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Characterisation of xylem conduits and their possible role in limiting the vase life of cut *Acacia holosericea* (Mimosaceae) foliage stems

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Abstract. Early desiccation limits the vase life of *Acacia* cut flowers and foliage and may be attributable to poor hydraulic conductivity (K_h) of the cut stems. *Acacia holosericea* A.Cunn. ex G.Don has been adopted as the test species to investigate the postharvest water relations of the genus *Acacia*. To understand potential constraints on K_h, xylem conduits in cut *A. holosericea* stems were anatomically characterised by light and scanning and transmission electron microscopy. Vessels with simple perforation plates and tracheids were the principal water conducting cells. Bordered vestured intervessel pits were present in xylem vessel elements. The majority of conduits (89%) were short at 1–5 cm long. Only 2% were 15–16 cm in length. Mean xylem conduit diameter was 77 ± 0.9 μm and the diameter profile showed a normal distribution, with 29% of diameters in the range of 70–80 μm. Simple perforation plates can offer relatively low resistance to water flow. On the other hand, bordered vestured pits and short xylem conduits can confer comparatively high resistance to water flow. Overall, the presence of bordered vestured pits, together with a high proportion of short xylem conduits and high stomatal densities (232 ± 2 mm^{-2}) on unifacial phyllodes, could contribute to early dehydration of *A. holosericea* cut foliage stems standing in vase water. Further research will relate these anatomical features with changes in K_h and transpiration of cut foliage stems.

Additional keywords: hydraulic resistance, longevity, stomatal distribution, velvet leaf wattle, water uptake.

Introduction

Acacia (family Mimosaceae) is the largest genus of flowering plants in Australia, containing over 950 species (Cowan 2001). Several select *Acacia* species are grown as cut flower and foliage crops in Europe (Sedgley and Parletta 1993; Horlock *et al*. 2000). The limited commercial success of this genus in foreign markets and the vast speciation in Australia suggest greater potential for *Acacia* as cut flower and foliage products on local and international markets (Ratnayake and Joyce 2010). However, an inherently short postharvest life is characteristic of many attractive *Acacia* species. Their cut flowers and foliage desiccate rapidly, rendering cut stems unattractive within just 2–6 days (Williamson and Milburn 1995; Jones *et al*. 1998). The short vase life of *Acacia* is associated with a rapid decline in postharvest water uptake by the cut stems (Williamson and Milburn 1995; Jones *et al*. 1998; Ratnayake *et al*. 2011).

Insufficient water uptake by cut flowers and foliage is typically caused by decreased hydraulic conductivity (K_h) in the xylem (van Doorn 1997). K_h is influenced by the properties of the conducted fluid as well as by the anatomy of the conducting xylem (Zimmermann 1983; van Ieperen *et al*. 2000). The xylem is structurally complex and comprised of tracheary elements (i.e. vessel elements and tracheids), xylem fibres and xylem parenchyma cells (Evert 2006). Vessel elements and tracheids are elongated cells with highly lignified secondary walls and are non-living at maturity. Vessel elements have perforation plates on their end walls that allow interconnected elements to form a xylem vessel. These perforation plates may have single or multiple perforations (Zimmermann 1983). The latter can be scalariform (i.e. the perforation plate is elongated with transverse bars arranged in a parallel series), reticulate or foraminate (i.e. circular holes). Tracheids do not have perforated end walls but, like xylem vessels, have pits on their common walls (Evert 2006).

When water flows within vessels or between vessels and tracheids, it crosses pit membranes, which are modified regions of the primary cell walls (Milburn 1979; Zimmermann

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1983). A pit comprises the pit chamber (i.e. empty spaces connecting the lumen of each cell) and the pit membrane (i.e. the partition between the adjacent pit cavities; Zimmermann 1983; Sano and Jansen 2006). The pit chamber is due to an absence of secondary wall deposition during cell wall thickening. Pit membranes contain microchannels that ramify among tightly interwoven cellulose microfibrils in a matrix of hemicellulose and pectin polysaccharides (Brett and Waldron 1996). Differences in pit characteristics confer differences in conductive efficiency that can limit the movement of the pit membrane in the pit cavity, mechanical strength, and resistance to cavitation (Choat *et al*. 2008).

