

ECOPHYSIOLOGICAL SHADE ADAPTATION IN THE BASAL ANGIOSPERM, *AUSTROBAILEYA SCANDENS* (AUSTROBAILEYACEAE)

Taylor S. Feild,^{1,*} Peter J. Franks,[†] and Tammy L. Sage*

*Department of Botany, University of Toronto, Ontario, Canada; and [†]James Cook University, Cairns, Queensland, Australia

Austrobaileya scandens, the sole species of the family Austrobaileyaceae, has been the subject of renewed interest following its placement near the root of the extant angiosperm phylogenetic tree. We present field observations on the growth habit, leaf anatomy, and physiological performance (photosynthesis and stem xylem hydraulics) of *Austrobaileya* from a premontane rain forest in northern Queensland. *Austrobaileya scandens* appears to possess functional characters that are commonly associated with flowering plants and ferns adapted to low light, including absence of palisade mesophyll tissue, low leaf photosynthetic rates, and possibly strong reliance on vegetative reproduction for recruitment. Also in line with many but not all shade-tolerant species, the photosynthetic apparatus of *A. scandens* expressed little physiological ability to upregulate CO₂ assimilation rate to increased light availability under greenhouse conditions. Broadly, these features appear to contribute to increasing the collection of light for photosynthesis under light-limiting conditions and the establishment and persistence in forest understory habitats. The ecology and physiology of *A. scandens* is different from hypotheses that the earliest angiosperms were early-successional xeric shrubs, disturbance-loving herbs characterized by high capacity for photosynthesis and water transport, or aquatic herbs. Our observations of *A. scandens*, in the context of other early-diverging lineages of flowering plants, indicate that first angiosperms were woody plants that exploited wet, relatively dark and disturbed (albeit at small scales) habitats.

Keywords: *Austrobaileya*, basal angiosperms, leaf optics, magnoliids, shade adaptation, understory disturbance, xylem hydraulics.

Introduction

Austrobaileya scandens C. T. White, the only representative of the family Austrobaileyaceae, occurs as an understory to subcanopy woody vine (liana) endemic to a few remaining patches of undisturbed, wet (3500–10,000 mm rainfall yr⁻¹) premontane to montane cloud forests (600–1100 m) across tropical, far-north Queensland Australia, including the Atherton and Mount Carbine tablelands (Endress 1980, 1983). *Austrobaileya* has been the subject of renewed interest following its well-supported placement among several lineages near the root of the extant flowering plant phylogenetic tree (Mathews and Donoghue 1999; Qiu et al. 1999, 2000; Soltis et al. 1999; Doyle and Endress 2000; Graham and Olmstead 2000). *Austrobaileya* forms the basal branch of a clade referred to as the ITA clade, or Austrobaileyales, which includes three other lineages, *Trimenia*, *Illicium*, and *Schisandra-Kadsura* (Mathews and Donoghue 1999; Qiu et al. 1999, 2000; Soltis et al. 1999; Graham and Olmstead 2000). Branches containing the monotypic family Amborellaceae and water lilies (Nymphaeales) diverge from the main angiosperm line before the ITA clade, and these three lineages together form an evolutionary grade at the base of the flowering plant phylogenetic tree.

Comparing the ecophysiological traits of these living members of basal angiosperm lineages may be helpful in evaluating

the potential ecological characters of the first angiosperms, which have remained enigmatic (Axelrod 1952; Takhtajan 1969; Stebbins 1974; Doyle 1977; Doyle and Donoghue 1986, 1993; Cronquist 1988; Donoghue and Doyle 1989; Taylor and Hickey 1992, 1996; Wing and Boucher 1998; Doyle and Endress 2000; Endress 2001). Currently, only limited information is available on the ecophysiological characters and environmental context associated with *Austrobaileya* and other early-diverging angiosperm groups. As part of an ongoing analysis of the ecology of extant “basal” flowering plants (Feild et al. 2000, 2001), we present field observations on the growth habit, leaf anatomy, and physiological performance (photosynthesis and stem xylem hydraulics) of *Austrobaileya* from a premontane rain forest in northern Queensland.

Material and Methods

Plant Species, Study Area, and Light Environment

All observations and measurements of *Austrobaileya* were made on individual lianas from a natural population located at the western base of Mount Bartle Frere, off Gourka Pocket Road (16°32', 145°35', 600 to 700 m above sea level). The study site was situated in an intact, simple notophyll vine forest (for a description of vegetation and soil characteristics, see Tracey 1982). The site is characterized by a tropical/subtropical climate, with mean monthly temperatures ranging from 20° to 25°C (Tracey 1982). Mean annual rainfall in this forest ranges from 6000 to 9000 mm yr⁻¹ (Tracey 1982), with little seasonal variation. Physiological studies were conducted over

¹ Author for correspondence; e-mail feild@botany.utoronto.ca.

two periods, from April 5 to April 20 and from December 5 to December 20, 2000.

Leaf Anatomy

Fully expanded leaves from the same branches used for physiological work were preserved in a modified FAA solution (20% ethyl alcohol [95%], 15% glacial acetic acid, 15% formaldehyde, and 50% water; Arens 1997) for stomatal observation and leaf cross-sectional anatomy. Measurements of stomatal size (μm) and density (number mm^{-2}) were made from epidermal peels taken from the middle portions of three individual leaves. Portions of leaf tissue were embedded in paraffin, sectioned in cross section with a microtome, and stained with toluidine blue using standard techniques described in Ruzin (1996). For observations of leaf cuticular properties, fresh *Austrobaileya* leaves were cryofixed and freeze substituted as described by Lam et al. (2001). Following freeze substitution, samples were critical-point dried, sputter-coated in gold, and prepared for SEM as described by Pontieri and Sage (1999).

Chlorophyll *a* Fluorescence Measurements

Chlorophyll *a* fluorescence emission was measured using a pulse-amplitude-modulated fluorometer (PAM-2000, Walz, Efeltrich, Germany). Minimal fluorescence emission (F_o) was determined using a nonactinic measuring beam, following exposure to 10 s of far-red illumination (at 710 nm) to ensure maximal reoxidation of photosystem II (PSII) electron carriers. An 800-ms saturation pulse (at ca. 3000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) was then used to determine the maximum fluorescence yield (F_m). Dark-adapted values for F_m and F_o were measured on leaves placed in darkness for a minimum of 4 h to calculate maximum PSII photon yield (F_v/F_m ; $F_v = F_m - F_o$; Krause and Weis 1991).

