

## ACCLIMATION OF LEAF ANATOMY, PHOTOSYNTHETIC LIGHT USE, AND XYLEM HYDRAULICS TO LIGHT IN *AMBORELLA TRICHOPODA* (AMBORELLACEAE)

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Recent phylogenetic analyses place *Amborella trichopoda*, a semiclimbing premontane rain forest shrub endemic to New Caledonia, as sister to all other extant flowering plants. Here we present new observations on leaf anatomy and morphology, leaf photosynthetic physiology, and xylem hydraulic function for *Amborella* in the field. We focus on experimental measurements of the degree of photosynthetic and xylem hydraulic acclimation to sunlight by examining *Amborella* plants growing in deeply shaded understories (0.7% full sunlight) compared with individuals from exposed montane ridge crests in New Caledonia. *Amborella* leaves exhibit few anatomical differences between sun and shade. Instead of primarily adjusting leaf anatomical characters to irradiance, *A. trichopoda* leaves exposed periodically to full sunlight modify their orientation, becoming folded up along the midvein, presumably to avoid increases in light interception. Chlorophyll *a* fluorescence measurements demonstrate that the photosynthetic apparatus of *A. trichopoda* is shade adapted, based on a low capacity for excess light energy dissipation and low photosynthetic electron transport rate. In addition, these characteristics exhibit limited upregulation in response to increased irradiance. In parallel, there are small or no differences in leaf area-specific hydraulic conductivity between sun and shade shoots of *A. trichopoda*. Taken together, *A. trichopoda* appears to possess limited developmental and physiological flexibility to light flux density. The bearing of these observations, in the context of other early-diverging clades of angiosperms identified by recent phylogenetic studies, on the physiological ecology of the first angiosperms is discussed.

**Keywords:** *Amborella*, angiosperm phylogeny, basal angiosperms, shade adaptation, xylem hydraulics.

### Introduction

A long-standing interest of botanists concerns the growth habit and ecology of the earliest flowering plants (Bews 1927; Corner 1949; Axelrod 1952; Stebbins 1965; Takhtajan 1969; Doyle and Hickey 1976). Classically, the first angiosperms were portrayed as large or small trees of wet tropical rain forests, analogous to modern Magnoliales and Winteraceae, with thick, heavy branches, primitive vesselless wood, and slow maturation and growth rates (Bews 1927; Axelrod 1952; Takhtajan 1969; Thorne 1974; Cronquist 1988). The evolution of shrubby, lianous, and herbaceous growth habits was then suggested to have accompanied the radiation of angiosperms into cooler and drier areas (Bews 1927; Axelrod 1952; Takhtajan 1969). By contrast, Stebbins and other botanists argued that angiosperms initially occurred as small-statured shrubs (“weed trees”; see Doyle and Hickey 1976) along exposed, erosion-prone water courses where amounts of light, water, and nutrients were high but at pulsed availability (Stebbins 1965, 1974; Doyle and Hickey 1976; Hickey and Doyle 1977). These conditions were suggested to favor the evolution

of traits promoting rapid growth rates and shorter generation time (Stebbins 1965, 1974; Doyle and Hickey 1976; Hickey and Doyle 1977). According to this hypothesis, angiosperms later shifted to wetter, drier, and cooler environments where arborescent and herbaceous forms evolved. Other botanists agreed that the capacity for rapid growth and maturation were critical elements of the early angiosperm radiation but suggested the first angiosperms utilized a weedy, rhizomatous growth form characterized by limited wood production or a perennial herbaceous habit (cf. the “paleoherbs”; Donoghue and Doyle 1989), which were likely reminiscent of extant Chloranthaceae, Piperaceae, and *Aristolochia* (Taylor and Hickey 1992, 1996; Wing and Boucher 1998). Inferences about the ecology and evolution of the first angiosperms require an accurate understanding of phylogeny, in particular the branching order of lineages near the base of the tree (Donoghue and Doyle 1989; Doyle and Donoghue 1993). However, past attempts at reconstructing the first-branching lineages of angiosperms are fraught with uncertainty, with different lines of phylogenetic evidence advocating wide-ranging hypotheses for which extant taxa should be placed near the angiosperm root (Doyle and Donoghue 1986; Donoghue and Doyle 1989; Loconte and Stevenson 1990; Taylor and Hickey 1992; Chase et al. 1993; Qiu et al. 1993; Donoghue and Mathews 1998).

Recent molecular phylogenetic analyses have converged on the placement of *Amborella trichopoda* Baill., the sole representative of Amborellaceae, as the sister group to all other

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extant flowering plants, and resolve water lilies (Nymphaeales) and a clade comprising *Austrobaileya*, *Trimenia*, and *Illiciales* (*Illicium*, *Kadsura*, and *Schisandra*) as the next two lineages diverging from the main angiosperm line (Mathews and Donoghue 1999; Parkinson et al. 1999; Qiu et al. 1999, 2000; Soltis et al. 1999, 2000; Barkman et al. 2000; Graham and Olmstead 2000). A well-resolved picture of basal angiosperm relationships provides a new opportunity to examine the suites of ecological and physiological characters potentially present in the first angiosperms, through comparative work on extant basal taxa. Because of its basal position, understanding the ecology of *Amborella* may bear on the possible early directions of ecological and physiological evolution occurring in angiosperms. Currently, limited information is available on the physiological ecology or environmental context of *A. trichopoda* (Feild et al. 2000).