The plant hydraulic flow path is typically composed of innumerable xylem conduits (i.e. vessels and tracheids). At any given height, there may be thousands of conduits in a single transverse section. Conduits within a single longitudinal section vary in length, with length–frequency profiles generally skewed towards shorter conduits (Zimmermann and Jeje 1981). Examples of long vessel lengths include 1.3 m for *Ilex verticillata* L. (A.Gray) (Zimmermann and Jeje 1981) and 3.9 m for *Eucalyptus obliqua* L.Her. (Skene and Balodis 1968). In *Acacia subulata* Bonpl., the maximum length was 12–13 cm (Ritman and Milburn 1988). Such lengths are still rather short compared with ring porous trees, such as *Quercus rubra* L. and *Fraxinus americana* L., which can have very long vessels of ~10 m (Zimmermann and Jeje 1981). Vessel length and diameter have important implications for K_h (Comstock and Sperry 2000). The longest vessels are typically the widest and are therefore able to transport greater volumes of water in the plant according to the Hagen–Poiseuille equation: $R = 128\,\eta L/\pi D^4$; where, R is the resistance to flow, η is the dynamic viscosity of the fluid (viscosity of water is 1.01×10^{-3} N s m^{-2} at 20°C; Chang 2000), L is the length and D is the diameter of the xylem conduit. Thus, the resistance to flow is inversely proportional to the fourth power of the xylem conduit diameter. Measurements of vessel lengths have been reported using a range of methods, including compressed air (Zimmermann and Jeje 1981), paint infusion (Zimmermann and Jeje 1981), India ink (Milburn 1979) and red latex particle suspension (Nijsse *et al*. 2001).

Postharvest research has been carried out on members of the genus *Acacia* with a view to improving vase life. In this context, vase solution additives such as the antioxidant citric acid significantly improved the vase life of *Acacia baileyana* F. Muell. (Williamson *et al*. 2002). The generally positive effects of a range of peroxidase and/or phenoloxidase enzyme inhibitors on the water relations of cut *Acacia holosericea* A. Cunn. ex G. Don (velvet leaf wattle) foliage stems suggested the possible involvement of these enzymes in wound-induced xylem occlusion (Çelikel *et al*. 2011). Moreover, postharvest pulse and vase solution treatments with Cu^{2+} resulted in marked extensions of the vase life of cut *A. holosericea* (Ratnayake *et al*. 2011). There is promise of effective postharvest treatments for *Acacia*, but xylem anatomical features that have potential implications for K_h and the vase life of cut stems have not been elucidated. Scanning electron micrographs of vessel wall structures were published by Scurfield *et al*. (1970) for some 22 plant genera studied. Vestures in *Acacia melanoxylon* R.Br. were described as 'prominently bead-like'. However, no transmission electron micrographs were provided for this sole *Acacia* species.

A. holosericea is a native Australian *Acacia* adapted to the dry tropics (Cossalter 1986). Cut foliage stems of this attractive species have a typically short vase life of 4–7 days (Ahmad *et al*. 2011). However, its silvery phyllodes make it a promising novel cut foliage crop. It has therefore been adopted as a 'model' *Acacia* species to investigate the postharvest water relations of the genus *Acacia* in detail (Ratnayake and Joyce 2010; Ahmad *et al*. 2011; Ratnayake *et al*. 2011). We examined xylem conduit structure and also the stomatal distribution on *A. holosericea* phyllodes in order to discern possible anatomical limitations to water uptake and transpirational water loss, which, in turn, might be related to short vase life of the cut foliage stems.