The dependency of photosystem II efficiency (or effective quantum yield; ϕ_{PSII}) to increasing irradiance (photosynthetically active radiation [PAR]; $\mu\text{mol m}^{-2} \text{s}^{-1}$) was determined for attached leaves and the green bark of *Austrobaileya*. The variable ϕ_{PSII} expresses the number of active PSII reaction centers (i.e., those with the primary quinone acceptor oxidized) and the efficiency of these centers to process absorbed light for photochemistry (Genty et al. 1989); ϕ_{PSII} was calculated as $\Delta F/F'_m = (F'_m - F)/F'_m$, where F is the fluorescence emission of the leaf under ambient light and F'_m is the maximal light-adapted fluorescence yield of the leaf where a saturating pulse is superimposed on the prevailing light (Genty et al. 1989); ϕ_{PSII} was determined at eight different irradiances from 2% to 100% full sunlight irradiance (20–2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$), starting from lowest to highest light intensity. Measurements of PAR were made near the leaf surface by the microquantum sensor of the PAM-2000 leaf clip, calibrated against a Li-190 light sensor (Li-COR, Lincoln, Nebr.). PAR values were corrected for the 2-mm difference between the distance of the photodiode and leaf plane as described by Rascher et al. (2000). PAR was provided in a controlled manner by using the internal halogen lamp of the fluorometer and was directed to the leaf or bark surface using a wide (50 mm) fiber optic bundle. At each light level, the leaf was illuminated until F values were stable for at least 15 s, which required 30–60 s. Then, a saturating pulse was applied to calculate ϕ_{PSII} . Photosynthetic tissues of *Aus-*

trobaileya were measured only after they were exposed to morning light for 3–4 h to allow for full photosynthetic induction and stomatal opening. Measurements were made between 1030 and 1200 hours to avoid time-dependent effects on photosynthetic performance. We also checked that leaves and bark were dry before measurements.

The apparent rate of photosynthetic electron transport (ETR) through PSII was calculated as $\text{ETR} = \phi_{\text{PSII}} \times \text{PAR} \times 0.5 \times \alpha$, where 0.5 accounts for the fact that two photons are required per electron passed through PSII (assuming linear electron flow and even distribution of absorbed quanta between PSII and photosystem I [PSI]) and α is leaf absorbance (Bilger et al. 1995). The average value of α for green leaves of 0.84 (Björkman and Demmig 1987) was used here, and it was assumed that excitation energy was evenly distributed between PSII and PSI (Bilger et al. 1995). The units of ETR are $\mu\text{mol m}^{-2} \text{s}^{-1}$. It should be noted that values of ETR will not be precise for two reasons. First, absorbance differences between bark and leaf tissues were not measured. Second, differences in internal cross-sectional anatomy, such as the frequency of intercellular air spaces and cell size and shape (Smith et al. 1997), could modify the internal distribution of light under which chloroplasts monitored by chlorophyll fluorescence function. However, chlorophyll fluorescence measurements should still provide a good relative index of photosynthetic activity of bark versus leaf photosynthesis because leaf and bark tissues of *Austrobaileya* plants were healthy and similar in color, which probably indicates similar pigment complements.

We examined the acclimation of leaf photosynthetic properties of *Austrobaileya scandens* to increasing irradiance (e.g., at levels greater than those encountered by plants growing in the forest understory) by growing three cuttings taken from three individual field-grown plants under controlled environmental conditions. Cuttings were established in sand using a mist bench and heating pad system. After 1 yr, they were transferred into 5-L pots with a standard potting soil mix and grown at 100 ± 20 SD $\mu\text{mol m}^{-2} \text{s}^{-1}$, 20°–25°C, and at 40%–85% relative humidity. Physiological measurements (A and F_v/F_m) were made on leaves that flushed from buds that had developed under this light regime. Then, the three plants were transferred to a brighter light regime, at 375 ± 55 SD $\mu\text{mol m}^{-2} \text{s}^{-1}$, with the same temperature and humidity conditions as the first treatment and A and F_v/F_m measured on fully-expanded leaves produced in this environment (after ca. 9 mo). Here it is important to note that our results are potentially confounded by ontogenetic effects, since leaves developed under high light were measured following the transfer of plants exposed to low light conditions. Logistical constraints on the availability of *Austrobaileya* plants prevented us from establishing controls that remained starting low light environment to determine how photosynthetic characteristics varied with development. Another limitation of our analysis of the photosynthetic plasticity of *Austrobaileya* conducted under greenhouse conditions is that microclimatic conditions are dissimilar to field conditions in some ways, including vapor pressure deficit regime and availability of belowground resources. However, photosynthetic properties of understory field-developed leaves and greenhouse leaves developed under moderate light were not statistically different from each other (table 1), indicating that

Table 1
Leaf Photosynthetic Characteristics of *Austrobaileya scandens*
from Different Light Environments in the Field and
under Controlled Greenhouse Conditions

Light environment or treatment	Diffuse PAR	F_v/F_m	A_{max} at 90%
Mount Bartle Frere:			
Understory (1.2 m)	12 ± 3	0.796 ± 0.008 ^A	3.84 ± 0.03 ^A
Subcanopy (4 m)	45 ± 15	0.781 ± 0.006 ^B	...
Greenhouse:			
Moderate light	100 ± 20	0.772 ± 0.003 ^B	3.91 ± 0.04 ^A
High light	375 ± 55	0.575 ± 0.058 ^C	3.71 ± 0.06 ^B

Note. PAR = photosynthetically active radiation. Diffuse PAR measurements ($\mu\text{mol m}^{-2} \text{s}^{-1}$) were made at leaf level with a portable light meter ($n = 10$ measurements per leaf sample for 40 measurements total per light environment or treatment, \pm SD). Averages of maximum photosystem II photon yield (F_v/F_m ; relative units \pm SD), maximum leaf photosynthetic rate at 90% light saturation (A_{max} at 90%; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, \pm SD) were taken from measurements on four leaves from four individual plants. See "Material and Methods" for site descriptions and growth conditions for greenhouse *A. scandens* plants. It was not possible to measure light-saturated carbon assimilation rates at 4 m above the forest floor with gas-exchange equipment; therefore, these data were necessarily omitted. Photosynthetic parameters with a different letter are significantly different from each other at least at the $P < 0.05$ level of significance (Mann-Whitney nonparametric test).

differences in microclimate did not significantly impact leaf photosynthetic performance.

The light environment for *Austrobaileya* plants used in experimental work was characterized using local measurements of PAR with a handheld light meter (Li-190, Li-COR). Measurements were made at leaf level and in the subcanopy (ca. 4 m) from 1000 and 1230 hours on representative clear periods ($n = 5$) and cloudy days ($n = 15$).

Leaf Gas-Exchange Measurements

A field-portable, open-flow photosynthesis system (model LI-6400, Li-COR) was used for measurements of leaf water vapor and carbon dioxide fluxes for *A. scandens* leaves. The measurement cuvette on this system enclosed a 6-cm² area of leaf. Air was passed through the cuvette (c_a ; $\mu\text{mol CO}_2 \text{ mol}^{-1}$) at a constant flow rate (set in the range 200–700 mL min⁻¹), and CO₂ concentration inside the cuvette was controlled by the CO₂ mixer. Humidity of the air entering the leaf cuvette was adjusted manually by passing a portion of the air upstream of the chamber through a desiccant tube. This manual manipulation allowed leaf-to-air vapor pressure difference (VPD) to be controlled to within 3% of the target value. Leaf temperature for all measurements was maintained at $28^\circ \pm 1.5^\circ\text{C}$ using the system's Peltier units for all observations. PAR was controlled using a mixed red and blue light-emitting diode (LED) light source. Leaf physiological parameters calculated with this system were CO₂ assimilation rate (A ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance to water vapor (g_s ; $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), and leaf intercellular CO₂ concentration (c_i ; $\mu\text{mol mol}^{-1}$).

