

STRUCTURE AND FUNCTION OF TRACHEARY ELEMENTS IN *AMBORELLA TRICHOPODA*

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Recent phylogenetic analyses have placed the root of flowering plants near *Amborella trichopoda*, a woody plant restricted to cloud forest habitats in New Caledonia. A distinctive feature of *A. trichopoda* is its reported lack of xylem vessels. Here we present observations of pit membrane structure and end wall morphology for primary and secondary tracheary cells of *A. trichopoda* as well as field measurements of stem hydraulic properties of *A. trichopoda* compared with five cloud forest species from New Caledonia. Observations of stem radial sections revealed that the primary wall material in the protoxylem and metaxylem elements was intact. No large porosities (such as those that have been observed in the pit membranes of Nymphaeales) were observed. However, a few elliptical pits of tracheary cells in the secondary xylem appeared to lack pit membranes. These observations are consistent with our measurements of functional conduit length, which indicate that the longest open conduits are equal to the length of two secondary xylem elements joined end to end. Thus, the xylem of *A. trichopoda* appears to be functionally vesselless, with the caveat that connections between individual vascular elements may occasionally be open (i.e., lacking in at least one pit membrane). Sapwood area and leaf area-specific hydraulic conductivities of *A. trichopoda* are similar to those of conifers and angiosperms, with and without xylem vessels, growing in understory cloud forest environments. These findings bear on discussions of the morphology and ecology of the first flowering plants as well as on the possible causes of their diversification.

Keywords: *Amborella*, basal angiosperms, xylem hydraulic conductivity, vessel evolution.

Introduction

Botanists have long been fascinated by the origin and radiation of flowering plants (Takhtajan 1969; Thorne 1974; Doyle 1978; Doyle and Donoghue 1986; Cronquist 1988; Donoghue and Doyle 1989; Taylor and Hickey 1992; Crane et al. 1995; Wing and Boucher 1998). Recent phylogenetic analyses of multiple molecular data sets have resolved the root of flowering plants near *Amborella*, water lilies (i.e., Nymphaeales), and a clade containing *Austrobaileya*, Trimeniaceae, and Illiciaceae plus Schisandraceae (Mathews and Donoghue 1999; Parkinson et al. 1999; Qiu et al. 1999; Soltis et al. 1999). These results bear directly on the evolutionary direction of morphological change (e.g., in carpel morphology, habit, breeding system, tracheary elements) and on ecological shifts that may have contributed to the rapid diversification of angiosperms in the Early to Middle Cretaceous (Doyle and Donoghue 1986; Crane and Ligard 1989; Taylor and Hickey 1992; Doyle and Donoghue 1993; Sanderson and Donoghue 1994; Crane et al. 1995; Doyle 1998; Wing and Boucher 1998; Mathews and Donoghue 1999). One important implication of placing *Amborella* as the sister group of all other angiosperms

is that it increases the likelihood that the first flowering plants were woody plants with vesselless xylem (i.e., containing only tracheids for water transport; contra Young 1981; Donoghue and Doyle 1989; Baas and Wheeler 1996; see also Feild et al. 1998).

Amborella trichopoda Baill., the only species of Amborellaceae, is an understory to subcanopy shrub (maximally 8 m tall) growing in middle-elevation (600–800 m above sea level) montane tropical rain forests of central New Caledonia, Australia (Jérémy 1980). Although it is reported to lack vessels (Bailey and Swamy 1948; Bailey 1957; Metcalfe 1987), the discovery of “incipient” vessel elements in some water lilies (Schneider and Carlquist 1995a, 1995b) and in other reputed vesselless angiosperms (e.g., *Sarcandra*, Chloranthaceae; Carlquist 1987; Takahashi 1988) underscores the need for a careful reexamination of *A. trichopoda* pit membrane structure and the end wall morphology of tracheary cells. Currently, no information is available on the water-transport capacity or relevant ecological traits of *A. trichopoda* in relation to woody plants living in the same communities. In this article, we provide the first observations of pit membrane structure for tracheary cells of *A. trichopoda* using scanning electron microscopy (SEM). We also report field measurements on the water-transport capacity of *A. trichopoda* stems compared to those of vessel-bearing trees and vesselless conifers and vesselless angiosperms (represented by Winteraceae) in New Caledonia. Our findings bear on discussions of the morphology

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and ecology of the first flowering plants and on the possible causes of their diversification.

Material and Methods

Study Areas and Species Examined

For anatomical and physiological studies, we sampled seven shrubs (each 1–2 m tall) of *Amborella trichopoda* from a large population (ca. 500 plants) growing from 600 to 800 m on Plateau de Dogny (22°35'S; 165°55'E) near Sarameá, New Caledonia, on July 27, 1999. Leaves on the measured branches were free of epiphylls, thereby ensuring that these leaves would be operating at maximum hydraulic capacity. The climate at this site is tropical, characterized by a wet season that extends from December to March, a short dry season that extends from April to May, a wet season that extends from June to August, and another dry season that extends from September to November (Jaffré 1980). Annual rainfall and temperature variation on Plateau de Dogny have not been measured; however, rainfall totals in Sarameá may approach 2500 mm a year (METEO-France 1994). The forest is often enshrouded in mist, and cloud moisture contributes a significant but unknown amount to overall moisture input (T. S. Feild, personal observation). Temperatures in New Caledonia are mild year-round; the mean monthly temperature in Nouméa (140 km away) is highest in February (26.2°C) and lowest in August (19.9°C; Jaffré 1980).

To put the hydraulic properties of *A. trichopoda* in context, we also measured the stem hydraulic properties of two conifers (*Agathis lanceolata* Warburg, [Araucariaceae] and *Retrophyllum comptonii* C. N. Page [Podocarpaceae]), two members of the vesselless angiosperm family Winteraceae (*Belliolum crasifolium* [Baillon] van Tieghem and *Zygogynum bailonii* van Tieghem), and one vessel-forming angiosperm (*Nothofagus condonandra* [Baill.] van Steenis [Nothofagaceae]) growing in an understory environment of a similar tropical montane rain forest on Mt. Dzumac (22°3'S; 166°26'E; 980 m), Province Sud, New Caledonia; measurements took place from late July to early August 1999. Mt. Dzumac was chosen because it was possible to establish a laboratory for measuring stem hydraulics, thus avoiding the transport of stems to Nouméa for measurement purposes. Climate data are limited for this site, with annual rainfall totaling 1816 mm in 1996 and 2742 mm in 1997; ca. one-half of this rainfall occurred from December through March (Enright and Goldblum 1998).