Materials and methods

Plant material

Terminal *Acacia holosericea* A. Cunn ex G. Don foliage stems with fully expanded mature phyllodes were harvested from 2- to 3-year-old plants at The University of Queensland's Gatton Campus nursery (152°20′E, 27°33′S) from September 2007 to October 2009. Plants were raised from seeds initially sown in steam pasteurised propagation media containing perlite, vermiculite and sphagnum peat. Seedlings were maintained in a propagation house at >85% relative humidity and ~25°C with an overhead mist system for 1 month and then transferred to 140-mm diameter (1.4-L) pots filled with steam pasteurised composted pine bark mix. Basal fertiliser (quantity per pot) comprised nitrogen (1.6 g), phosphorous (0.3 g), potassium (0.5 g), magnesium (0.19 g), calcium (0.26 g), sulfur (0.29 g), iron (0.48 g) and trace amounts ($\leq$ 0.0008 g) of boron, copper, manganese, molybdenum and zinc. Pots were maintained under full sun and irrigated with town water by overhead sprinkler irrigation twice a day for 5–6 min each time. Cut stems were harvested at around 0500 hours, immediately stood upright in deionised (DI) water and transported within 15 min of harvest to the laboratory while covered with a plastic film shroud. Stem segments of the required lengths and diameters of 5–7 mm, were excised with sharp secateurs under DI water from 35–40-cm long 1-year-old terminal shoots. Any side shoots or phyllodes on stem segments were removed under DI water.

Scanning electron microscopy

Approximately 5 mm longitudinal × 7 mm transverse cylindrical stem segments were excised and immediately fixed in a 0.1 M phosphate-buffered (PB; pH 6.8) fixative containing 3% (v/v) glutaraldehyde and 4% paraformaldehyde (electron microscopy grade, ProSciTech, Thuringowa Central, Qld, Australia) at 4°C for 48 h (O'Brien and McCully 1981). Further processing was with a microwave processor (18°C; Pelco Biowave 34700 microwave with SteadyTemp Cooler, Ted Pella, Redding, CA, USA). Samples were rinsed three times with 0.1 M PB and further rinsed in the microwave for 3 min (80 W, 74.5 kPa vacuum). Stem segments were dehydrated in a graded series of 30, 50, 60, 70, 80, 90 and then twice in 100% (v/v) ethanol for 3 min at each step (250 W, without vacuum). Some stem segments were then split longitudinally along natural fracture lines by

freeze-fracturing in liquid nitrogen. All stem samples were dried in 1 : 1 ethanol : hexamethyldisilazane (HMDS; ProSciTech) and three times in 100% HMDS for 3 min at each step (250 W, without vacuum) and further dried overnight in 100% HMDS. Dried stem pieces were mounted on individual aluminium stubs using conductive double-sided adhesive carbon tabs and carbon paint (ProSciTech). The stubs were then sputter coated with platinum for 3 min (EIKO IB-5 Sputter Coater, EIKO Engineering, Hitachinaka, Japan). Coated surfaces were viewed with a JSM-6300F field emission scanning electron microscope (Japan Electron Optics Laboratory (JEOL), Tokyo, Japan) at an accelerating voltage of 8 kV. Electron micrographs were digitally recorded using Image Slave software ver. 2.12 (The Dindima Group, Vic., Australia). Some micrographs were obtained from longitudinal stem sections processed under low vacuum (viz. low vacuum scanning electron microscopy (SEM) for 10 min at 50 Pa) for freeze drying. Freeze-dried samples were sputter coated with carbon for 7 s at 22 mAmp (Cressington Carbon Coater 208 with an SPI-module, SPI Supplies, West Chester, PA, USA). Coated sections were viewed with a JSM-6460 LA scanning electron microscope (JEOL) at an accelerating voltage of 10 kV under high vacuum. Digital images were recorded using the Smiles View Software ver. 2.12 (The Dindima Group). Samples were observed for specific xylem conduit characters such as type of perforation plate, structure of the xylem vessel lumen wall and structure of the intervessel pits.

Light microscopy: Method 1

Cylindrical stem segments (~5 mm longitude × ~5 mm transverse) were excised from three replicate stems. They were fixed in formalin acetic acid alcohol (FAA; O'Brien and McCully 1981) at room temperature (~25°C). After 24 h, the samples were transferred to 50% ethanol and processed by the Histotechnology Laboratory, Queensland Institute of Medical Research (QIMR), The University of Queensland, Herston. Processing (dehydration and paraffin embedding) and slide preparation were after Ruzin (1999).