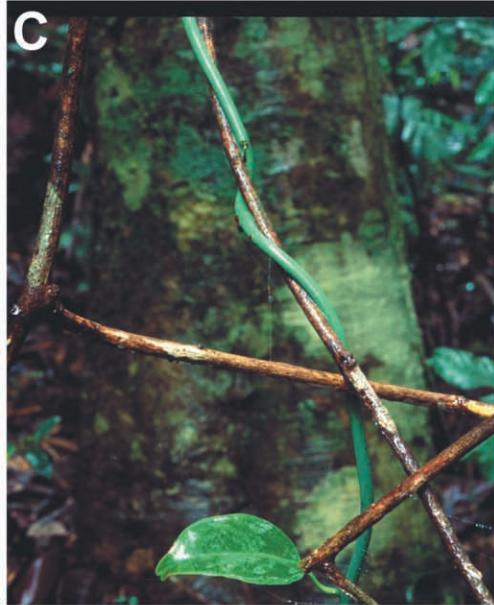
We selected one vigorously growing branch from each of four individual *Austrobaileya* lianas that occurred in the forest

understory layer, 75–150 cm above the forest floor. From these branches, a single fully expanded leaf that was free of insect damage and epiphylls was chosen for physiological work. Leaf gas-exchange measurements were conducted on relatively cloudless periods from 0930 to 1230 hours to ensure that leaves were adequately induced before experiments. Although plant water status was not measured, it is assumed to have been high because of the noticeably saturated soil and frequent heavy rain just before measurements. CO₂ concentrations were ca. 380 $\mu\text{mol CO}_2 \text{ mol}^{-1}$ in the understory layer, determined by running the Li-Cor 6400 with an empty chamber while bypassing the CO₂ and H₂O scrubbers. For photosynthetic light response curves, a leaf was sealed gently in the cuvette and exposed initially to 200 $\mu\text{mol m}^{-2} \text{ s}^{-1}$, 380 $\mu\text{L L}^{-1}$ CO₂, and 1 kPa VPD. After steady-state fluxes of CO₂ and H₂O were reached, which required 20–35 min, irradiance was increased to 2000 $\mu\text{mol m}^{-2} \text{ s}^{-1}$. Leaf A and g_s were then calculated under steady-state conditions for nine irradiances in the range 2000–0 $\mu\text{mol m}^{-2} \text{ s}^{-1}$. Leaves took 20–50 min to equilibrate to each new irradiance. This information, obtained for four leaves from four different plants, provides an indication of the extent of stomatal limitation to photosynthesis in *Austrobaileya*. To determine the responses of *Austrobaileya* stomata to atmospheric drought, we examined changes in g_s following a step change in VPD from 1.0 to 2.0 kPa. For each experiment, a single leaf was enclosed in the cuvette and exposed to 1300 $\mu\text{mol m}^{-2} \text{ s}^{-1}$, 380 $\mu\text{L L}^{-1}$ CO₂, and 1.0 kPa VPD. After steady-state fluxes were reached, the incoming air stream was dried to 2.0 kPa over 5 min, and the time course of g_s followed until steady-state conditions were reached.

Stem Xylem Hydraulics

A portable steady-state flowmeter (SSFm), which utilizes the pressure drop across a tube (PEEK capillary tubing, Upchurch Scientific, Seattle) of known conductance arranged in series with an excised stem segment, was used to determine hydraulic flux through a stem segment under positive pressure (5–20 kPa) as described in detail previously (Feild et al. 2001). A weak solution of bleach (10% volume aqueous solution) was flowed through the SSFM, with the pressure transducer removed, once a week to prevent algal and bacterial growth. To control for the effects of ions on stem hydraulic conductance (Zwieniecki et al. 2001), the osmolarity of the measuring solution was kept constant using a filtered (to 0.1- μm pore size) 15 mmol KCl solution.

Undamaged, leaf-bearing branches of *Austrobaileya* were collected from 10 plants at Mount Bartle Frere. Because the stem xylem hydraulic properties can be sensitive to light environment (Maherali et al. 1997), care was taken to select individuals only from uniform conditions. Branches were cut at lengths two to three times longer than that of the longest vessel length as determined from low pressure (<0.15 MPa) air injection using a hand bicycle pump (Zimmerman and Jeje 1981). Maximum vessel lengths were ca. 13 cm long and did not vary for shoots sampled during April and December. Branches were sampled in the early morning (0800–0930 hours) on wet, cloudy days from the same plants examined for photosynthetic and gas-exchange properties. After severing, we triple bagged branches in plastic and transported them



to a laboratory. Here, stem segments were cut from branches under water at a standardized length (20 cm) and a narrow range of diameters and diameter (3.5–5 mm, excluding bark) to minimize size-dependent variation in tracheid geometry and hydraulic conductance (Maherali et al. 1997). Both ends of the segment were shaved with a fresh razor blade under water. Leaves along the axis of the stem were excised under water, and the cut petioles were sealed with a fast-solidifying glue (Loctite Super Bonder 409, with accelerator 712, Loctite Corporation, Newington, Conn.) to prevent water flow through these open vascular pathways. All stem segments were measured within 2 h after initial sampling in the field. Stem xylem hydraulic conductivity (K_{H} ; $\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$) was defined as the mass flow rate of water through an excised stem segment (kg s^{-1}) divided by pressure drop per unit length (MPa m^{-1} ; Sperry et al. 1988). Sapwood area-specific hydraulic conductivity (K_{S} ; $\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$) was determined by dividing stem K_{H} by the sapwood cross-section area. Sapwood cross section was determined just below the supported leaf area for each stem segment using a light microscope at $\times 200$ with a calibrated ocular micrometer, and the surface area contributed by the pith was subtracted. Leaf area-specific hydraulic conductivity (K_{L} ; $\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$) was determined by dividing stem K_{H} by the total leaf area supported by (i.e., distal to) that branch. For leaf area measurements, individual leaves of each branch were traced (flat) onto paper. These leaf silhouettes were then cut out and measured with a leaf-area meter (Li-1000, Li-Cor). Huber value (H) for stem segments used in hydraulic conductivity measurements was expressed as the ratio of sapwood cross-sectional area (m^2) to total leaf area downstream of that stem segment (m^2).

