feild *et al*. 1998). The waxy materials of stomatal plugs extend into the stomatal antechamber and appear to phys-

ically prevent the guard cells from fully closing (Bailey & Nast 1944; Bongers 1973). This feature may explain the observed insensitivity of *D. winteri* stomata to light, CO_2, and VPD as well as the transpiration patterns exhibited by *D. granadensis* in the field (Figs 2 & 4; Feild *et al*. 1998). The functional significance of stomatal plugs appears to be related to the occurrence of *Drimys* in areas that are generally wet (montane cloud forests and temperate rainforests). One of the inevitable consequences of frequent rainfall and cloud cover is prolonged wetting of leaf surfaces (Brewer & Smith 1997; Eichert, Goldbach & Burkhardt 1998). Stomatal plugs strongly decrease leaf wet ability, preventing the formation of a continuous water film on the leaf surface that would otherwise impede CO_2 diffusion into the leaf (Feild *et al*. 1998). This conclusion is supported by the maintenance of transpiration by *D. granadensis* during windy periods associated with cloud and mist compared with other species where transpiration rates were inhibited.

In areas with high and reliable rainfall, the inhibition of stomatal regulation by the presence of stomatal plugs is probably to have little cost. However, on rare sunny days or periods of drought, an inability to control water loss rates may place extreme demands on the water balance of *D*.
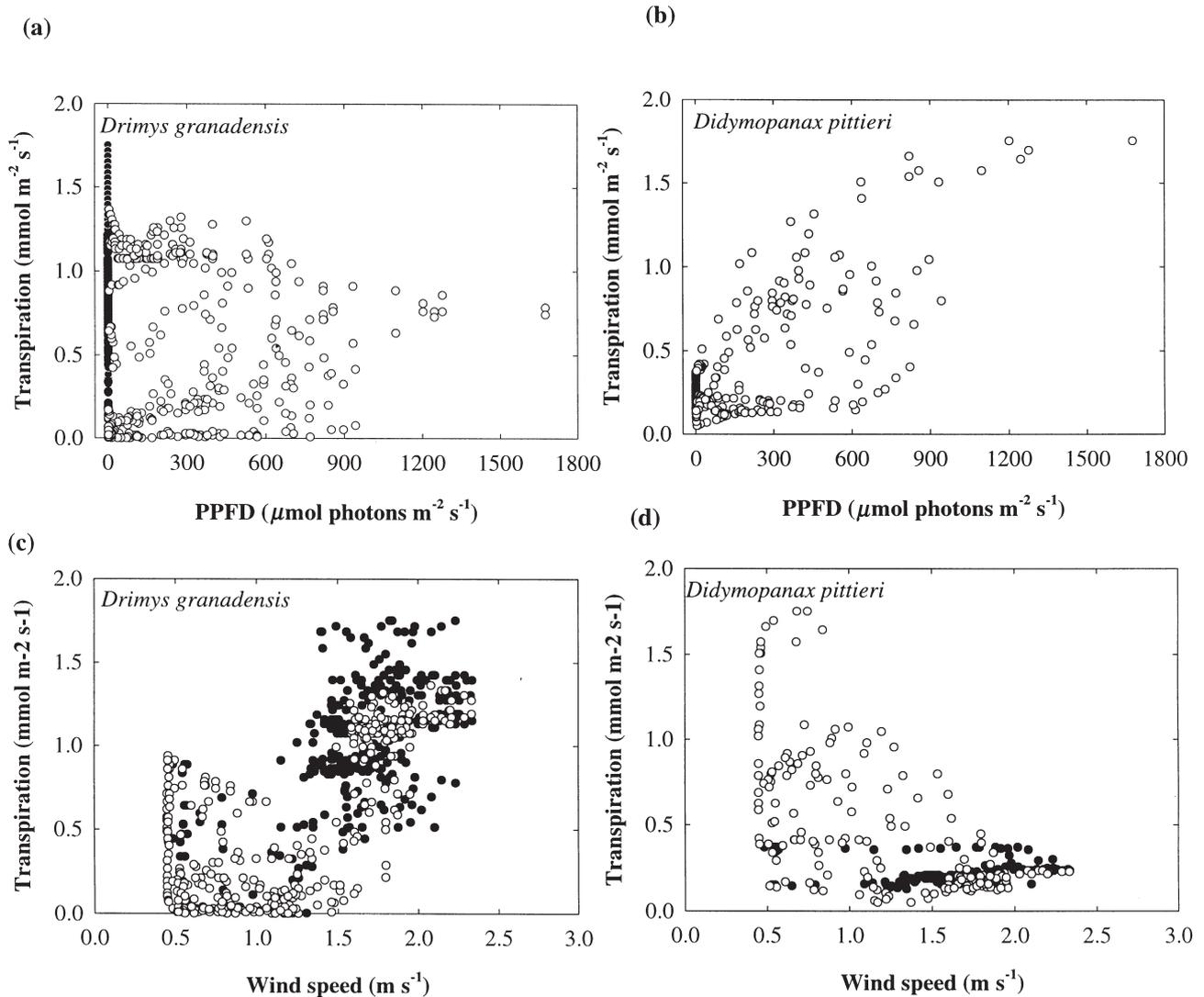
(a)