Given the contrasting views as to the probable ecology and ancestral environments (in particular, inferred light regimes) of early angiosperms, it is instructive to examine the physiological and morphological reactions of leaves and stems in basal angiosperm groups to varying light intensity. Here, new observations on leaf anatomy and morphology, leaf photosynthetic physiology, and xylem hydraulic function are presented for *A. trichopoda* in New Caledonia. We focus on experimental measurements of photosynthetic and xylem hydraulic acclimation to light by examining *A. trichopoda* plants growing in deeply shaded understories in comparison to plants from exposed montane ridges.

## Material and Methods

### Study Areas

*Amborella trichopoda* occurs as a common dioecious, vesselless shrub (to 8 m tall) in wet premontane rain forests (400–1000 m above sea level) of central and northern New Caledonia (Jérémie 1980; Feild et al. 2000). *Amborella trichopoda* was studied in two large populations in central New Caledonia that span the range of light environments *A. trichopoda* encounters in the field. Fieldwork was conducted from November 8 to November 30, 2000. Ten understory shrubs of *A. trichopoda* were examined at 600–700 m in a premontane rain forest (ca. 200 m below Plateau de Dogny; 21°37'S lat., 165°53'W long., near Sarraméa, Province Sud), ca. 130 km northwest from Nouméa. Here *A. trichopoda* is an abundant understory to subcanopy shrub, <8 m tall in 20-m-tall forest. The forest canopy is angiosperm dominated (Lauraceae, Myrtaceae, and Sapotaceae) with an understory composed largely of *Angiopteris* (Marrattiaceae) ferns and angiosperms (*Psychotria* [Rubiaceae] and *Zygogynum* [Winteraceae]). A second population was studied in a montane rain forest, ca. 850 m above sea level (near Massif l'Aoupinié; 21°11'S lat., 165°18'W long., near Goapin, Province Nord), ca. 110 km northwest from the first *Amborella* population. At this elevation and in contrast to the Plateau de Dogny region, *A. trichopoda* frequently occurs as an exposed shrub to 4.5 m tall along roadcuts on ridge crests, which we refer to as “sun plants,” and as an understory shrub in undisturbed forest patches above and below the road. Plants sampled from the forest understory at Massif l'Aoupinié and Plateau de Dogny

are referred to as “shade plants.” The conifer *Agathis corbassonii* de Laubenfels (Araucariaceae) and a diverse assemblage of angiosperms, including *Metrosideros* and *Elaeocarpus* species, form a 12–15-m-tall canopy at Massif l'Aoupinié. At this site, 10 *A. trichopoda* plants from along the exposed roadside environment and 10 plants from the understory for comparison were studied.

Both field sites are characterized by a tropical climate with mean monthly temperatures ranging from 20° to 25°C (ORSTOM 1981). Rainfall at Plateau de Dogny varies from 1700 to 2500 mm yr<sup>-1</sup> with a moderately pronounced seasonal pattern. During the warm wet season (November to mid-May), rainstorms and cloud and mist occur almost every day, and tropical depressions and cyclones are frequent. This season accounts for nearly 70% of the yearly rainfall. From June to August, rainfall is somewhat lower (rainstorms 5 d a week typically). This period is followed by a dry season (mid-September to mid-November) characterized by generally clear weather and rainstorms 1–2 d a week, with monthly rainfall averages between 70 and 120 mm (ORSTOM 1981). Southeastern trade-wind-driven cloud moisture increases as the season progresses, which augments to an unknown amount the moisture input in the forest (ORSTOM 1981). Rainfall on Massif l'Aoupinié is appreciably greater, ca. 3500–4000 mm a year. During the dry season, trade-wind-driven cloud moisture inputs appear to be much greater on Massif l'Aoupinié due to greater elevation and local topography; on all days that Plateau de Dogny was cloud free during the dry season, Massif l'Aoupinié was enshrouded in wind-driven cloud and mist.

### Characterization of Light Environment

The light environment of *Amborella* was characterized using local measurements of photosynthetically active radiation (PAR; 400–700 nm,  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) intensity with a handheld Li-189 photodiode-based meter (Li-COR, Lincoln, Nebr.). Measurements were taken between 1100 and 1230 hours on representative clear periods ( $n = 3$ ) and cloudy days ( $n = 14$ ) above leaves of the same branches sampled for anatomical and physiological observations.

### Leaf and Stem Anatomy and Morphology

Fully expanded leaves from the same exposed and understory *Amborella* 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 morphological and anatomical measurements. The following anatomical data were taken from leaf transverse sections made by hand using a double-edged razor blade: (1) total leaf thickness, (2) adaxial epidermis thickness, and (3) hypodermis thickness. Leaf sections were cleared for 1 h using a 50% solution of bleach and water and stained with toluidine blue. From epidermal peels, stomatal size (the maximum diameter,  $\mu\text{m}$ ) and stomatal density (number  $\text{mm}^{-2}$ ) were determined at 400 $\times$  and 200 $\times$ , respectively, for six leaves sampled randomly from six sun and six shade branches. Anatomical measurements were made on three different sections from the same leaf and using two leaves from each individual plant. Observations of tracheid diameters of *A. trichopoda* were made on transverse sections taken from stems

preserved in 50% ethyl alcohol. Average maximal tracheid diameter (cell lumens were typically elliptical in cross section; Bailey and Swamy 1948; Feild et al. 2000) were based on 25 measurements at  $400\times$  on all *A. trichopoda* stems used for xylem hydraulic conductivity calculations.