Results

Growth Habit Morphology

Austrobaileya scandens occurs as a large (up to 9 m tall and 4 m wide) and often gangly liana that is characterized by the production of numerous, sparsely leaved pendent to twisted shoots that ramify in understory and subcanopy environments (fig. 1). *Austrobaileya* seedlings establish in shaded forest understory habitats of undisturbed primary forests, and they do not appear to recruit along roadsides, forest edges, pastures, or large forest gaps. Diffuse understory light levels above leaves of *Austrobaileya* seedlings as well as understory leaves range from 4 to 8 $\mu\text{mol m}^{-2} \text{s}^{-1}$ on clear days and from 11 to 15 $\mu\text{mol m}^{-2} \text{s}^{-1}$ on cloudy days. Sunfleck irradiances are variable, ranging from 500 to 1100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, with an average value of $880 \pm 357 \text{ SD } \mu\text{mol m}^{-2} \text{s}^{-1}$. Seedlings appear to be infrequent, with three and five seedlings observed in two $20 \times 20\text{-m}$ plots below mature plants that were observed to produce fruits. A characteristic feature of *A. scandens* seedlings

is their production of several pendent shoots (up to five) that arise from basal buds located on a swollen transition zone between the root and shoot. The bark of these shoots, including those of adult plants, remains green, often up to 3 m (fig. 1C, 1D).

Two major shoot types that differ in their climbing behavior are produced by *Austrobaileya*. One shoot type, referred to as “stolons,” grow as scandent, low-climbing stems (fig. 1B, 1D). Stolons often exhibit extensive horizontal “exploration” of the forest understory at lengths of up to 4 m from the base of the shoot manifold. Stolons tend to be small in diameter (<2 cm) and are characterized by low amounts of lateral branching, such that they resemble leafless green dowels meandering through the understory. *Austrobaileya* stolons were absent from understory clearings and tree falls, and they were instead observed growing through the canopies of small understory shrubs in the intact, deeply shaded understory. When branching did occur, small irregularly shaped canopies consisting of a few arched branches were developed (fig. 1B). Stolons reroot adventitiously when they become buried and disoriented by falling canopy debris. New stems may iterate upward, or the stolon may grow partially underground and develop a cluster of new scandent shoots. We often found that what appeared to be small seedlings were instead emerging suckers connected to subterranean or layered stems from adult plants. Although mature stolons are flexible, they are occasionally snapped by limb falls. Broken shoots apparently resprout readily, as indicated by the occurrence of “bayonet joints” at these locations (fig. 1D).

In contrast to stolons, a second shoot type, which we call “twiners,” grows upward and grapples onto stems of other plants by circumnutated growth (fig. 1C). Twiner shoots may develop from understory dowels that traverse through the understory or may be produced from the base of the plant (fig. 1A). The petioles of *A. scandens* leaves are also reflexed, which may further aid in grappling onto supports. However, we note that *Austrobaileya* lianas appear to lack any other structural features assisting ascension into the subcanopy (e.g., tendrils, root adhesion pads, or spines). Upward-growing shoots can become large in diameter (up to 7 cm), but most are between 2 and 3 cm in diameter. The light environments that twiners climb into appear to be only marginally better lit than the understory layer, because twiners reach heights of 4–9 m in a forest characterized by a dense and tall subcanopy and canopy layer (25–30 m) as well as frequent cloud cover. Measurements of midday levels of diffuse light at 4 m above the ground were low, averaging $45 \pm 15 \text{ SD } \mu\text{mol m}^{-2} \text{s}^{-1}$ ($n = 40$) on cloudy days compared with $300 \pm 10 \text{ SD } \mu\text{mol m}^{-2} \text{s}^{-1}$ ($n = 40$) in open areas on cloudy days. In the subcanopy, *Austrobaileya* twiners develop a canopy architecture similar to those in the understory, consisting of few leaves and a network of scandent

Fig. 1 Growth habit of *Austrobaileya scandens* from a tropical montane rain forest in Queensland, Australia. A, Overall habit for a small plant with one upward-growing shoot (a twiner) and laterally ramifying stolon. Note the nonoverlapping leaf arrangement on ascending and horizontal shoots and the persistent green bark of the stems. B, Scandent habit of a sapling, consisting of a poorly biomechanically supported stem. C, Twining shoot of *Austrobaileya*. D, Detail of the understory stolons, with low self-shading leaves and photosynthetic bark. Note the regrowth from a snap in the middle shoot that possesses a “bayonet junction.” E, Leaf bicoloration, with the leaf undersurface lighter in color relative to the upper surface. Scale bar in E = 3 cm.

shoots that frequently hang a meter or more below branches of the supporting host tree. In December, fruits were observed dangling from these subcanopy shoots and were not on understory ones.

Leaf Morphology and Anatomy

Leaves of *Austrobaileya* are entire and leathery in texture and are produced in an opposite to subopposite, nonoverlapping pattern at nearly horizontal orientations, including on scandent shoots (Bailey and Swamy 1949; Metcalfe 1987; fig. 1). *Austrobaileya* leaves also possess small drip tips (0.5–1.5 cm in length; fig. 1E) at their apices. Leaves are conspicuously bicolored, with the leaf lower surface lighter in color than that of the upper surface (fig. 1E). The upper surface of deeply shaded leaves, and more frequently the green bark of *Austrobaileya* stolons, possesses a subtle bluish hue (not illustrated).

Stomata are predominantly developed on the leaf under-surface (abaxial) and on the green bark of shoots, with occasional stomata produced on the leaf upper surface (adaxial). Abaxial stomatal densities of understory leaves sampled in the field averaged 35 ± 5 SD stomata mm^{-2} ($n = 4$ leaves). Average stomatal size was 32 ± 5 SD μm ($n = 40$) in the longest dimension for these leaves. The guard cells of *A. scandens* are not visible from the surface and are enclosed by a cuticular housing (vestibule) that is slightly raised above the epidermis (fig. 2A–2C). The vestibule is perforated by a slitlike opening ca. $6 \mu\text{m}$ in diameter (fig. 2A–2C). In cross section, the vestibule is variably shaped, resembling a cylinder to an inverted urn, creating a relatively wide chamber (ca. $10 \mu\text{m}$ in diameter) that can be closed off by reflexed extensions from the walls of the guard cells (fig. 2B). The guard cells are situated ca. $18 \mu\text{m}$ below the top of the leaf epidermis (fig. 2B). The lower epidermis is densely covered with cuticular ridges and striations that form concentric circles around the cuticular vestibules (fig. 2A, 2D). Internally, the mesophyll of *A. scandens* primarily consists of irregularly shaped, spongy mesophyll cells and lacks a hypodermal layer and tubular palisade cells (fig. 2E). Spongy parenchyma cells of *A. scandens* are loosely packed, with abundant internal airspaces from the middle to the leaf undersurface (fig. 2E).