Light microscopy: Method 2

Small cubes of ~1 mm^3 were excised from the xylem of stems. They were fixed in 0.1 M phosphate-buffered (pH 6.8) 3% (v/v) glutaraldehyde (electron microscopy grade, ProSciTech) at 4°C for 24 h. The microwave tissue processor described under the SEM protocol was used to further process samples. Fixed stem pieces were rinsed in three sequential changes of 0.1 M PB. Samples were then fixed twice serially in 0.1 M sodium cacodylate-buffered (pH 7.2) 1% (v/v) osmium tetroxide (spectroscopy grade, ProSciTech) for 6 min (80 W, 74.5 kPa vacuum) followed by rinsing twice in ultrapure water (Dinh *et al*. 2011). Samples were dehydrated in a graded ethanol series (30, 40, 50, 60, 70, 80, 90 and twice in 100% (v/v) ethanol) for 3 min at each step (250 W, without vacuum). The dehydrated samples were infiltrated with London Resin (LR) White resin (Medium grade; ProSciTech) in a sequential graded combination of resin : 100% ethanol and twice in 100% resin for 6 min each. Samples in 100% resin were held in a rotator overnight, followed by infiltrating with fresh resin for 30 min at 250 W under high vacuum. The procedure of resin infiltration under high vacuum and holding in a rotator overnight was repeated for a further 2 days. Afterwards, the samples were embedded in fresh LR White resin and cured overnight at 60°C. Embedded samples were sectioned with a Leica Ultracut ultramicrotome (Leica EM UC6, Leica Microsystems GmbH, Vienna, Austria). Semithin sections of 0.5 µm were obtained and placed on glass microscope slides, heated at 70°C until dry and stained with toluidine blue O (TBO; 0.5% (w/v) toluidine blue in 1% (w/v) borax (sodium tetraborate); Sigma–Aldrich, Castle Hill, NSW, Australia) for 10 s (O'Brien and McCully 1981). Excess stain was removed by rinsing with distilled water. Slides were observed under brightfield illumination with an Olympus BX61 light microscope equipped with a digital camera and imaging software (DP Controller software; Olympus Optical, Tokyo, Japan).

Transmission electron microscopy

Samples were prepared and embedded in resin as described under light microscopy (LM) Method 2. The resin blocks were further trimmed around the area of interest to give a block face of ~0.5 × 0.5 mm^2. Ultrathin sections of ~90 nm that included the pit area of xylem conduits were cut with a diamond knife (Diatome, Biel, Switzerland) on the ultramicrotome. Sections that expanded with chloroform vapour were collected onto Formvar coated glow discharged copper grids (200 square mesh; ProSciTech) and later stained with alcoholic uranyl acetate (5% (w/v) uranyl acetate in 50% (v/v) ethanol) for 2 min and in lead citrate (Reynold's lead stain; ProSciTech) for 1 min (O'Brien and McCully 1981). The sections were viewed with a JEM-1010 transmission electron microscope (JEOL) at an accelerating voltage of 80 kV and images were digitally recorded using iTEM software (Olympus Soft Imaging Systems GmbH, Münster, Germany). Samples were observed particularly for the structure of intervessel pits and vessel–parenchyma pits.

Determination of xylem conduit length

Terminal 40- to 50-cm long 1-year-old stems of *A. holosericea* with mature foliage were cut from field grown plants at Karalee (152°56′E, 27°31′S), Queensland, Australia. Harvest was at 0530 hours to ensure maximum water status. Harvested stems were immediately stood upright in DI water and transported within 2 h to the laboratory. During transport, the cut stems were covered with a plastic film shroud to minimise moisture loss. Upon arrival at the laboratory, the lowermost phyllodes on stems were removed and a 2-cm length was recut from the stem ends under DI water to obtain ~40-cm long stems. Carbon particles in Hunt's Speedball India ink (Speedball Art Products, Statesville, NC, USA) were used for conduit length determination, as pores in porous pit membranes are smaller than the carbon particles (Milburn 1979). India ink was filtered three times through Whatman No. 1 filter paper (Livingstone International, Rosebery, NSW, Australia) and the filtrate diluted with DI water to 40 times its original volume (Crombie *et al*. 1985). Conduit length determination was based on Chiu and Ewers (1993) and Nijsse *et al*. (2001), but using India ink instead of a latex particle suspension. Data were analysed according to