(b)

(c)

(d)

Figure 3. Diurnal transpiration (mmol m^{-2} s^{-1}) in relation to light (PPFD, mmol photons m^{-2} s^{-1}) for D. granadensis (*n* = 2; a) and D. pittieri (n = 1; b). The relation between transpiration and canopy wind speed (m s^{-1}) for D. granadensis and D. pittieri are depicted in (c) and (d), respectively. Open circles are measurements during daylight hours, filled circles indicate measurements taken during darkness. Data are for the same days depicted in Figs 1 and 2.

granadensis. Preliminary studies on the vulnerability of *Drimys* xylem to drought-induced embolism show that approximately 50% of the tracheids embolized at an air-injection pressure of 2·5 MPa (T. S. Feild, unpublished results). Assuming that xylem embolism by drought occurs by air-seeding, maximum xylem tensions measured in *D. granadensis* (− 0·7 MPa during bright sunny periods) indicate a fairly large safety margin even during periods of maximum transpiration (Sperry 1995). Alternatively, leaves of *Drimys* could potentially adjust the amount of stomatal occlusion by wax in response to evaporative demand the leaf develops under allowing for a typical response of stomatal aperture to PPFD, CO_2, and VPD. For example, small trees of *Tasmannia lanceolata* (Winteraceae) that grow in the understory and subcanopy of eucalypt gallery forests where they encounter frequent leaf wetting from canopy

drip, have wax rodlets associated with their guard cells. In contrast, shrubby forms of this species growing on exposed ridge tops in drier alpine heathlands, lack epicuticular waxes associated with the stomatal apparatus and presumably are able to their close stomata in response to drought (Bongers 1973; T. S. Feild unpublished results). Whether such population-level variability in the occlusion of stomata by wax occurs in *D. granadensis*, or other species of *Drimys*, is unknown.