Physiological Performance

Leaf gas-exchange measurements made on understory field plants showed that net leaf CO_2 uptake rose rapidly with increasing PAR, with the 90% light-saturation point at 220 ± 8 SD $\mu\text{mol m}^{-2} \text{s}^{-1}$ ($n = 4$ leaves) or ca. 11% full sunlight (assuming a PAR of $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$; fig. 3A). Net leaf CO_2 uptake at light saturation was 3.84 ± 0.3 SD $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$ ($n = 4$ leaves; fig. 3A). Average rates of CO_2 released from darkened *A. scandens* leaves were 0.89 ± 0.053 SD $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$, and the photosynthetic light compensation point, the irradiance where photosynthetic carbon reduction rate equals the rate of mitochondrial respiration, was $8 \mu\text{mol m}^{-2} \text{s}^{-1}$. Paralleling the light response of leaf CO_2 uptake, photosynthetic electron transport rate (ETR), determined with chlorophyll *a* fluorescence, increased rapidly with increasing PAR (saturating at ca. $275 \mu\text{mol m}^{-2} \text{s}^{-1}$) to a maximum rate of 48 ± 3 SD $\mu\text{mol electrons m}^{-2} \text{s}^{-1}$ ($n = 4$ leaves) and then declined at irradiances greater than $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ ($n =$

4 leaves). Electron transport rates for the green bark of *Austrobaileya* saturated at ca. $250 \mu\text{mol m}^{-2} \text{s}^{-1}$, with maximum ETR values ca. 60% of leaf ETR at 28 ± 6 SD $\mu\text{mol electrons m}^{-2} \text{s}^{-1}$ ($n = 4$ leaves). Values for light-saturated ETR were largely invariant along a 1-m length (data not shown).

Maximum g_s for field *A. scandens* leaves, measured at 0.5 kPa VPD, averaged 0.160 ± 0.003 SD $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ ($n = 4$ leaves). When the chamber light was switched off, stomatal conductance declined to a minimum average value of 0.007 ± 0.002 SD $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ ($n = 4$ leaves), which took 2.7–3.3 h to reach steady state. Following a step change in VPD, from 1.0 to 2.0 kPa, g_s of *Austrobaileya* leaves declined from 0.158 ± 0.007 SD $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ to 0.059 ± 0.011 SD $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ ($n = 4$ leaves; fig. 4). This meant that transpiration rate at 2.0 kPa VPD was less than it was at 1.0 kPa, constituting a pronounced response to dry air (fig. 4). However, this response was not fast. *Austrobaileya* leaves required between 2.8 and 3.2 h to achieve steady-state transpiration rate after the step change in VPD (fig. 4).

Mean leaf area-specific hydraulic conductivity (K_L) of *A. scandens*, which describes the ability of stem xylem tissue to support a given amount of leaf area with water, averaged $1.8 \times 10^{-4} \pm 0.84$ $\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$ ($n = 10$). K_L is a function of the relative allocation to xylem cross-sectional surface area versus leaf area (H ; Huber value) and the intrinsic efficiency of the xylem to conduct water (sapwood-specific conductivity, K_s). Huber values for *A. scandens* stems averaged $0.65 \times 10^{-4} \pm 0.34$ SD $\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$ ($n = 10$), while K_s averaged 2.3 ± 1.50 SD $\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$ ($n = 10$).

Acclimation of Leaf Photosynthesis to Light

Average values of maximum PSII photon yield (F_v/F_m) declined in leaves that developed under increasing light levels, from 0.796 in understory leaves of field-grown plants to 0.575 in greenhouse plants under the high-light treatment (table 1). F_v/F_m values were not significantly different between subcanopy leaves and leaves that developed under moderate light intensities in a greenhouse, and maximum CO_2 assimilation rate did not differ between understory and moderate light-grown plants (table 1). Light-saturated leaf CO_2 assimilation rates were ca. 6% lower under high light compared with rates measured for understory and moderate light-grown *A. scandens* leaves (table 1).

Discussion

Austrobaileya lianas appeared to explore light-limited habitats, with diffuse PAR ranging from 0.75% in the understory to 11% full sunlight in the subcanopy layer. This growth pattern contrasts with previously examined tropical and temperate angiosperm vines that were climbers that reached the forest canopy by responding to light gaps with rapid growth or thicket-forming colonizers of sunny forest edges and open areas (Peñalosa 1984; Castellanos 1991; Hegarty and Caballé 1991; Avalos and Mulkey 1999; Tibbits and Ewers 2000). In support, observations of the growth habit, anatomy, and physiology of *Austrobaileya scandens* indicate that this species persists and grows in dimly lit forest understory habitats with