Milburn and Covey-Crump (1971). Briefly, five 30-cm long cut stem segments were fixed to a manifold system (Durkin 1979). Diluted India ink solution was fed into the cut stem segments via the proximal (basal) end under 10 kPa pressure through silicon tubing. The ink suspension was allowed to pass through the stem segment until flow completely ceased. Due to the positive pressure, ink particles travelled towards conduit ends and gradually increased in local concentration by lateral water loss via pits. The conduits became packed with ink particles. Once water flow ceased, it was assumed that all cut conduits had been stained. Numbers of ink-filled conduits were counted in serial 1-cm long stem sections. Hand sectioning from the distal end was performed using single-edged razor blades to obtain transverse sections. The number of ink-filled xylem conduits was then counted in each transverse section from digital images obtained with an Olympus BH-2 compound LM (Olympus Optical).

Determination of xylem conduit diameter

Plant materials were obtained as described previously for xylem conduit length measurements. Stem samples were processed as described previously under LM Method 1. Xylem cell diameters were measured in 100 vessels demarcated by xylem ray cells. The selected vessels were in randomly chosen transects of three replicate transverse sections (TS). For each xylem vessel, diameters at the widest and narrowest points were measured perpendicular to each other, and the average xylem diameter calculated. Stem transverse sections were observed under an Olympus BX-51 compound LM (Olympus Optical) and digital images and measurements were taken using QCapturePro software ver. 5.1 (QImaging Corporation, Surrey, Canada). Normality of diameter data was determined by the Anderson–Darling test using Minitab Release 14.1 (Minitab, Sydney, NSW, Australia) statistical software.

Stomatal distribution

Three replicate samples of 4 mm × 3 mm area from each surface were obtained from the middle regions of phyllodes. Samples were fixed and processed for SEM as previously described for stem samples. Both surfaces of phyllode samples were examined under a JSM-6300F field emission scanning electron microscope (JEOL) and digital images were recorded. Guard cell length (stomatal length; µm) and stomatal density (stomata mm^{-2}) measurements were made on digital images with iTEM software (Olympus Soft Imaging Systems). The size of the stomatal aperture was not measured due to the closure of stomata when exposed to fixatives. Nevertheless, stomatal length is a parameter of stomatal size because when stomata open or close, the short axis of the guard cells can change in dimensions, but the long axis remains the same (Beaulieu *et al.* 2008).

Results

Xylem anatomy

Stem cross-sections of *A. holosericea* current season shoots are generally triangular (Fig. 1). The stem is bounded by a cuticle over a single epidermal cell layer. The chlorenchymatous cortex is demarcated internally by a sclerenchyma ring of variable