Xylem Anatomical Observations and Functional Conduit Length

Observations on *A. trichopoda* tracheary element dimensions (diameter and length) were made on stems preserved in 50% ethyl alcohol. Observations were made within 3 mo of preservation. Before sectioning, stem portions were rehydrated (in a stepwise manner) from 50% to 30% ethyl alcohol for 1 h and then to distilled water for 1 h. Tracheary element lengths were measured on macerated samples of stem xylem using standard procedures (O'Brien and McCully 1981). Average tracheary cell diameter and length were determined from 100 measurements on macerated cells from three stems of similar diameter (17–20 mm) from three plants. For observations of

pit membrane ultrastructure, transverse, tangential, and radial sections were cut from *A. trichopoda* stems. These sections were then mounted on aluminum stubs, air-dried, sputter-coated with platinum, and examined with a digital scanning electron microscope (LEO 495) at 5 kV. The length of the longest functional conduits was determined by forcing air at low pressure (ca. 0.01 MPa) through stem segments (Zimmermann and Jeje 1981). Fresh stems were fitted to a hand pump and slowly pressurized to 0.01 MPa. The stem segments were repeatedly shortened by 5 mm (using a razor blade) until the first bubbles appeared through the downstream end. This method is based on the assumptions that extracellular passageways permeable to air are not present within the stem and that gas cannot pass through wet pit membranes except at relatively high (typically greater than 1.5 MPa) pressures. In contrast, a conduit (tracheid or vessel) cut open at both ends can conduct gas at low (less than 0.01 MPa) pressure (Zimmermann and Jeje 1981).

Stem Hydraulic Measurements

We used a low-pressure steady state flowmeter (SSFM) method to measure stem hydraulic conductivity (K_H ; Tyree et al. 1993; Zwieniecki et al. 2000; Feild and Holbrook, in press). This method, which uses the drop in pressure across a tube of known conductance to measure the flow rate into the stem segment, was chosen over the traditional methods for measuring stem K_H (in which an analytical balance is used) because of the remoteness of the field sites. Briefly, an elevated reservoir (30–60 cm) was used to drive water through a 1.25-m-long piece of capillary tubing (PEEK, Upchurch Scientific, Seattle) and the stem segment, arranged in a series. The reservoir water was filtered through a 0.01- μ m filter before reaching the stem. Two pressure transducers (PX236, Omega Engineering, Stamford, Conn.) were used to measure the pressure at each end of the capillary tube, allowing us to determine both the pressure drop across the capillary tube and the delivery pressure to the stem segment. The capillary tube could be easily interchanged with PEEK capillary tubes of different inner diameters (and lengths) so that the conductance of the tube was approximately equal to that of the stem. The conductance of each tube was determined in the laboratory by comparing the pressure drop across the tube at a known temperature to the outflow rate measured by an analytic balance (± 0.01 mg). Five delivery pressures (0.001, 0.0025, 0.0045, 0.007, and 0.010 MPa) were used, and a linear calibration equation was determined for each capillary tube. Capillary tubes were calibrated before travel to New Caledonia and then immediately upon return to check for drift. After correcting for temperature, a 2% change was observed in the calibration value over a 2-mo period. Stems were measured under delivery pressures ranging from 0.003 to 0.006 MPa. In this range, mass flux is linearly related to applied pressure for stems (Sperry et al. 1988; Chiu and Ewers 1993). Data were recorded when steady state pressure readings were achieved, generally ca. 10 min after connecting the stem segment to the flowmeter. During all measurements, stem temperature was measured with a copper-constantan thermocouple (30 American wire gauge, Omega Engineering) so that the effect of temperature on viscosity of

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

As a result of logistical constraints, we were unable to provide a high-pressure flush to establish maximum stem hydraulic conductivity (Sperry et al. 1988). Instead, branches were sampled at times when losses of hydraulic conductivity attributable to embolisms were likely to be at a minimum (i.e., early morning [0800–0930 hours] on wet, cloudy days). For *A. trichopoda*, branches were cut in the field, triple-bagged in plastic, and transported immediately to a laboratory in Nouméa. On Mt. Dzumac, we were able to sample branches and immediately process them in a laboratory that had been set up in a tent. Stem segments that were longer than the longest functional conduit were cut from branches underwater (Sperry et al. 1988). Maximum conduit length for all species had been previously determined, as described above, from a minimum of three branches for each species. Both ends of the stem segment were recut with a fresh razor blade before attachment to the SSFM. Sapwood area specific 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 measured with a micrometer along the major and minor axes of the distal stem end, and the surface area contributed by the pith was subtracted (Sperry et al. 1988). Measured branches were <2 cm in diameter, and all of the wood was considered to be sapwood (i.e., functional for water transport). Leaf specific conductivity (K_L ; $\text{kg MPa}^{-1} \text{m}^{-1} \text{s}^{-1}$) was determined by dividing K_H by the total leaf area supported distal to the measured segment. For leaf area measurements, individual leaves of each branch were traced (flat) onto paper. These leaf silhouettes were subsequently measured with a leaf area meter (Li-1000, Li-Cor, Lincoln, Nebr.). Huber value was expressed as the ratio of sapwood area (in m^2) to leaf area (in m^2).