Our results on the water economy of *D. granadensis* are relevant to recent discussions on whether Winteraceae are primitively versus secondarily vesselless (Young 1981; Carlquist 1983; 1996; Donoghue 1989; Donoghue & Doyle 1989; Doyle 1998; 2000; Feild *et al*. 1998; 2000). Although phylogenetic analyses of angiosperms place *Amborella*, a vesselless tropical cloud forest shrub, at the base of the
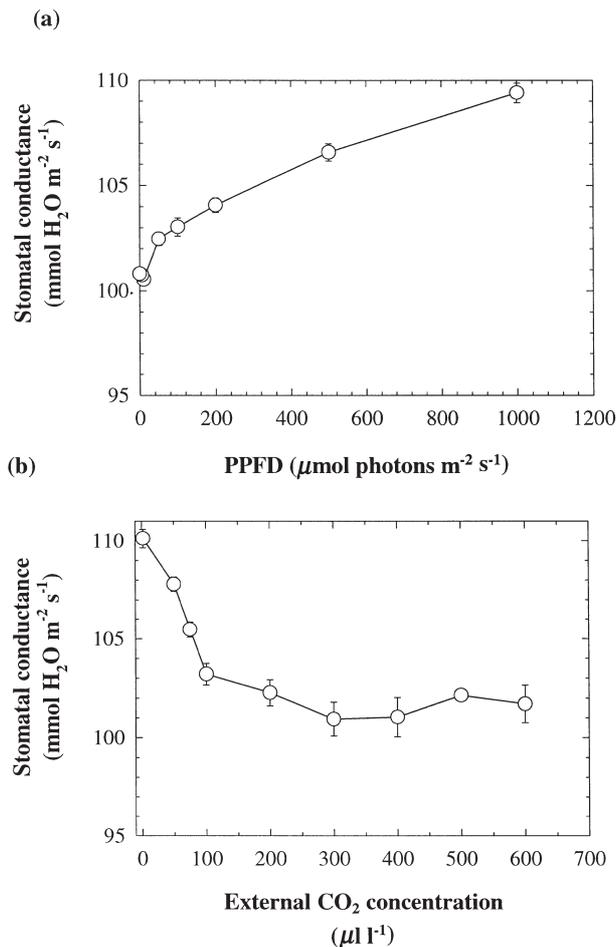
Figure 4. Response of *D. winteri* stomatal conductance (g_s mmol H_2O m^{-2} s^{-1}) to step changes in PPFD (a) and CO_2 concentration (b). Points are steady-state values ($n = 5$, means ± SEM from five plants) after a change in PPFD or CO_2 concentration under constant temperature (25 °C) and vapour pressure deficit (0·6 kPa).

angiosperm phylogeny, Winteraceae are nested among taxa with vessels (Doyle 1998; 2000; Soltis *et al.* 1998; Mathews & Donoghue 1999; Savolainen *et al.* 2000). Specifically, Winteraceae are linked with Canellaceae (16 spp. of vessel-bearing tropical dry forests and lowland rainforest trees, Kubitzki 1993) forming a 'winteroid' clade that is the sister-group to Magnoliales or Piperales; positions which are several nodes up from the angiosperm root (Doyle 1998; Soltis *et al.* 1998; Mathews & Donoghue 1999). There have been some objections to the phylogenetic argument that Winteraceae are secondarily vesselless because loss of vessels was believed to require the unlikely shift from a greater to less effective water conducting system (Carlquist 1983; 1996; Cronquist 1988; Baas & Wheeler 1996). Our measurements of hydraulic capacity and transpiration by *D. granadensis* provide direct evidence that if vessels were lost in Winteraceae, it need not have resulted in a drastic decrease in hydraulic performance.

Extension of the Winteraceae fossil pollen record into the Early Cretaceous suggests that this group has had a more complex ecological history than can be deduced from their current distribution (Walker, Brenner & Walker 1983; Doyle *et al.* 1990; Doyle 2000; Feild *et al.* 2000). In particular, fossil distributions imply that the temperate, post-Southern Gondwana distribution of Winteraceae came about by a southward migration and diversification of plants that occupied lowland tropical, possibly dry environments similar to those of modern Canellaceae sometime in the Lower Cretaceous (110 million years ago, Doyle *et al.* 1990; Doyle 2000). These data support the hypothesis that vessels were lost by ancestors of Winteraceae as they passed through cool wet, possibly cold environments (Doyle *et al.* 1990; Doyle 2000; Feild *et al.* 2000). Determining whether vessels were lost in Winteraceae will require further resolution of their fossil history and paleoecology as well as additional studies examining the functional consequences of vesselless wood (Brodribb & Hill 1999). The results of this study emphasize the need for critical functional tests on extant species for refining our understanding of the likelihood of a particular evolutionary transition.

ACKNOWLEDGMENTS

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