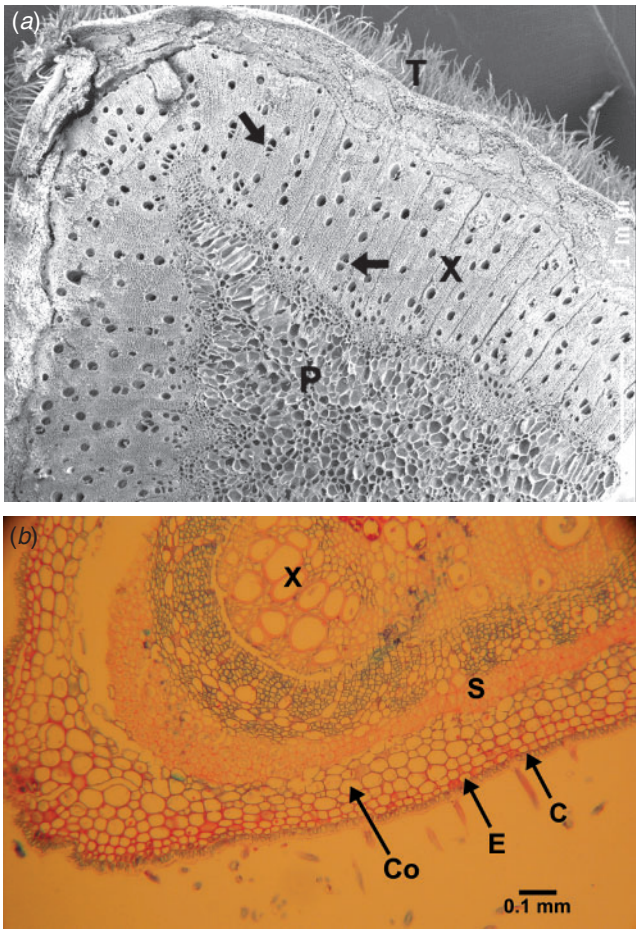

Fig. 1. *Acacia holosericea* stem transverse sections from 1-year-old shoots. (*a*) Transverse face observed by scanning electron microscopy. Approximately 50% of the xylem cross-sectional area is shown. Note the presence of a large number of solitary vessels and comparatively fewer paired (arrowed) vessels. (*b*) Light microscopy image produced via Method 1. The arrangement of different cell types is shown. C, cuticle; Co, collenchyma; E, epidermis; P, pith; S, sclerenchyma; T, trichomes; X, xylem.

width (Fig. 1*b*). This ring encloses the phloem, cambial layer and lignified secondary xylem, which comprises vessels, tracheids, fibres, axial parenchyma and narrow vascular rays (Fig. 2*a*, *b*) along with the internal pith parenchyma (Figs 1, 2*a*). Vascular rays are uniseriate and storied (Fig. 2*a*), occasionally being in contact with vessels. Vessels are more commonly in contact with the single layer of vasicentric axial parenchyma cells (Fig. 2*a*, *b*).

Most vessels are solitary and adjacent elements are joined by simple perforation plates that are visible as almost unobstructed openings with small rims at the margins of the lumen (Figs 2*b*, 3*a*). Vessels occasionally appear as pairs in transverse section (Figs 1*a*, 2*a*), particularly where they overlap, and the walls are occupied by bordered pits (Figs 2*d*, 3*b*, 4*a*). The secondary wall is absent from the pit area, but an arched chamber develops in the first-formed secondary wall, leading to a narrower pit aperture in the inner wall surface (Fig. 3*c*, *d*). The pits are approximately oval in surface view, with a maximum