#### *Chlorophyll a Fluorescence Measurements*

Chlorophyll *a* fluorescence emission from *Amborella* leaves was measured using a portable pulse-amplitude modulated fluorometer (PAM-2000, Walz, Effeltrich, Germany) in the field to calculate fluorescence parameters related to photosynthetic light use (Bilger et al. 1995). For determination of maximum photosystem II (PSII) photon yield, the ratio of variable fluorescence ( $F_v = F_m - F_0$ ) to maximal fluorescence ( $F_m$ ) yields were determined following dark adaptation. Leafy branches of *A. trichopoda* were sampled at midday, placed in moist plastic bags, and then darkened for 6 h to ensure relaxation of photoprotective energy dissipation processes (Björkman and Demmig 1987; Demmig-Adams and Adams 1992; Horton et al. 1996). Minimal fluorescence yield ( $F_0$ ) was determined using a nonactinic measuring beam after illuminating the leaf with 5 s of far-red light to maximally oxidize the quinone electron acceptors of PSII (Feild et al. 1998). Then the leaf was exposed to a saturating pulse of white light at  $5000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  for 800 ms to determine  $F_m$ .

The light responses of photosynthetic light-use efficiency by exposed and understory leaves of *A. trichopoda* were determined by measuring the light response of the effective photon yield of PSII ( $\Phi_{\text{PSII}}$ ), 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 where a saturating pulse (same conditions as above) is superimposed on the prevailing light intensity (Genty et al. 1989; Bilger et al. 1995).  $\Phi_{\text{PSII}}$  was used to calculate electron transport through PSII (denoted ETR) as described by Bilger et al. (1995):  $\text{ETR} = \Phi_{\text{PSII}} \times \text{PAR} \times 0.84 \times 0.5$ , where PAR is the light intensity during measurement. A value of 0.84 was used for leaf absorbance (Björkman and Demmig 1987), and 0.5 is used assuming that absorbed light is evenly distributed between PSII and PSI (Bilger et al. 1995). We assumed that leaf absorbance values were similar between sun and shade leaves of *A. trichopoda*. All measurements were made between 0930 and 1200 hours. For exposed branches, natural variation in ambient PAR was used to reconstruct a relationship between incident PAR (measured with a PAM-2000 leaf clip quantum sensor) and  $\Phi_{\text{PSII}}$ . In the understory, ambient light was controlled by using the internal halogen lamp of the PAM-2000. At each light intensity, the leaf was illuminated until  $F$  values were stable for at least 30 s (taking ca. 2–4 min), and then a saturating pulse was applied to measure  $\Phi_{\text{PSII}}$ . Light response data were curve fitted using Sigma Plot 2000 (Jandel Scientific, Chicago).

The responses of PSII to a brief (8 min) exposure to a high-intensity light treatment (ca.  $1800 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  PAR, which is typically encountered by exposed shoots of *A. trichopoda* in the field) were investigated. Because of nonreliable light conditions and the inability to illuminate understory leaves with a high-intensity light treatment, detached shoots of *A. trichopoda* were used for these experiments. Shoots, ca. 25 cm long, were cut from branches in the morning

(0930–1000 hours), placed in a moist bag, and transported to a lower elevation where clear, sunny conditions occurred. Next, the proximal end of the branch was cut back 10 mm underwater to remove tracheids embolized by the first cut in air and the leaves gently dried with a paper towel to remove condensation. Branches were exposed to indirect light (ca.  $100\text{--}150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ), while the end of the branch was kept under filtered water for a minimum of 30 min to allow leaf surfaces to dry completely and sufficient time for stomatal opening (Valladares et al. 1997). Then, the reservoir was moved into a sunny clearing for 8 min while  $\Phi_{\text{PSII}}$  was measured on three distal leaves for each branch at 1, 2, 3, 5, and 8 min under the high-intensity light treatment. After 8 min, the branch was rapidly transferred to a dark room and  $F'_v/F'_m$  (denoting PSII photon yield following exposure to actinic light; Genty et al. 1989) measured on a single leaf after 30 s, 1, 2, 5, and 10 min in the dark. For measurements taken at 20, 30, 60, and 90 min in the dark,  $F'_v/F'_m$  was recorded for the other two leaves on which  $\Phi_{\text{PSII}}$  had been determined under high light. Rapid rates of water uptake were observed in detached shoots where the petioles had been placed into graduated vials, indicating that leaves were transpiring freely after detachment.