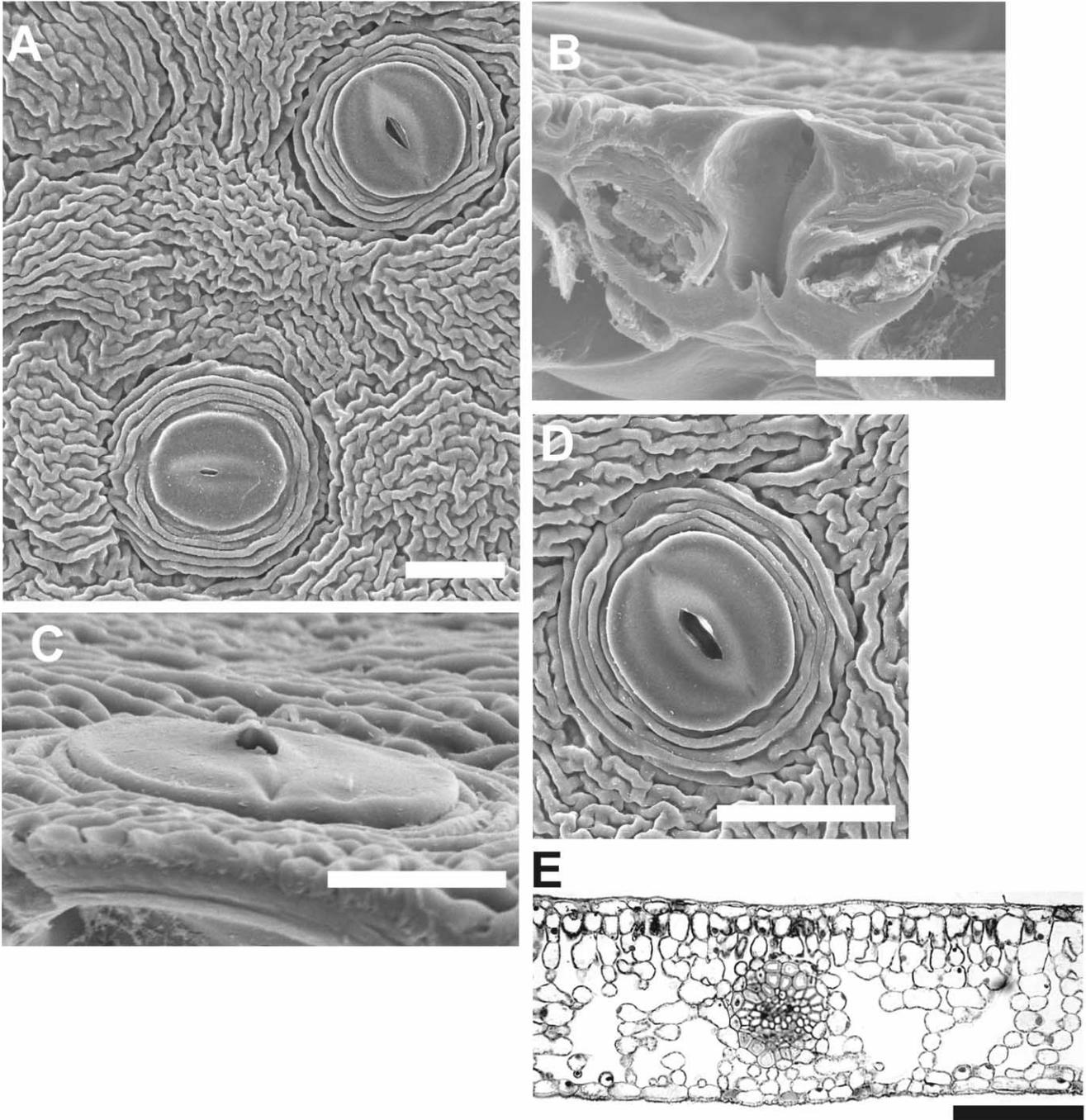


Fig. 2 Leaf and cuticular anatomy of *Austrobaileya scandens*. *A*, Micrograph of the leaf abaxial cuticle. Note the highly striated surface and the concentric rings of cuticular striations around the stomatal complex. The stomatal guard cells are shielded by a cuticular vestibule. Scale bar = 20 μm . *B*, Cross section of the stomatal complex (cuticular vestibule and guard cells). Note the air volume above the guard cells and the reflexed extensions from the guard cells that seal off the vestibule from the intercellular air space. Scale bar = 13 μm . *C*, Cuticular vestibules appear to be in the same plane as the epidermis. Scale bar = 13 μm . *D*, Detail of the stomatal complex. Scale bar = 20 μm . *E*, Cross section of the leaf. Note the lack of tubular palisade cells and increasing abundance of air spaces from the top (adaxial) surface to the bottom (abaxial) surface. Scale bar = 185 μm .

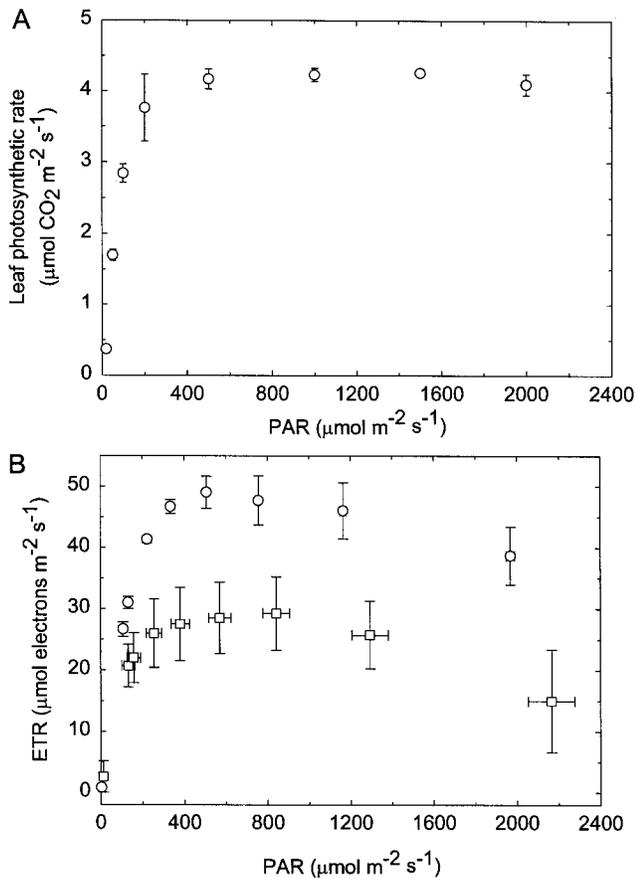


Fig. 3 Photosynthetic properties of *Austrobaileya* leaves and bark. A, Steady-state light response of leaf CO_2 assimilation. B, Steady-state responses of photosystem II electron transport ($\mu\text{mol electrons m}^{-2} \text{ s}^{-1}$) of leaves (open circles) compared with bark (open squares). Experimental conditions for measurements are described in detail in “Material and Methods.” Sample sizes for all measurements are four leaves from four different plants. Error bars denote SD for each plot.

high whole-plant resiliency to disturbances by falling canopy debris and successfully harvesting of low PAR.

Resulting from extensive vegetative spread across the forest floor and subcanopy utilizing creeping stolons and ascending twiners, *Austrobaileya* possesses considerable meristem redundancy in space and is a “diffuse” target for canopy debris. This reduces the risk that individual adult plants will be killed from the impact of a single disturbance event (Cook 1983; Peñalosa 1984; Gartner 1989; Greig 1993; Mack 1998; Bond and Midgely 2001). Similarly, *Austrobaileya* seedlings become highly branched and develop a reserve supply of basal buds at small size. When plants do become damaged by limbs or buried by leaves, they are able to recuperate readily with stem and basal sprouting as well as by occasional “layering” when pinned shoots reroot adventitiously. In some plants, the newly rooted stem may eventually rot away from the parent plant, leaving a physiologically independent ramet (Gartner 1989; Greig 1993). Consistent with the ability for profuse vegetative sprouting, allocation to carbohydrate storage in root tissues of *Austrobaileya* is high, with a large proportion of ray tissue

relative to water-conducting vessels and the presence of compound starch grains in root tracheids (Carlquist 2001). The morphological flexibility that allows *A. scandens* to survive and regrow following understory damage may contribute to persistence in a preferred site even as the site’s habit becomes changed by a dynamic overstory (Gartner 1989; Greig 1993). However, it is clear that there are limits to *Austrobaileya*’s disturbance tolerance, since this species does not appear to regenerate in sunny, disturbed rain forest patches produced by cyclones, human-related activities, or tree falls. Future studies on the frequency and spatial patterning of clonal reproduction compared with sexual reproduction on *Austrobaileya* are needed.

In relation to understory light interception, *Austrobaileya*’s scandent habit, consisting of shoots that grow upward and eventually lean over, and the cylindrical surfaces afforded by its photosynthetic stems (fig. 3B) may aid the capture of diffuse light, which is incident in all directions. The nonoverlapping and horizontal pattern of leaf placement on scandent shoots (fig. 1A, 1D) may minimize self-shading and increase the capture efficiency of sunflecks at high solar angles. Inside *Austrobaileya* leaves, the loosely packed and irregularly shaped spongy mesophyll may, as in other shade-adapted taxa, enhance understory light harvesting by creating numerous light-reflecting air-water interfaces that lengthen the pathways over which most photons migrate within the leaf (fig. 2F; Bailey and Swamy 1949; Terashima and Saeki 1983; Metcalfe 1987; Vogelmann and Martin 1993; DeLucia et al. 1996; Vogelmann et al. 1996; Smith et al. 1997). Path-lengthening effects, in turn, may increase the likelihood that intercepted photons of green, yellow, and far-red wavelengths, which are poorly absorbed by chlorophyll and abundant in understory light environments (Lee 1997), will be utilized for leaf carbon gain, since these are retained within the leaf for longer times (Vogelmann et al. 1996). Air-water interface reflections are likely to be responsible for leaf bicolouration in *Austrobaileya* because air spaces are concentrated near the leaf undersurface (fig. 2F).