Results

The primary xylem of *Amborella trichopoda* consisted of a three to seven cell-thick band around the large pith of the stem (figs. 1, 2). Primary xylem conduits were thin walled (mean = 1.2 μm ; standard deviation [SD] = 0.2 μm) and varied considerably in diameter from 29 (protoxylem elements) to 69 μm (metaxylem cells). The mean diameter of primary xylem conduits was 48 μm (SD = 11.6 μm). Consistent with earlier reports, we also found numerous large (ca. 100- μm) cavities near the primary xylem band in *A. trichopoda* stems (figs. 1, 2; see Bailey 1957). Secondary tracheary cells occurred in relatively uniform files (in terms of lumen diameter and wall thickness) radiating from the center of the stem (fig. 1). The diameter of secondary xylem tracheary elements of *A. trichopoda* stems ranged from 20 to 49 μm (mean = 28 μm ; SD = 5.8 μm). Secondary walls were thicker than primary xylem walls by a measure of as much as 5 μm (mean = 4.2 μm ; SD = 0.2 μm). Lengths of secondary xylem tracheary elements ranged from 1049 to 2400 μm (mean = 1524 μm ; SD = 210 μm).

We observed substantial variation in the morphology, frequency, and position of tracheary element pitting in both the primary and secondary xylem. In the primary xylem, the latest-formed metaxylem elements had numerous variably sized and shaped pits (fig. 2A, 2B). Metaxylem pits were elongate slits and occurred in one or more rows along the length of the cells

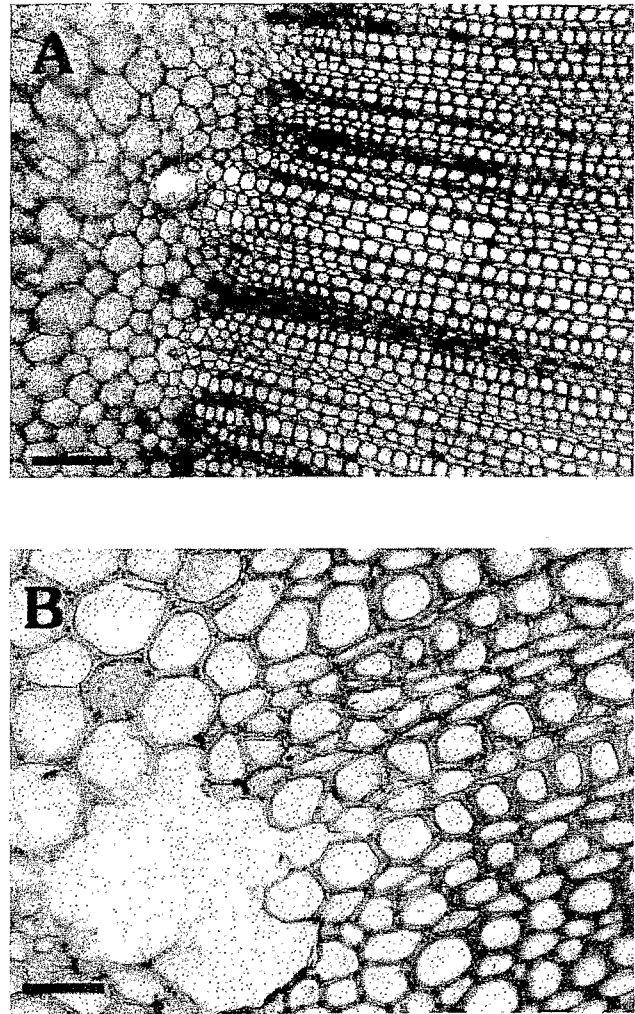


Fig. 1 Stem cross sections of *Amborella trichopoda* showing the organization of primary and secondary tracheary elements. A, Overall vascular pattern of *A. trichopoda*, showing relatively homogeneous cell diameters and thicker walls of the secondary tracheary cells as compared to the thinner-walled primary xylem strands located near the pith. B, Detail of the primary to secondary xylem transition and structure of the relatively large lacuna near the pith. Scale bar = 150 μm in A and 30 μm in B.

(fig. 2B). In the secondary xylem, pit shape ranged from elliptical to scalariform (fig. 2C), and these pits were also arranged in one or more rows along the radial walls. It was not uncommon to find both elliptical and scalariform pits clustered at the ends of secondary xylem tracheary elements (fig. 2D). Radial sections revealed that scalariform pitting occurred sporadically in *A. trichopoda* stems; there was no tendency for scalariform pitting to be expressed in younger versus older xylem.

SEM observations of *A. trichopoda* xylem structure were generally consistent with a tracheid-based conducting system. Primary wall material between the gyres of the thickening bands in the primary xylem did not contain any visible perforations. Except for what appeared to be damage during sample preparation (i.e., obvious tears or cuts), the pit membranes