#### *Xylem Hydraulic Measurements*

Xylem hydraulic conductivity ( $K_H$ ) was defined as the mass flow rate of water through an excised stem segment per unit pressure gradient normalized to stem length ( $\text{kg H}_2\text{O MPa}^{-1} \text{m}^{-1} \text{s}^{-1}$ ; Sperry et al. 1988). A portable steady-state flowmeter (SSFm), which utilizes the drop in pressure 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 the stem segment under positive pressure (5–20 kPa). A pressure transducer (PX136, 30 kPa/5 mV range, Omega Engineering, Stamford, Conn.) was fitted so that the pressure of the hydraulic head and at the stem fitting could be easily read with a four-digit multimeter. 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 ( $\pm 0.0001$  g). Five hydraulic head pressures, from 0.001 to 0.01 MPa, were used and a linear calibration equation determined for each capillary tube. Capillary tubes were calibrated before and then immediately after travel to New Caledonia to check for drift. After correcting for temperature, no drift in tube conductance was observed over a 2-mo period. A weak solution of bleach (10% vol. 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 (ca. 10 mmol dissolved ionic substances) by using sterilized, filtered (to  $0.2 \mu\text{m}$  using a hand-filter pump, MSR, or syringe filter, Gelman Acrodisc LC PVDF) bottled spring water (Mont Dore Spring Water, Mont Dore, New Caledonia). During all hydraulic measurements, temperatures of measured stems were monitored with copper-constantan thermocouples so that changes in flow rate resulting from changes in the

**Table 1**  
**Midday Light Environment Characteristics for the Three Study Areas for *Amborella trichopoda***  
**in Two New Caledonia Tropical Montane Rain Forests**

Variable	Massif l'Aoupinié		Plateau de Dogny, understory
	Exposed	Understory	
Exposed light level, clear ( $n = 3$ )	1961 ± 150	na	na
Diffuse light level, cloudy ( $n = 6$ )	240 ± 23	13 ± 2	13 ± 3
Diffuse light level, clear ( $n = 3$ )	na	17 ± 6	19 ± 10
Sun fleck light levels ( $n = 3$ )	na	987 ± 329	910 ± 341

Note. Averages of photosynthetically active light intensity (PAR, 400–700 nm,  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) with sample sizes ( $n$ , with number of days sampled, based on 12 measurements on each day) are indicated for each microsite; na = not applicable.

viscosity of water could be removed by normalizing all hydraulic measurements to 25°C (Sperry et al. 1988).

Undamaged leaf-bearing branches of *A. trichopoda* were collected from 10 sun and 10 shade plants at Massif l'Aoupinié and 10 shade plants at Plateau de Dogny. Because the stem xylem hydraulic properties are sensitive to light environment (Schultz and Matthews 1993; Maherali et al. 1997; Cochard et al. 1999), care was taken to select individuals from uniform conditions. Exposed branches were sampled from roadside *A. trichopoda* shrubs growing in small clearings (400 m<sup>2</sup>), which had several stems exposed to full sun in nearly all directions, so we could be certain that these had developed under full sun conditions. Branches from understory environments were cut from plants growing in the intact understory.

Branches were cut from plants of *A. trichopoda* at times when losses in stem  $K_H$  resulting from embolisms were probably at a minimum (i.e., early morning [0800–0930 hours] on wet, cloudy days). After severing, branches were triple bagged in plastic and transported to the field laboratory. Here, stem segments were cut from branches underwater at a standardized length (50 mm) and diameter (3.5–5 mm, excluding bark) to minimize size-dependent variation in tracheid geometry and hydraulic conductance (Carlquist 1975; Maherali et al. 1997). Both ends of the segment were shaved in cross section with a fresh razor blade underwater. Leaves along the axis of the stem were excised underwater and the cut petioles sealed with a fast-solidifying glue (Loctite Super Bonder 409, with accelerator 712, Loctite, 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. Sapwood area-specific hydraulic conductivity ( $K_S$ ;  $\text{kg MPa}^{-1} \text{m}^{-1} \text{s}^{-1}$ ) 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 200× with a calibrated ocular micrometer, and the surface area contributed by the pith was subtracted (Sperry et al. 1988). Leaf area-specific hydraulic conductivity ( $K_L$ ;  $\text{kg MPa}^{-1} \text{m}^{-1} \text{s}^{-1}$ ) was determined by dividing stem  $K_H$  by the total leaf area supported (i.e., distal) by 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, Lincoln, Nebr.). Huber value (HV) was expressed as the ratio of sapwood area (m<sup>2</sup>) to leaf area (m<sup>2</sup>). Values for  $K_L$ ,  $K_S$ , and HV were compared between field sites using a Student's *t*-test

## Results

Forest understory light environments at Plateau de Dogny and Massif l'Aoupinié were comparable, with similar mean diffuse light intensity measured at midday on cloudy and clear days (table 1). Sun fleck light intensities were ca. 8% brighter in the forest understory of Massif l'Aoupinié compared to Plateau de Dogny (table 1). By contrast, mean midday light level during clear periods above exposed shoots of *Amborella trichopoda* plants sampled along the ridge crest at Massif l'Aoupinié varied from 1800 to 2120  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PAR. However, cloud-free periods were brief, varying from 2–15 min due to belts of trade-wind-driven cloud flowing over the ridge crest for the majority of the day. Midday light levels during cloudy periods were brighter than ambient understory light intensities at both field sites (table 1) and ranged from 150 to 270  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PAR, depending on the extent of cloud cover.