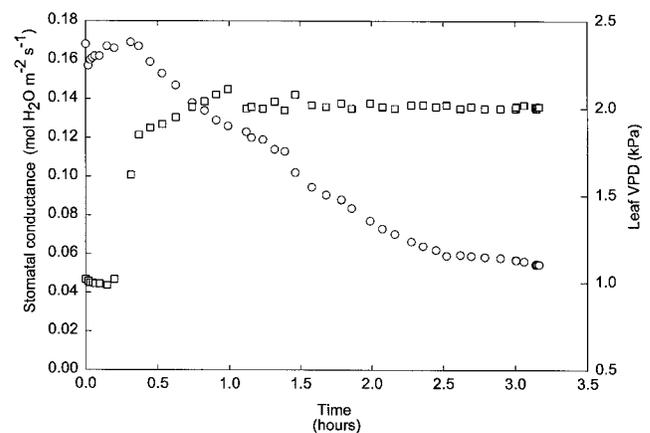


Fig. 4 Response kinetics for *Austrobaileya scandens* stomata (circles) to a step change in leaf-to-air vapor pressure deficit (VPD; kPa; squares) from 1 kPa to 2 kPa. A representative response is depicted for a single leaf of *A. scandens*. Experimental conditions are detailed in “Material and Methods.”

Leaf bicoloration may also be associated with the highly striated and grooved texture of the abaxial cuticle, in contrast to the smoother adaxial cuticular surface (data not shown), which may reflect light back into the leaf that leaks through the mesophyll (Lin and Ehleringer 1983; DeLucia et al. 1996; Smith et al. 1997).

Another foliar feature generally associated with plants adapted to low light is low stomatal density, with values for *A. scandens* in the lower 10% of densities measured for a diverse range of species from temperate and wet tropical forests (Abrams and Kubiske 1990; Bongers and Popma 1990). However, *Austrobaileya* lacks many of the classically “extreme” shade characteristics that occur in some rain forest understory mosses, ferns, and flowering plants, including epidermal lens cells and red (anthocyanic) leaf undersurfaces (Lee 1997). Interestingly, though, strongly shaded leaves and stems of *Austrobaileya* develop a bluish iridescent hue that may originate from thin-film interference (Lee 1997). Although the physiological significance (if any) of blue iridescence remains to be clarified, it is restricted to plants that occur in deep-shade environments. The ultrastructural basis for blue leaf/bark coloration in *Austrobaileya*, whether at the chloroplast or epidermal scale, requires further study (Lee 1997).

Photosynthetic and xylem water transport measurements were also consistent with *Austrobaileya*'s occurrence in shady habitats. *Austrobaileya* developed leaves that photosynthesized at low maximum rates, and light compensation and saturation points were low compared with sun-adapted species (Boardman 1977; Langenheim et al. 1984; Givnish 1988; Turnbull 1991; Chazdon 1992). Instead, light-saturated CO₂ assimilation and photosynthetic electron transport rates of *A. scandens* leaves were nested in the lower range of values found for shade-adapted cloud forest angiosperm and conifer taxa as well as in the lower range for understory temperate lianas and sun-adapted tropical liana taxa (Carter and Teramura 1988; Castellanos 1991; Avalos and Mulkey 1999; Brodribb and Feild 2000). Also in line with many (but not all) shade-tolerant taxa (Björkman 1981; Langenheim et al. 1984; Walters and Field 1987; Turnbull 1991; Chazdon 1992), the photosynthetic apparatus of *A. scandens* expressed little physiological ability to upregulate CO₂ assimilation rate to increased light availability under greenhouse conditions (table 1). Under two treatment conditions, except under irradiances that surpassed those normally encountered in the field (table 1), photosynthetic rates did not differ. At diffuse light levels ca. 70% greater than those occurring in the forest subcanopy, leaves of *A. scandens* may have become photoinhibited, exhibiting a sustained depression in maximum PSII quantum efficiency (F_v/F_m ; table 1) and visual leaf chlorosis (data not shown). The advantages of these photosynthetic properties in low light environments is that they permit efficient light harvesting while minimizing the carbon respiratory burden for constructing and maintaining an otherwise nitrogen-rich photosynthetic apparatus (Björkman 1981; Givnish 1988; Evans 1989; Walters and Reich 2000).

The hydraulic capacity of *A. scandens* stems to supply a supported leaf area with water (leaf-specific hydraulic conductivity; K_L) were generally lower than those measured for sun-adapted angiosperm cloud forest tree species and tropical angiosperm lianas, which is indicative of the low transpirational demand that *Austrobaileya* likely experiences. However,

K_L values were similar to temperate and tropical conifers as well as the vesselless angiosperms (Winteraceae and *Amborella trichopoda*; Tyree and Ewers 1996; Brodribb and Hill 1999; Brodribb and Feild 2000; Feild et al. 2000, 2001). Functionally, low K_L requires that plants like *Austrobaileya* develop low leaf water potentials in order to achieve equivalent transpiration rates as species with high K_L and thus, all else being equal, increasing the risk of water stress-induced cavitation (Zimmermann 1983; Tyree and Ewers 1991; Brodribb and Feild 2000). Consequently, the hydraulic properties of *A. scandens* wood may play a significant role in confining it to shady habitats. The observed coupling of low shoot hydraulic supply with low photosynthetic potential in *A. scandens* is consistent with recent work revealing a close link between photosynthetic capacity (and hence evapotranspirational demand) of leaves and the hydraulic supply of supporting stems (Brodribb and Feild 2000).