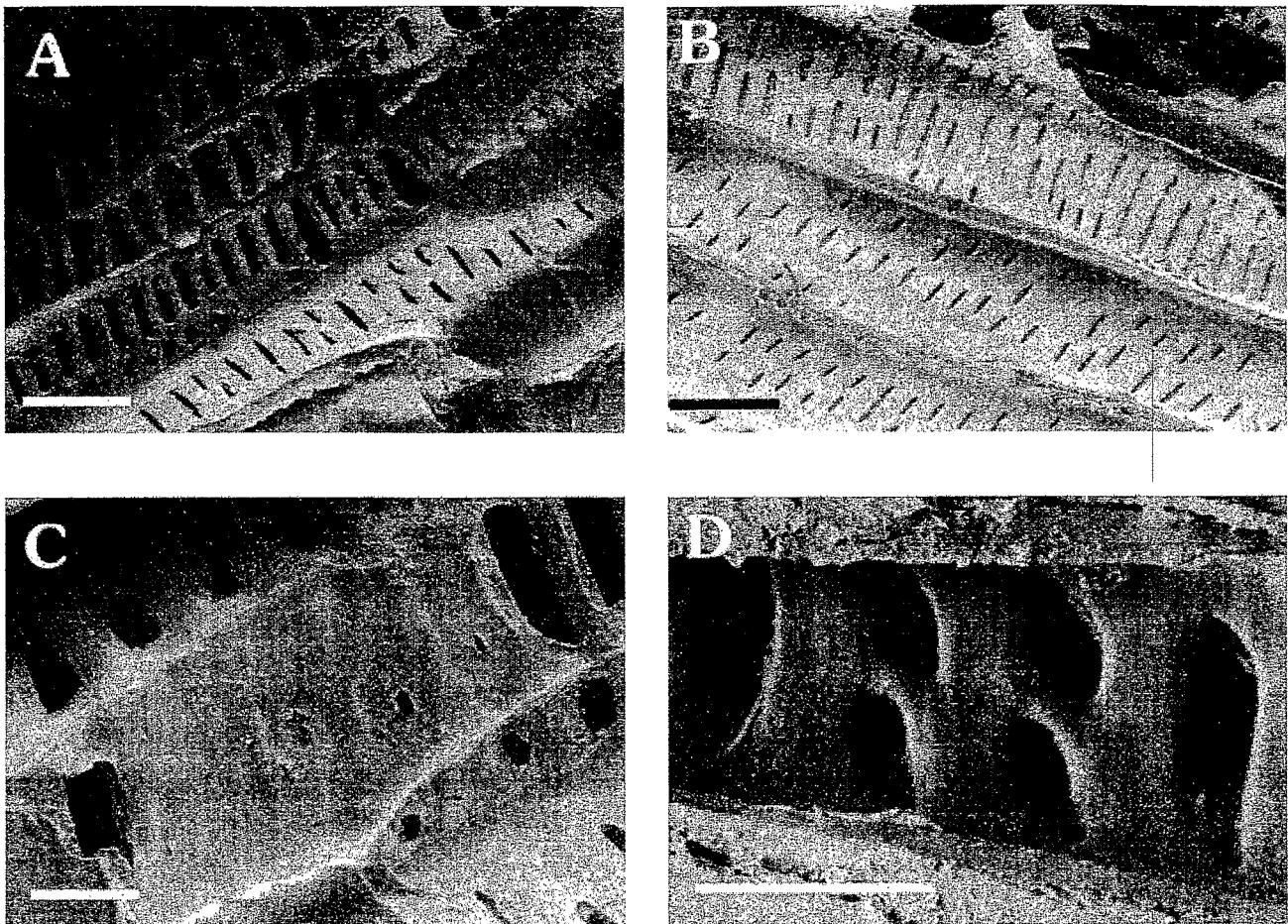


Fig. 2 SEM photographs of the protoxylem to metaxylem transition and tracheary element pitting morphology of primary and secondary xylem of *Amborella trichopoda* stems from radial sections. A, Detail of the transition from protoxylem elements (helical rings) to metaxylem cells in the primary xylem. The primary wall material between the helical bands of protoxylem cells was torn away during sectioning. Note the abundance of pits on metaxylem elements located in the center. B, Latest-formed metaxylem tracheary cells with numerous slitlike pits containing intact pit membranes. C, Detail of intact primary wall material (pit membrane) of metaxylem tracheary cells; irregularly shaped holes are likely to be tears in the pit membrane resulting from sectioning. D, Co-occurrence of elliptical and scalariform pits on the end wall portions of secondary tracheary cells. Scale bar = 15 μm in A and B and 10 μm in C and D.

of all primary and most secondary xylem elements were smooth and intact (fig. 3A–3C). A few elliptical and scalariform pits exhibited visible pores, although none of these exceeded 0.2 mm in diameter (fig. 3A). In a few instances, groups of bordered pits appeared to lack pit membranes altogether (fig. 3D). Although the potential for artifacts arising from sample preparation can never be fully eliminated, we believe that the disappearance of pit membranes (a disappearance not accompanied by any visible remnant) is unlikely to have been caused by either air-drying or sectioning. Consistent with the observation that intertracheid membranes were largely intact, low-pressure air (0.01 MPa) bubbled through the xylem of *A. trichopoda* when stem sections were shortened to a length that was less than the summed average length of two secondary xylem elements joined end to end (i.e., ca. 5 mm). This indicates that functional conduits in *A. trichopoda* are, at most, composed of two xylem elements. In addition, because open pits (i.e., bordered pits that lack a pit membrane) were not

frequently observed, the typical conduit is likely to consist of a single tracheary cell.

We observed threefold variation in stem hydraulic properties across the conifers, vesselless angiosperms, and vessel-bearing angiosperms sampled from the forest understory (table 1). The capacity of the xylem system to conduct water (K_s , sapwood normalized hydraulic conductivity) varied from 0.27 $\text{kg MPa}^{-1} \text{m}^{-1} \text{s}^{-1}$ in *Agathis lanceolata* to 0.86 $\text{kg MPa}^{-1} \text{m}^{-1} \text{s}^{-1}$ in *Nothofagus codonandra*. Within this range, the K_s of *A. trichopoda* stems was ca. 70% of the maximum K_s measured in *N. codonandra* (table 1). The hydraulic capacity of *A. trichopoda* wood was greater than that of both species of conifers and of the Winteraceae that we sampled (table 1). The ability of *A. trichopoda* stems to supply a supported leaf area with water (leaf-specific hydraulic conductivity, K_L) was ca. 60% of the K_L measured in vessel-forming *N. codonandra* (table 1). Leaf specific hydraulic conductivity is determined by the xylem hydraulic capacity (K_s) as well as by the relative in-