Shade leaves of *A. trichopoda* from Plateau de Dogny and Massif l'Aoupinié were flat and arranged in a nonoverlapping pattern on pendent shoots with limited lateral branching (fig. 1A). In addition, shade leaves of *A. trichopoda* leaves were bicolored, with the upper leaf surface a darker green compared to leaf lower surface (fig. 1B). By contrast, sun leaves of *A. trichopoda* from exposed rain forest ridges and roadsides of Massif l'Aoupinié were folded up along the midvein (conduplicate, fig. 1C, 1D). Leaf folding reduced the amount of leaf surface area exposed to the sky by ca. 50% (data not shown). Other morphological differences included increased brittleness and nervation (fig. 1D) in sun compared to shaded leaves. Stomata were only observed on the lower surfaces for shade and sun leaves of *A. trichopoda*. Internally, the mesophyll of sun and shade leaves of *A. trichopoda* consisted predominately of spongy tissue, with no palisade layer, and a conspicuous hypodermis near the top surface of the leaf. The thickness of this layer did not differ for sun and shade leaves (table 2). In addition, no differences were observed in adaxial epidermal cell layer thicknesses or guard cell lengths for sun and shade leaves of *A. trichopoda* (table 2). However, total leaf thickness and stomatal density, respectively, were 10% and 36% greater in sun leaves compared to shade leaves (table 2).

Maximum PSII photon yield ( $F_v/F_m$ ) was 4% lower in sun *A. trichopoda* leaves compared to those from shade (sun =  $0.79 \pm 0.01$  SD vs. shade =  $0.82 \pm 0.01$ ; Student's *t*-test;  $P < 0.001$ ). Electron transport through PSII (ETR) saturated

**A****B****C****D**

**Fig. 1** Shade-sun leaf morphology of *Amborella trichopoda* from tropical montane rain forest in central New Caledonia. **A**, Overall planate leaf arrangement of a shade shoot from Plateau de Dogny. **B**, Leaf bicoloration of understory leaves. Note the lighter color of the leaf undersurface relative to the top surface. **C**, Leaf-folding (conduplicate) pattern exhibited by sun shoots produced along a roadside at Massif l'Aoupinie. **D**, Detail of the leaf folding depicting increased nervation.

**Table 2**  
**Comparative Leaf Anatomy of Sun and Shade Leaves of**  
*Amborella trichopoda*

Variable	Sun	Shade
Leaf thickness ( $\mu\text{m}$ )	418 $\pm$ 15	378 $\pm$ 8*
Epidermis thickness ( $\mu\text{m}$ )	21 $\pm$ 5	23 $\pm$ 2 ns
Hypodermis thickness ( $\mu\text{m}$ )	28 $\pm$ 4	25 $\pm$ 4 ns
Stomatal size ( $\mu\text{m}$ )	29 $\pm$ 4	31 $\pm$ 3 ns
Stomatal density (number $\text{mm}^{-2}$ )	48 $\pm$ 2	31 $\pm$ 6***

Note. Values for variables represent averages  $\pm$  SD of 25 measurements from six fully expanded leaves from six individual plants; ns = not significant. Averages for shade leaves of *A. trichopoda* were determined from three mature leaves from three individuals at Plateau de Dogny and l'Aoupinié.

\*  $P < 0.05$  (Student's *t*-test).

\*\*\*  $P < 0.001$  (Student's *t*-test).