Angiosperm lianas are well known for their extraordinarily high xylem water transport efficiency, expressed as stem hydraulic conductivity relative to the cross-sectional area of conducting xylem (K_s ; sapwood-specific hydraulic conductivity), which is determined by the size and density of xylem conduits (Zimmermann 1983; Tyree and Ewers 1991). Freed of the mechanical constraints of self-support and thus the necessity of thick lignified xylem walls, climbers tend to produce slender stems possessing megaporous vessels that provide a high-conductance path to supply water to a large leaf area (Tyree and Ewers 1991; Givnish 1995). In contrast to other flowering plant lianas with stem hydraulic efficiencies ranging from 10 to 40 kg m⁻¹ MPa⁻¹ s⁻¹ (Gartner et al. 1990; Chiu and Ewers 1992; Cochard et al. 1994; Tyree and Ewers 1996; Tibbits and Ewers 2000), K_s of *Austrobaileya* xylem, averaging 2.3 kg m⁻¹ MPa⁻¹ s⁻¹, was considerably lower. Consistent with hydraulic flux measurements, the vessels of *Austrobaileya* wood appear to be more resistive in structure. Xylem characters potentially contributing to relatively low hydraulic conductivity include comparatively short maximum vessel length (ca. 13 cm in the stems measured), vessel elements with gradually tapered walls, frequent scalariform perforation plates with occasional pit membrane remnants, and, relative to other lianas, small vessels (30–80 μm in diameter, with diameters as large as 200 μm in large stems) with angular lumens (Bailey and Swamy 1949; Fisher and Ewers 1998; Carlquist 2001; Carlquist and Schneider 2002). Additional measurements on the comparative stem hydraulic characteristics of angiosperm trees, shrubs, and lianas that co-occur with *Austrobaileya* in the understory as well other liana taxa in the Austrobaileales (*Trimenia*, *Schisandra*, and *Kadsura* species) would be useful in placing hydraulic properties measured for *Austrobaileya* in an appropriate environmental and phylogenetic context.

Interestingly, *Austrobaileya* exhibited a pronounced stomatal response to VPD (e.g., g_s reduced by more than half in response to doubling VPD) and low leaf conductance to water vapor when stomata were presumably maximally closed in the dark (Franks and Farquhar 1999). High potential to curtail leaf water loss in response to atmospheric drought seems paradoxical in view of *Austrobaileya*'s wet habitat (Tracey 1982). Indeed, *Austrobaileya* wood lacks growth rings (Carlquist 2001), and older leaves are colonized by lichen and moss epiphylls, which are both signs of stable moisture supply. So, why

is *Austrobaileya* confined to wet, shady habitats? Although *Austrobaileya*'s stomatal response to VPD was considerable, the kinetics were slow and required around 3 h to reach steady state (fig. 4). Thus, *A. scandens* may be unable to adjust leaf water loss rates fast enough to avoid ensuing low leaf water potentials and possibly xylem cavitation under the high and fluctuating VPD conditions that occur in large forest gaps, roadsides, logged forests, and exposed canopy habitats. This may be physiologically compounded by a low stem hydraulic capacity relative to leaf area, which would constrain the development of high transpiration rates (Zimmermann 1983; Tyree and Ewers 1991; Brodribb and Feild 2000). Slow stomatal regulation to VPD, however, may be advantageous in understory and subcanopy environments where *A. scandens* grows by allowing time averaging of microclimatic variation, such as rapid excursions in VPD during sunflecks, which would otherwise trigger stomatal closure and increase stomatal limitations on carbon assimilation (Valladares et al. 1997). Future studies of non-steady-state gas-exchange dynamics of *Austrobaileya* would be required to test these predicted ecological advantages.

Mechanistically, the slow stomatal response to humidity and low minimum conductance of *Austrobaileya* leaves may be partially related to the anatomy of the stomatal complex. In particular, the cuticular vestibule, formed from overarching extensions of the epidermal cells, creates a relatively large volume of air above the guard cells that may be decoupled from the bulk phase VPD conditions. Consequently, the guard cells may respond to the locally high water vapor concentrations within the vestibule when the stomata are open, and slow rates of closure in response to atmospheric drought may reflect slow rates of devaporization of the vestibule as humidity conditions within it approach the bulk phase. Also, the cuticular vestibules, coupled with reflexed extensions of guard cell walls that effectively sealed off the stomatal cavity from the intercellular airspaces, may be responsible for the low values of leaf minimum conductance to water vapor that we observed in *Austrobaileya* leaves.

How do these observations bear on the ecological traits of the first angiosperms? The ecology and physiology of *Austrobaileya scandens* is different from hypotheses that the earliest angiosperms were early-successional xeric shrubs, disturbance-loving herbs characterized by high capacity for photosynthesis and water transport, and, most recently, aquatic herbs (Doyle and Hickey 1976; Doyle 1977; Hickey and Doyle 1977; Doyle and Donoghue 1986; Bond 1989; Taylor and Hickey 1992, 1996; Crane et al. 1995; Wing and Boucher 1998; Sun et al. 2002). Instead, our observations of *A. scandens* suggest that first angiosperms were woody plants that exploited wet, relatively dark and disturbed (albeit at small scales) habitats. The physiological ecology of *Austrobaileya* does not appear to be an isolated circumstance at the base of the extant phylogenetic tree and appears to correspond with characteristics found in other Austrobaileyales plants as well as *Amborella*. For example, *Amborella*, *Illicium*, *Kadsura-Schisandra*, and *Trimenia* appear to be geared for maximal physiological function under low light conditions, with low and easily light-saturated leaf photosynthetic rates, widespread absence of palisade mesophyll tissue, low stomatal densities, and low xylem water transport capacity (Metcalfe 1987; T. S. Feild, unpublished data).

Also, twining and scrambling vine growth habits occur frequently in the Austrobaileyales, as represented by *Trimenia* (formerly the two *Piptocalyx* species), *Kadsura*, and *Schisandra* (Metcalfe 1987). Although *Amborella*, *Illicium*, and most *Trimenia* taxa are free-standing shrubs or small trees, it is interesting to note that these plants display scandent tendencies, ranging the irregular, low apically dominant branching, occasional production of pendent shoots (*Amborella* and shrubby species of *Trimenia*), regeneration by vinelike underground runners (some *Illicium* species), and pseudorhizomatous seedling stages that are multibranched (T. S. Feild, unpublished observations).

At first pass, the water lily lineage, which has radiated in freshwater aquatic habitats that are generally open and brightly exposed (Schneider and Williamson 1993), seems highly divergent compared with the Austrobaileyales and *Amborella*, with the evolution of several vegetative specializations related to the biophysical conditions of underwater life coupled with high light availability. These features include herbaceousness, ventilating aerenchymatous pathways from leaves to roots, guard cells that lack a humidity response, high CO₂ fixation rates, and the presence of palisade mesophyll tissues (Sculthorpe 1967; Brewer and Smith 1995). However, at least initially, water lilies exploit extremely low light environments with the germination and seedling phase of their life history in submersed, dark muddy habitats. Consequently, there may be a shared set of functional characters among early-diverging angiosperms, in particular phytochrome-mediated developmental responses, that allow germination and development in both wet shady understories and muddy pond bottoms.

Additional comparative work, including assessment of growth habit and ecophysiological character states among basal angiosperms and studies on the morphology and biochemistry of Early Cretaceous angiosperm fossils, will be necessary to evaluate whether modern representatives of early-diverging angiosperms mirror the ecological roles and environmental conditions of early-flowering plants. Indeed, recent evidence of a much greater diversity of "magnoliid" forms in the Early Cretaceous (Friis et al. 2001) may lend support to the idea that the early lineages were once much more diverse, both structurally and in terms of the number of species. This finding also emphasizes the need to bring in functional analyses of early angiosperm fossils to test ecological inferences from living plants.

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