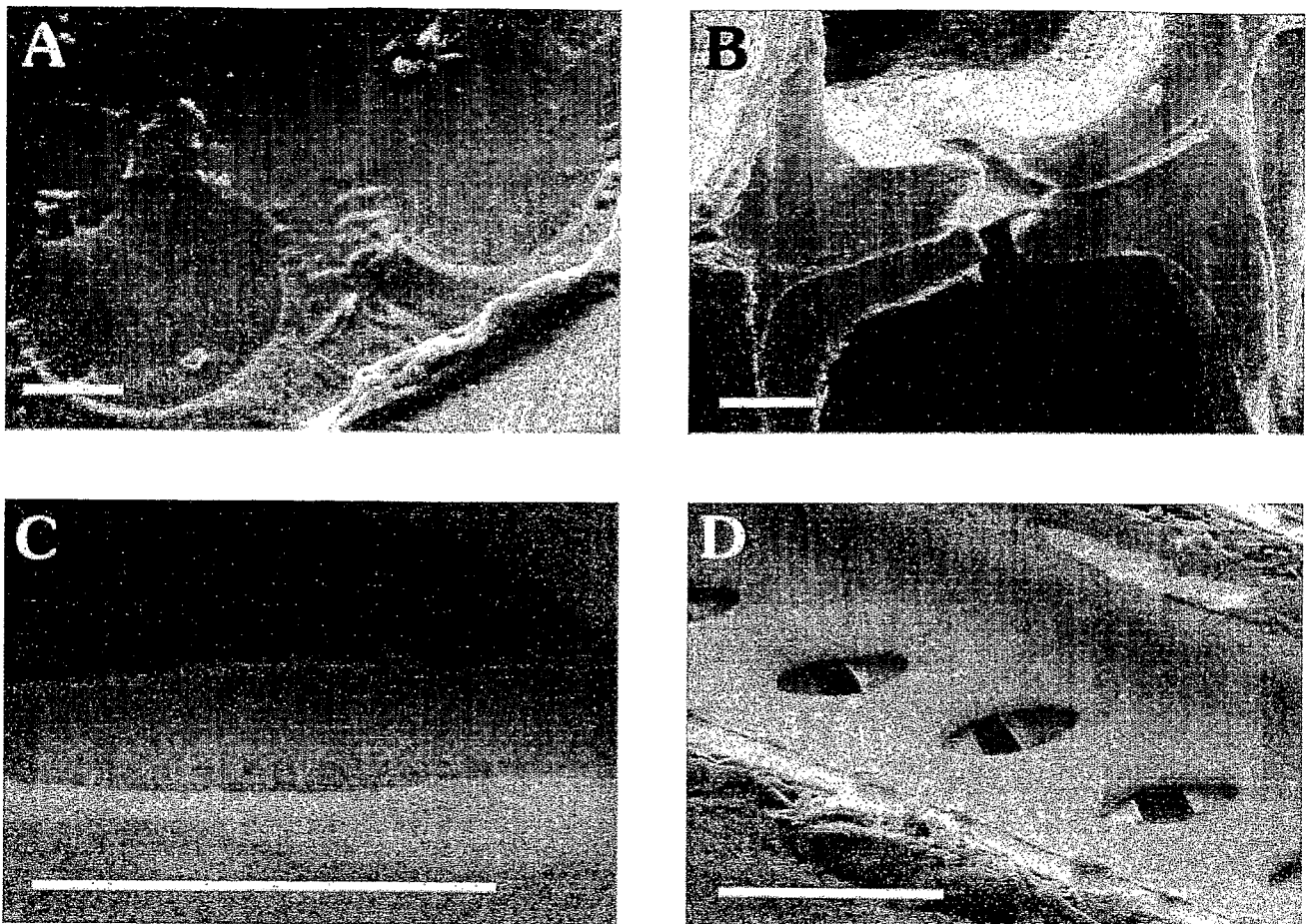


Fig. 3 SEM photographs of variation in pit membrane intactness of *Amborella trichopoda* stems observed from radial and cross-sectional views. A, Presence of intact pit membranes on elliptical pits located on secondary xylem tracheary elements. B, Cross section of an intact intertracheary elliptical pit of the secondary xylem. C, Detail of small porosities in the pit membranes of secondary tracheary cells. D, Pit membranes completely lacking within intertracheary elliptical pits in the secondary xylem. Scale bars = 8 μm in A and B, 2.5 μm in C, and 16 μm in D.

vestment of conducting xylem area (sapwood) to leaf area (i.e., Huber value; Zimmermann 1983; Tyree and Ewers 1991, 1996). We observed a relatively large variation in the ratio of sapwood to leaf area allocation among species; this ratio ranged from 2.0 in *A. lanceolata* to 3.3 in *Nothofagus codonandra*. The Huber values of *A. trichopoda* were well within the range of all plants measured (table 1).

Discussion

The presence or absence of xylem vessels is, in general, rather easily determined, because the characteristic features of vessels (absence of pit membranes, short length and wide diameter of vessel elements, interconnection of a large number of vessel elements, differentiated perforation plates, etc.) tend to be tightly correlated (Bailey and Tupper 1918; Frost 1930; Herendeen et al. 1999). Likewise, in most cases the functional consequences of these structural differences are quite obvious. In general, plants with vessels have greater water-transport efficiency because vessels are multicelled water-conducting

compartments, with the axial cell walls partially or wholly removed during development (Frost 1930; Carlquist 1975; Zimmermann and Jeje 1981; Zimmermann 1983). In contrast, tracheids are smaller in diameter and are single cells bounded by intact pit membranes (Zimmermann 1983). Pit membranes, although porous, can contribute a substantial resistance to water flow (Carlquist 1975; Zimmermann 1983; Bond 1989; Tyree and Ewers 1991, 1996). Consequently, vessel-forming plants have greater hydraulic conductance, because water passes through larger-diameter tubes and because fewer pit membranes are traversed during transport (Zimmermann 1983; Tyree and Ewers 1991, 1996). In addition, the evolutionary appearance of xylem vessels is accompanied by the development of vascular elements specialized for support (i.e., fibers) and an increased abundance of xylem parenchyma. The greater hydraulic efficiency of xylem vessels may allow for improvements in nonhydraulic aspects of stem function (e.g., support, storage) without a loss in overall conducting capacity.