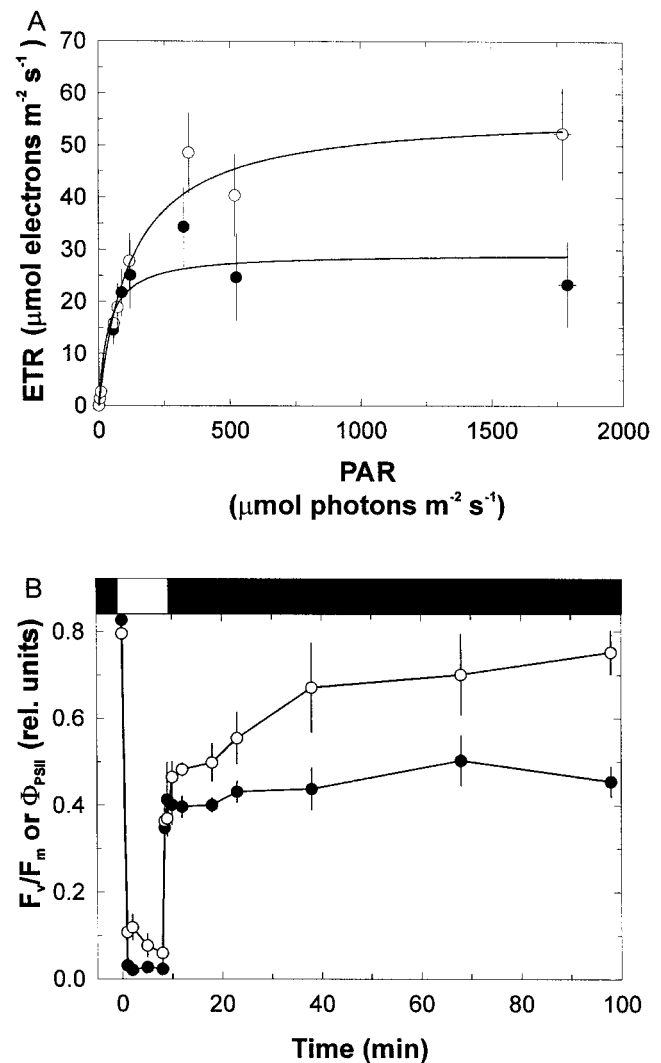
rapidly with increasing PAR, occurring at ca. 250 and 600  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PAR in shade and sun *A. trichopoda* leaves, respectively (fig. 2A). Light-saturated ETR was nearly twofold greater in sun leaves of *A. trichopoda* compared with those produced in shade (fig. 2A). In response to saturating light (1800  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PAR, ca. 85% full sun), effective PSII photon yield ( $\Phi_{\text{PSII}}$ ) was ca. 50% greater in sun leaves compared with shade leaves during the first 2 min under the high-illumination treatment and declined to ca. 30% greater in sun leaves after 8 min (fig. 2B). When light was removed,  $\Phi_{\text{PSII}}$  of sun and shade leaves, respectively, recovered to ca. 85% and 50% of the dark-adapted value after 30 min in darkness (fig. 2B). After 90 min of darkness,  $\Phi_{\text{PSII}}$  of sun and shade *A. trichopoda* leaves, respectively, were ca. 94% and 56% of the dark-adapted PSII quantum yield (fig. 2B).  $\Phi_{\text{PSII}}$  of sun and shade leaves remained near these values after 6 h of dark adaptation (data not shown).

Mean leaf area-specific hydraulic conductivity ( $K_L$ ) in sun shoots of *A. trichopoda* was higher than that of shade shoots from both field sites; however, these differences were only significant (table 3) between sun and shade shoots sampled at Massif l'Aoupinié.  $K_L$  did not differ significantly between shade shoots sampled at Massif l'Aoupinié and Plateau de Dogny (table 3).  $K_L$  is affected by the relative allocation to conducting xylem area and leaf area (Huber value) and the capacity of the xylem to conduct water ( $K_s$ , stem hydraulic conductivity normalized to xylem area; Zimmermann 1983; Tyree and Ewers 1991). Huber values of sun *A. trichopoda* branches tended to be greater than values for shade branches, but differences were not significant across all field sites (table 3). In addition,  $K_s$  values were similar for plants from all three study environments (table 2). Consistent with these measurements, no significant differences were observed in tracheid diameter between exposed and understory (Plateau de Dogny and Massif l'Aoupinié) stems (table 3).

## Discussion

The anatomy and physiology of *Amborella trichopoda* shoots indicate that this species is generally well suited to low light availability. Several anatomical and physiological traits present in *A. trichopoda* appear to contribute toward maxi-

mizing understory light capture, and these traits exhibit only limited or no adjustments to increases in light availability (table 2). Instead of primarily adjusting leaf anatomical characters to irradiance, *A. trichopoda* leaves exposed periodically to full sunlight modify their orientation, becoming folded up along the midvein presumably to avoid increases in light interception (Koller 2000; but see Mooney et al. 1977). However, the overall difference in total leaf thickness observed between sun and shade leaves suggests some adjustment of spongy mesophyll anatomy in response to light. For example, it is possible that differences in mesophyll shape, size, orientation, and/or density may differ between sun and shade leaves of *A. trichopoda* to



**Fig. 2** Comparative physiological light responses of the photosynthetic apparatus of sun and shade leaves of *Amborella trichopoda*. **A**, Response of PSII electron transport rate (ETR,  $\mu\text{mol electrons m}^{-2} \text{s}^{-1}$ ) to light intensity for sun leaves (open circles;  $n = 3$ ) compared to shade leaves (filled circles;  $n = 3$ ). **B**, Responses of PSII photon yield before, during, and after brief (8 min, time denoted by the white box) exposure to ambient midday light intensity (ca. 1800  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , PAR) for sun (open circles;  $n = 3$ ) and shade shoots (closed circles;  $n = 3$ ). Error bars denote SD for each plot.

**Table 3**  
**Stem Hydraulic and Anatomical Characteristics of *Amborella trichopoda* from Different Light Environments in the Field**

Variable	Massif l'Aoupinié		Plateau de Dogny, understory
	Exposed	Understory	
$K_L \times 10^{-4}$	1.13 ± 0.39 <sup>A</sup>	0.77 ± 0.39 <sup>B</sup>	0.92 ± 0.41 <sup>AB</sup>
$K_S$	0.54 ± 0.19 <sup>A</sup>	0.64 ± 0.21 <sup>A</sup>	0.67 ± 0.21 <sup>A</sup>
$HV \times 10^{-4}$	2.35 ± 1.32 <sup>A</sup>	1.23 ± 0.53 <sup>A</sup>	1.25 ± 0.64 <sup>A</sup>
Tracheid diameter	21 ± 3 <sup>A</sup>	23 ± 2 <sup>A</sup>	22 ± 2 <sup>A</sup>

Note. Sites with a different letter are significantly different from each other at  $P < 0.05$  level of significance (Student's  $t$ -test). Averages of leaf area-specific hydraulic conductivity ( $K_L$ ; kg H<sub>2</sub>O m<sup>-1</sup> s<sup>-1</sup> MPa<sup>-1</sup> [±SD]), stem conductivity per unit sapwood area ( $K_S$ ; kg H<sub>2</sub>O m<sup>-1</sup> s<sup>-1</sup> MPa<sup>-1</sup> [±SD]), and Huber value (HV; ratio of sapwood area [m<sup>2</sup>] to leaf area [m<sup>2</sup>] [±SD]), with sample sizes of 10 stems for each parameter at each field site. See "Material and Methods" for site descriptions.

explain the overall increase in leaf thickness with light flux density (Chazdon and Kaufmann 1993).

Anatomical features characteristic of shade-adapted plants were present in *Amborella* plants growing in both sun and shade. For example, the mesophyll tissue in leaves of *A. trichopoda* lacked palisade cells and consisted entirely of spongy cells that may facilitate the harvesting of diffuse and far-red-biased wavelengths that dominate understory light environments through increased internal light scattering (Vogelmann 1993; Vogelmann and Martin 1993; DeLucia et al. 1996). Other morphological traits of *Amborella* that are generally associated with tolerance to shade in understory plants include relatively low stomatal density and possibly leaf bicoloration (fig. 1B; Givnish 1988; Smith et al. 1997). Leaf bicoloration may increase light trapping by backscattering light passing through the mesophyll on its way out of the leaf (Smith et al. 1997). However, *Amborella* does not possess leaf features that are associated with extreme shade, such as spherical epidermal "lens" cells, blue coloration, or reddish leaf undersurfaces, as found in some ferns, mosses, and angiosperms (Lee 1997). At the architectural scale, the semiscandent habit of *A. trichopoda*, characterized by pendent, flexible branches may further contribute to the capture of diffuse irradiance (which is incident on leaf surfaces in all directions) while casting little shade on other branches below. Leaves of *A. trichopoda* are displayed in a nonoverlapping pattern that would further minimize self-shading.

Chlorophyll *a* fluorescence measurements demonstrated that the photosynthetic apparatus of *A. trichopoda* possesses several characteristics typical of shade-adapted plants (Boardman 1977; Chazdon and Field 1987; Givnish 1988; Demmig-Adams and Adams 1992; Zipperlen and Press 1996; Kitao et al. 2000; Valladares et al. 2000). We observed that the rate of photosynthetic electron transport through PSII (ETR) of shade leaves saturated at ca. 250 μmol photons m<sup>-2</sup> s<sup>-1</sup> (fig. 2A). Based on the range of sun fleck intensities measured in this study (table 1), shade *Amborella* leaves appear to possess a low ability to harvest sun flecks. However, more information is needed on the frequency distributions of irradiance and duration of sun flecks in the understory to evaluate their impor-

tance to carbon gain. In addition, leaves of *A. trichopoda* produced in the understory expressed a limited capacity for nonphotochemical dissipation of excess light energy, as inferred by the strong suppression and absence of a recovery in  $F_v/F_m$  after short exposure to full sunlight (fig. 2B). Although sun leaves of *A. trichopoda* produced an ETR twice that of shade leaves, this degree of sun acclimation of leaf photosynthetic rate is lower than values reported for other tropical and temperate forest angiosperm trees and shrubs (Chazdon and Field 1987; Zipperlen and Press 1996; Nicotra et al. 1997; Clearwater et al. 1999; Loik and Holl 1999; Valladares et al. 2000). Combined with the observation that ETR in sun leaves saturated at only 600 μmol photons m<sup>-2</sup> s<sup>-1</sup> (25% full sunlight), it appears that *Amborella* possesses a limited capacity to acclimate to high light. Also, in contrast to typical sun-adapted plants, which exhibit rapid recovery (ca. 5–15 min) in  $F_v/F_m$  to the dark-adapted state following collapse of the pH gradient across chloroplast membranes in darkness (termed "energization quenching," or qE; Krause and Weis 1991; Demmig-Adams and Adams 1992), sun leaves of *A. trichopoda* expressed a prolonged lowering in  $F_v/F_m$ , requiring ca. 80 min in the dark for 94% of the dark-adapted state (fig. 2B). This indicates that the major component of nonphotochemical energy dissipation in sun leaves of *A. trichopoda* is related to "photoinhibitory quenching" or qI-quenching processes (Horton et al. 1996; Richter et al. 1999; Ruban and Horton 1999). Photoinhibitory quenching may be linked to damage to PSII, which requires energy for the repair and synthesis of new PSII reaction centers (Krause and Weis 1991), or may reflect the engagement of a long-term downregulation of PSII light capture efficiency to partly replace the rapidly relaxing qE mechanism under conditions of prolonged high irradiance (Richter et al. 1999; Ruban and Horton 1999). Regardless of the mechanism, qI quenching lowers the efficiency of light harvesting under high irradiance (Horton et al. 1996). Correspondingly, values of light-saturated quantum yield of PSII electron transport for sun shoots of *A. trichopoda* were nested in the lower end of the range for conifers and other vesselless angiosperms (Winteraceae) and considerably lower than most vessel-bearing angiosperms (Brodribb and Feild 2000).