Our observations of *Amborella trichopoda*, combined with studies of other species representing early diverging angio-

Table 1

Stem Hydraulic Properties of *Amborella trichopoda* Compared to Tropical Conifers, Vesselless Angiosperms, and a Vessel-Forming Angiosperm Growing in the Understory of New Caledonian Tropical Montane Cloud Forests

Species	Family	$K_L \times 10^{-4}$	K_S	HV $\times 10^{-4}$
Conifers:				
<i>Agathis lanceolata</i> ($n = 4$)	Araucariaceae	0.46 \pm 0.06	0.27 \pm 0.02	2.0 \pm 0.3
<i>Retrophyllum comptonii</i> ($n = 4$)	Podocarpaceae	0.77 \pm 0.02	0.29 \pm 0.06	2.6 \pm 0.4
Vesselless angiosperms:				
<i>Amborella trichopoda</i> ($n = 10$)	Amborellaceae	1.6 \pm 0.5	0.62 \pm 0.04	2.2 \pm 1.7
<i>Belliolum crassifolium</i> ($n = 10$)	Winteraceae	0.69 \pm 0.08	0.31 \pm 0.10	2.9 \pm 0.8
<i>Zygogynum baillonii</i> ($n = 10$)	Winteraceae	1.8 \pm 1.2	0.54 \pm 0.21	3.2 \pm 1.4
Vessel-forming angiosperms:				
<i>Nothofagus codonandra</i> ($n = 6$)	Nothofagaceae	2.5 \pm 1.4	0.86 \pm 0.20	3.3 \pm 2.1

Note. Averages of leaf area-specific hydraulic conductivity (K_L ; kg MPa⁻¹ m⁻¹ s⁻¹ [\pm SD]), stem conductance per unit sapwood area (K_S ; kg MPa⁻¹ m⁻¹ s⁻¹ [\pm SD]), and Huber value (HV; ratio of sapwood area [m²] to leaf area [m²] [SD]), with sample sizes (n) indicated for each species.

sperm lineages (Carlquist 1987, 1990, 1992, 1996; Takahashi 1988; Schneider and Carlquist 1995a, 1995b), indicate that the set of characteristics that distinguishes typical tracheids from typical vessels may not be so tightly linked in these plants. Various combinations of xylem features that in effect delimit a spectrum of structural and functional conditions that are intermediate between tracheids, as exemplified by conifers and the vessel elements typical of most angiosperms, have been documented. For example, many water lilies (Schneider and Carlquist 1995a, 1995b) and the monocot *Acorus* (Carlquist and Schneider 1997) exhibit large pores in their pit membranes, whereas in the case of *Illicium* and most Chloranthaceae, remnants of pit membranes are present in scalariform perforation plates (Carlquist 1987, 1990, 1992). The functional consequences (e.g., for embolisms and hydraulic flux) of these structural differences have not yet been studied but may result in a range of intermediate hydraulic capacities among basal lines of angiosperms. Under these circumstances, rather than trying to categorize the first angiosperms as either having or lacking vessels (e.g., Bailey and Tupper 1918; Young 1981; Carlquist 1983, 1987; Donoghue 1989; Baas and Wheeler 1996), it may be more productive to simply describe the morphological characteristics and functional attributes observed in representatives of the early angiosperm lineages. Such a decomposition of states may allow the best inference of the structure and function of tracheary elements in ancestral flowering plants.

From this perspective, most characteristics of *A. trichopoda* match a stereotyped tracheid-bearing system. In contrast to secondary xylem in a typical vessel-bearing plant, there is little differentiation in cell diameter or wall thickness of tracheary elements. Likewise, perforation plates are not clearly demarcated, although secondary tracheary cells do show a tendency toward differentiation of end wall pitting. The majority of pit membranes are structurally and functionally intact. The porosities observed in a few pit membranes are similar in size to those reported in some Winteraceae (Carlquist 1983), but they are more than an order of magnitude smaller than those found in Nymphaeales and *Acorus* (Schneider and Carlquist 1995a, 1995b; Carlquist and Schneider 1997). Although bordered pits lacking pit membranes were occasionally observed, measurements of functional conduit length indicate that at most, two

tracheary cells are directly connected. Therefore, with respect to the spread of embolism, the *A. trichopoda* system may function more like a system that contains only tracheids. However, our observation that pit membranes are sometimes absent indicates that the capacity to hydrolyze pit membranes is present in secondary xylem of *A. trichopoda*.

Amborella trichopoda appears to be intermediate in terms of water transport. We have shown that the maximum xylem hydraulic capacity of *A. trichopoda* exceeds that of all other tracheid-containing plants growing in the understory of the tropical premontane rain forests sampled. In fact, *A. trichopoda* falls in the upper range of capacities reported previously for tropical and temperate conifers and Winteraceae (table 1; Tyree and Ewers 1991, 1996; Becker et al. 1999; Brodribb and Hill 1999; Feild and Holbrook, in press). However, most vessel-forming plants have stem conductivities that are at least two times greater than that of *A. trichopoda* (Tyree and Ewers 1991, 1996). To understand the significance of these findings, it is important to appreciate the environmental context. *Amborella trichopoda* is a shrub that is adapted to the wet understory of rain forests in New Caledonia (Bailey and Swamy 1948; Jérémie 1980). The absence of growth rings and the high abundance of epiphylls on older leaves indicate that *A. trichopoda* grows in uniformly wet conditions throughout the year (T. S. Feild, personal observations). In addition, *A. trichopoda* seedlings are most abundant in the understory and not in patches of rain forest that have been disturbed by tree falls, cyclone activity, and/or fire (T. S. Feild, personal observation).

What do these observations indicate about the ecological circumstances surrounding the origin of angiosperms and of vessels? The phylogenetic position and current ecology of *A. trichopoda* seem inconsistent with hypotheses that the first angiosperms were gap-colonizing "weed trees" or rhizomatous, herbaceous plants living in seasonally dry riparian or disturbed habitats (e.g., Stebbins 1974; Hickey and Doyle 1977; Doyle 1978; Taylor and Hickey 1992; Doyle and Donoghue 1993). We note, however, that the morphology and ecology of *A. trichopoda* may not have been retained from the ancestor of angiosperms; instead, they might have originated more recently within the *Amborella* lineage. A long period of time (probably more than 140 million yr) separates the origin of the *Amborella* branch from *A. trichopoda* as we know it

today. Furthermore, the rain forests in which *A. trichopoda* now grows in New Caledonia are not likely to have been climatically and geologically stable refugia for the *Amborella* line since its origin. Indeed, New Caledonia may have been entirely submerged (or nearly so) during the Paleocene to Eocene, and likewise, the emplacement of ultramafic substrates during the Eocene dramatically altered the landscape of New Caledonia (Brothers and Lillie 1988; Jaffré 1995). These observations imply that many of the so-called primitive angiosperms (e.g., Winteraceae, *Trimenia*, etc.) now present in New Caledonia must have arrived there more recently (Pole 1994). The same may be true of presumed relicts elsewhere, such as *Austrobaileya* (Endress 1983). In general, we caution against interpreting *A. trichopoda* as a model of the first angiosperm.