In parallel with the limited acclimation of the photosynthetic apparatus to light, little or no difference in leaf area-specific hydraulic conductivity ( $K_L$ ) was observed between sun and shade shoots of *A. trichopoda* (table 3). This indicates that sun and shade shoots of *A. trichopoda* plants have equivalent capacities to supply their leaves with water and that sun plants are incapable of upregulating the allocation of xylem conducting area to leaf area or the efficiency of their vascular transport system to light (Zimmermann 1983). Since  $K_L$  is coordinated to photosynthetic capacity through stomatal conductance (see Brodribb and Feild 2000; Hubbard et al. 2001), the small increases in leaf photosynthetic rate between sun and shade *Amborella* leaves may result from alterations in leaf morphology, including leaf thickness or stomatal density (table 1). It is also possible that small changes in mesophyll cell geometry may be important morphological characteristics, by altering internal leaf CO<sub>2</sub> transport, in explaining the small increase of photosynthetic electron transport rate to irradiance (Chazdon and Kaufmann 1993). However, leaf folding by *Amborella* plants in full sun is likely to reduce shoot evaporative

demand by decreasing thermal loading, thus allowing plants to sidestep some of the adverse effects of increased irradiance and vapor-pressure deficit.  $K_L$  values for both sun and shade *A. trichopoda* branches appear to be in the lower range of values reported previously for other New Caledonian rain forest angiosperms with vessel-bearing xylem and other tropical rain forests occurring in exposed environments (Tyree and Ewers 1996; Brodribb and Feild 2000). The ability of *Amborella* stems to supply leaves with water, for both sun and shade plants, is nested among the range of capacities measured for conifers and other vesselless angiosperms (e.g., Winteraceae) growing in sunny environments (Brodribb and Feild 2000; Feild et al. 2000). However, additional information on the comparative stem hydraulic characteristics of angiosperm taxa that co-occur with *Amborella* in the understory are needed.

Other ecological traits of *Amborella* appear to be linked to the occurrence in low-light understory habitats. The multi-stemmed habit and a strong propensity for sprouting in *Amborella* may be important for long-term persistence in understory habitats, where falling debris from canopy trees frequently damages plants (Greig 1993; Bond and Midgely 2001). In addition, seedlings of *A. trichopoda* do not appear to regenerate in sunny, disturbed rain forest patches opened by cyclones, fire, logging roadcuts, or tree falls. Instead, *Amborella* seedlings establish preferentially under closed forest canopies in semidisturbed microsites, such as along trails and on soil exposed by small washouts. Although numerous large plants of *A. trichopoda* occur along exposed roadcuts in Massif l'Aoupinié, these individuals do not appear to have established in these sites. Seedlings were not observed, in spite of what appears to be abundant seed production, in nearby clearings or underneath these individuals. Instead, we suggest that during the course of *Agathis* logging, understory and subcanopy *Amborella* plants became exposed to more sunny conditions. However, more attention is needed on understanding the seedling establishment requirements and demography of *A. trichopoda*.

The extant ecology of *Amborella* differs from previous models for the early ecological evolution of angiosperms that linked their initial success to the evolution of a weedy "progenetic" (e.g., accelerated time to first reproduction) life history, including rapid and efficient reproduction, high rates of resource use, or herbaceous habits in response to unstable or semiarid

environments (Stebbins 1965, 1974; Doyle and Hickey 1976; Hickey and Doyle 1977; Retallack and Dilcher 1981; Doyle and Donoghue 1986; Crane 1987; Bond 1989; Taylor and Hickey 1992, 1996). Instead, observations of *A. trichopoda* suggest that the first flowering plants were woody, adapted to shady wet environments, and possessed limited structural and physiological flexibility to light. However, in some characters, *Amborella* does appear similar to Stebbins's (1965) original conception of the first angiosperms in that they possess a multistemmed shrubby habit and female plants appear to reach reproductive maturity at a relatively small size. *Amborella* saplings bear seed when they are as small as 65 cm tall, which may indicate some capacity for rapid attainment of reproductive maturity. The physiological ecology of *Amborella* corresponds to that found in other early-diverging clades of flowering plants (i.e., ITA clades, including *Austrobaileya*, Illiciales, and *Trimentia*), with a suite of shared ecological and functional characters among these clades. For example, *Amborella* and ITA plants possess low photosynthetic and xylem hydraulic capacities, occur in moist, shady environments, and have a strong propensity for vegetative reproduction; in addition, scandent habits are widespread (T. S. Feild, M. J. Donoghue, and N. M. Holbrook, unpublished data). If the physiological features present today in *Amborella* and ITA lineages are used as a guide for our interpretation of those present in the first angiosperms, then it appears that success in shady, wet forest understory habitats may account for their initial ecological toehold in Cretaceous landscapes. However, further work is needed on the ecology of basal angiosperms, in particular, on seedling demography and plasticity to light, as well as a clearer understanding of their fossil history to determine how reliably extant representatives of the lineages reflect the ecological and physiological conditions present in the first flowering plants.

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