Finally, we note that the inference of ancestral conditions in angiosperms must take into account the lineage that includes all angiosperms except *Amborella*, within which the water lily lineage and the *Austrobaileya*-*Trimeniaceae*-*Illiciales* lineage appear to have diverged rather early. The water lily lineage may represent an early shift into freshwater aquatic habitats accompanied by loss of secondary growth (and possibly loss of typical vessels). These plants may now be specialized to the point that their vascular and ecological traits have little bearing on conditions in the first angiosperms. The *Austrobaileya*-*Trimeni-*

aceae-*Illiciales* clade is noteworthy for the widespread occurrence of woody vines (Smith 1947; Bailey and Swamy 1949; Carlquist 1984). This occurrence may reflect a primary radiation as lianas, perhaps accompanied by the acquisition (or stabilization) and further elaboration of a suite of vessel characters associated with the transport of water through slender stems. Vines generally have a high intrinsic capacity for water transport because of the presence of large-diameter vessels and long vessels (Ewers et al. 1991; Gartner 1991). Evolution of the climbing habit in this lineage may have been foreshadowed to some extent by *A. trichopoda*, which has a scandent habit (as in *Austrobaileya*), marked by the production of leaning branches that come close to touching the ground, and reflexed petioles (Jérémie 1980).

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Literature Cited

- Baas P, EA Wheeler 1996 Parallelism and reversibility in xylem evolution—a review. *IAWA Bull* 17:351–364.
- Bailey IW 1957 Additional notes on the vesselless dicotyledon, *Amborella trichopoda*. *J Arnold Arbor Harv Univ* 35:374–378.
- Bailey IW, BGL Swamy 1948 *Amborella trichopoda* Baill., a new morphological type of vesselless dicotyledon. *J Arnold Arbor Harv Univ* 29:245–254.
- 1949 The morphology and relationships of *Austrobaileya*. *J Arnold Arbor Harv Univ* 30:11–26.
- Bailey IW, WW Tupper 1918 Size variation in tracheary cells. I. A comparison between the secondary xylems of vascular cryptogams, gymnosperms and angiosperms. *Proc Am Acad Arts* 54:149–204.
- Becker PM, MT Tyree, M Tsuda 1999 Hydraulic conductances of angiosperms versus conifers: similar transport sufficiency at the whole plant level. *Tree Physiol* 19:445–452.
- Bond WJ 1989 The tortoise and the hare: ecology of angiosperm dominance and gymnosperm persistence. *Biol J Linn Soc* 36: 227–249.
- Brodribb T, RS Hill 1999 The importance of xylem constraints in the distribution of conifer species. *New Phytol* 143:365–372.
- Brothers RN, AR Lillie 1988 Regional geology of New Caledonia. Pages 325–374 in AEM Nairin, GF Stehli, S Uyeda, eds. *The ocean basin and margins*. Vol 7B. Plenum, New York.
- Carlquist S 1975 Ecological strategies of xylem evolution. University of California Press, Berkeley. 259 pp.
- 1983 Wood anatomy of *Bubbia* (Winteraceae); with comments on origin of vessels in dicotyledons. *Am J Bot* 70:578–590.
- 1984 Wood anatomy of *Trimeniaceae*. *Plant Syst Evol* 144: 103–118.
- 1987 Presence of vessels in wood of *Sarcandra* (Chloranthaceae); comments on the vessel origin in angiosperms. *Am J Bot* 74: 1765–1771.
- 1990 Wood anatomy of *Ascarina* (Chloranthaceae) and the tracheid-vessel element transition. *Aliso* 12:667–684.
- 1992 Pit membrane remnants in perforation plates of primitive dicotyledons and their significance. *Am J Bot* 79:660–672.
- Carlquist S, EL Schneider 1997 Origins and nature of vessels in monocotyledons. I. *Acorus*. *Int J Plant Sci* 158:51–56.
- Chiu ST, FW Ewers 1993 The effect of segment length on conductance measurements in *Lonicera fragrantissima*. *J Exp Bot* 44: 175–181.
- Crane PR, EM Friis, KR Pedersen 1995 The origin and early diversification of angiosperms. *Nature* 374:27–34.
- Crane PR, S Lidgard 1989 Angiosperm diversification and paleolatitudinal gradients in Cretaceous floristic diversity. *Science* 246: 675–678.
- Cronquist A 1988 The evolution and classification of flowering plants. New York Botanical Garden, Bronx. 555 pp.
- Donoghue MJ 1989 Phylogenies and the analysis of evolutionary sequences, with examples from seed plants. *Evolution* 43:1137–1156.
- Donoghue MJ, JA Doyle 1989 Phylogenetic analysis of angiosperms and the relationships of Hamamelidae. Pages 17–45 in PR Crane, S Blackmore, eds. *Evolution, systematics, and fossil history of the Hamamelidae*. Vol 1. Clarendon, Oxford.
- Doyle JA 1978 Origin of angiosperms. *Annu Rev Ecol Syst* 9: 365–392.
- 1998 Phylogeny of vascular plants. *Annu Rev Ecol Syst* 29: 567–599.
- Doyle JA, MJ Donoghue 1986 Seed plant phylogeny and the origin of angiosperms: an experimental cladistic approach. *Bot Rev* 52: 321–431.
- 1993 Phylogenies and angiosperm diversification. *Paleobiology* 19:141–167.
- Endress PK 1983 Dispersal and distribution in some small archaic relict angiosperm families (Austrobaileyaceae, Eupomatiaceae, Himantandraceae, Idiospermoideae-Calycanthaceae). *Sonderbd Natwiss Ver Hambg* 7:201–217.
- Enright NJ, D Goldblum 1998 Stand structure of the emergent conifer *Agathis ovata* in forest and maquis, Province Sud, New Caledonia. *J Biogeogr* 25:641–648.
- Ewers FW, JB Fisher, K Fichtner 1991 Water flux and xylem structure

- in vines. Pages 127–160 in FE Putz, HA Mooney, eds. The biology of vines. Cambridge University Press, Cambridge.
- Feild TS, NM Holbrook In press Xylem sapflow and stem hydraulics of the vesselless angiosperm *Drimys granadensis* (Winteraceae) in a Costa Rican elfin forest. *Plant Cell Environ*.
- Feild TS, MA Zwieniecki, MJ Donoghue, NM Holbrook 1998 Stomatal plugs of *Drimys winteri* (Winteraceae) protect leaves from mist but not drought. *Proc Natl Acad Sci USA* 95:14256–14259.
- Frost FH 1930 Specialization in secondary xylem of angiosperms. I. Origin of vessel. *Bot Gaz* 89:67–92.
- Gartner BL 1991 Stem hydraulic properties of vines vs. shrubs of western poison oak, *Toxicodendron diversilobum*. *Oecologia* 87: 180–189.
- Herendeen PS, EA Wheeler, P Baas 1999 Angiosperm wood evolution and the potential contribution of paleontological data. *Bot Rev* 65: 279–300.
- Hickey LJ, JA Doyle 1977 Early Cretaceous fossil evidence for angiosperm evolution. *Bot Rev* 43:3–104.
- Jaffré T 1980 Etude écologique du peuplement végétal des sols dérivés de roches ultrabasiques en Nouvelle Calédonie. *Travaux de Documents* 124. ORSTOM, Paris. 273 pp.
- 1995 Distribution and ecology of the conifers of New Caledonia. Pages 171–196 in NJ Enright, RS Hill, eds. Ecology of the southern conifers. Smithsonian Institution Press, Washington, D.C.
- Jérémie J 1980 Amborellaceae. Flore Nouvelle Calédonie *Dépendances* 10:157–160.
- Mathews S, MJ Donoghue 1999 The root of angiosperm phylogeny inferred from duplicate phytochrome genes. *Science* 286:947–950.
- Mercalfé CR 1987 Anatomy of the dicotyledons. Vol 3. Magnoliales, Illiciales and Laurales. 2d ed. Clarendon, Oxford.
- METEO-France 1994 Climat de Nouvelle Calédonie. METEO-France en Nouvelle Calédonie, Nouméa.
- O'Brien TP, ME McCully 1981 The study of plant structure: principles and selected methods. *Terrestrial plants*, Melbourne. 357 pp.
- Parkinson CL, KL Adams, JD Palmer 1999 Multigene analyses identify the three lineages of extant flowering plants. *Curr Biol* 9: 1485–1488.
- Pole M 1994 The New Zealand flora—entirely long-distance dispersal? *J Biogeogr* 21:625–635.
- Qiu Y-L, J Lee, F Bernasconi-Quadroni, DE Soltis, PS Soltis, M Zanis, EA Zimmer, Z Chen, M Savolainen, MW Chase 1999 The earliest angiosperms: evidence from mitochondrial, plastid, and nuclear genomes. *Nature* 402:404–407.
- Sanderson MJ, MJ Donoghue 1994 Shifts in diversification rate with the origin of angiosperms. *Science* 264:1590–1593.
- Schneider EL, S Carlquist 1995a Vessels in the roots of *Barclaya roundifolia* (Nymphaeaceae). *Am J Bot* 82:1343–1349.
- 1995b Vessel origins in Nymphaeaceae: *Euryale* and *Victoria*. *Bot J Linn Soc* 119:195–193.
- Smith AC 1947 The families Illiciaceae and Schisandraceae. *Sargentia* 7:1–224.
- Soltis PS, DE Soltis, MW Chase 1999 Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology. *Nature* 402: 402–404.
- Sperry JS, JR Donnelly, MT Tyree 1988 A method for measuring hydraulic conductivity and embolism in xylem. *Plant Cell Environ* 11:35–40.
- Stebbins GL 1974 Flowering plants: evolution above the species level. Harvard University Press, Cambridge, Mass. 399 pp.
- Takahashi A 1988 Morphology and ontogeny of stem xylem elements in *Sarcandra glabra* (Thunb.) Nakai (Chloranthaceae): additional evidence for the occurrence of vessels. *Bot Mag* 101:387–395.
- Takhtajan AL 1969 Flowering plants: origin and dispersal. Smithsonian Institution Press, Washington, D.C. 310 pp.
- Taylor DW, LJ Hickey 1992 Phylogenetic evidence for the herbaceous origin of angiosperms. *Plant Syst Evol* 180:137–156.
- Thorne RF 1974 A phylogenetic classification of the Annoniflorae. *Aliso* 8:147–209.
- Tyree MT, FW Ewers 1991 The hydraulic architecture of trees and other woody plants. *New Phytol* 119:345–360.
- 1996 Hydraulic architecture of wood tropical plants. Pages 217–243 in SS Mulkey, RL Chazdon, AP Smith, eds. Tropical forest ecophysiology. Chapman & Hall, New York.
- Tyree MT, B Sinclair, P Lu, A Granier 1993 Whole shoot hydraulic resistance in *Quercus* species measured with a new high-pressure flowmeter. *Ann Sci For* 50:417–423.
- Wing SL, LD Boucher 1998 Ecological aspects of the Cretaceous flowering plant radiation. *Annu Rev Earth Planet Sci* 26:379–421.
- Young DA 1981 Are the angiosperms primitively vesselless? *Syst Bot* 6:313–330.
- Zimmermann MH 1983 Xylem structure and the ascent of sap. Springer, Berlin. 143 pp.
- Zimmermann MH, AA Jeje 1981 Vessel-length distribution in stems of some American woody plants. *Can J Bot* 59:1882–1892.
- Zwieniecki MA, L Hutyra, MV Thompson, NM Holbrook 2000 Dynamic changes in petiole conductivity in red maple (*Acer rubra* L.), tulip tree (*Liriodendron tulipifera* L.), and northern fox grape (*Vitis labrusca* L.). *Plant Cell Environ* 23:407–414.