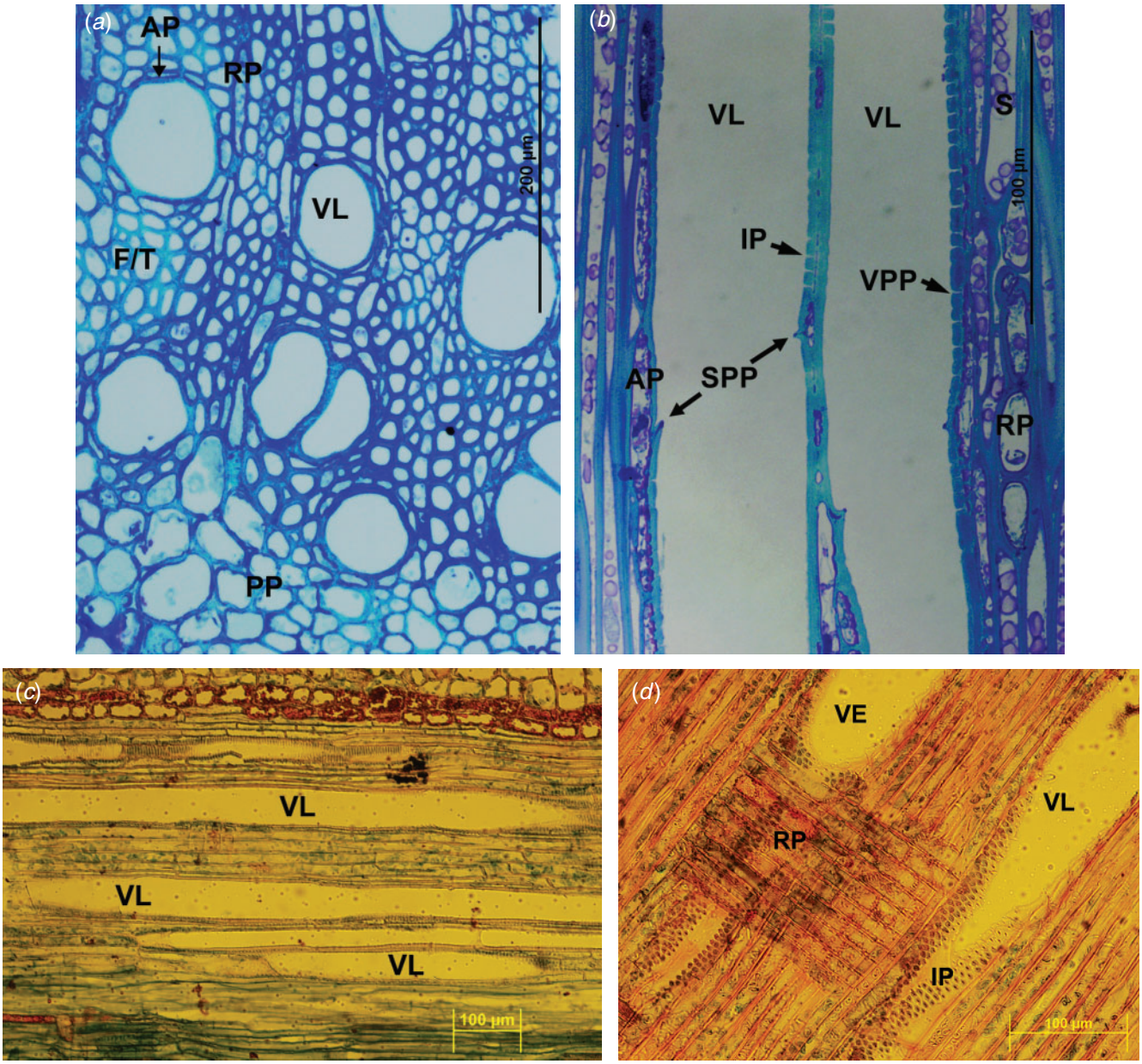

Fig. 2. Light microscope images of *Acacia holosericea* secondary xylem. (*a*) Transverse section prepared via light microscopy (LM) Method 2. (*b*) Radial longitudinal section prepared via LM Method 2. (*c*, *d*) Tangential longitudinal sections prepared via LM Method 1. AP, axial parenchyma; F, xylem fibres; IP, inter-vessel pits; PP, pith parenchyma; RP, ray parenchyma; S, storage parenchyma; SPP, simple perforation plate; T, tracheids; VE, vessel element; VL, vessel lumen; VPP, vessel–parenchyma pits.

dimension of 2–2.5 µm (Fig. 3*d*). Branched or coralloid protuberances or vestures (Ohtani *et al.* 1984) occur on the inner lining of the bordered pit chamber, giving rise to a warty appearance of the chamber lining (Fig. 3*f*). Dislodgement of the overlaying secondary cell wall revealed a relatively homogenous fibrous pit membrane (Fig. 3*e*). Half-bordered pits connect the vessels to the parenchyma cells (Fig. 4*b*).

Xylem conduit length and diameter

The majority of conduits (89%) were short at 1–5 cm long and ~2% of conduits were long at 15–16 cm (Fig. 5*a*). The longest conduit length measured was 16 cm. Mean xylem conduit diameter was 77.0 ± 0.9 µm ($n = 300$). Minimum and maximum diameters were 35.3 µm and 127.6 µm, respectively. According to the Anderson–Darling normality test the diameter classes were normally distributed (test statistics (A^2) = 0.37; $P = 0.417$; Fig. 5*b*).

Stomatal distribution

The epidermis of isobilateral phyllodes in *A. holosericea* had paracytic stomata on both surfaces (i.e. amphistomatic; Fig. 6). The stomata were arranged in a random or diffuse distribution.

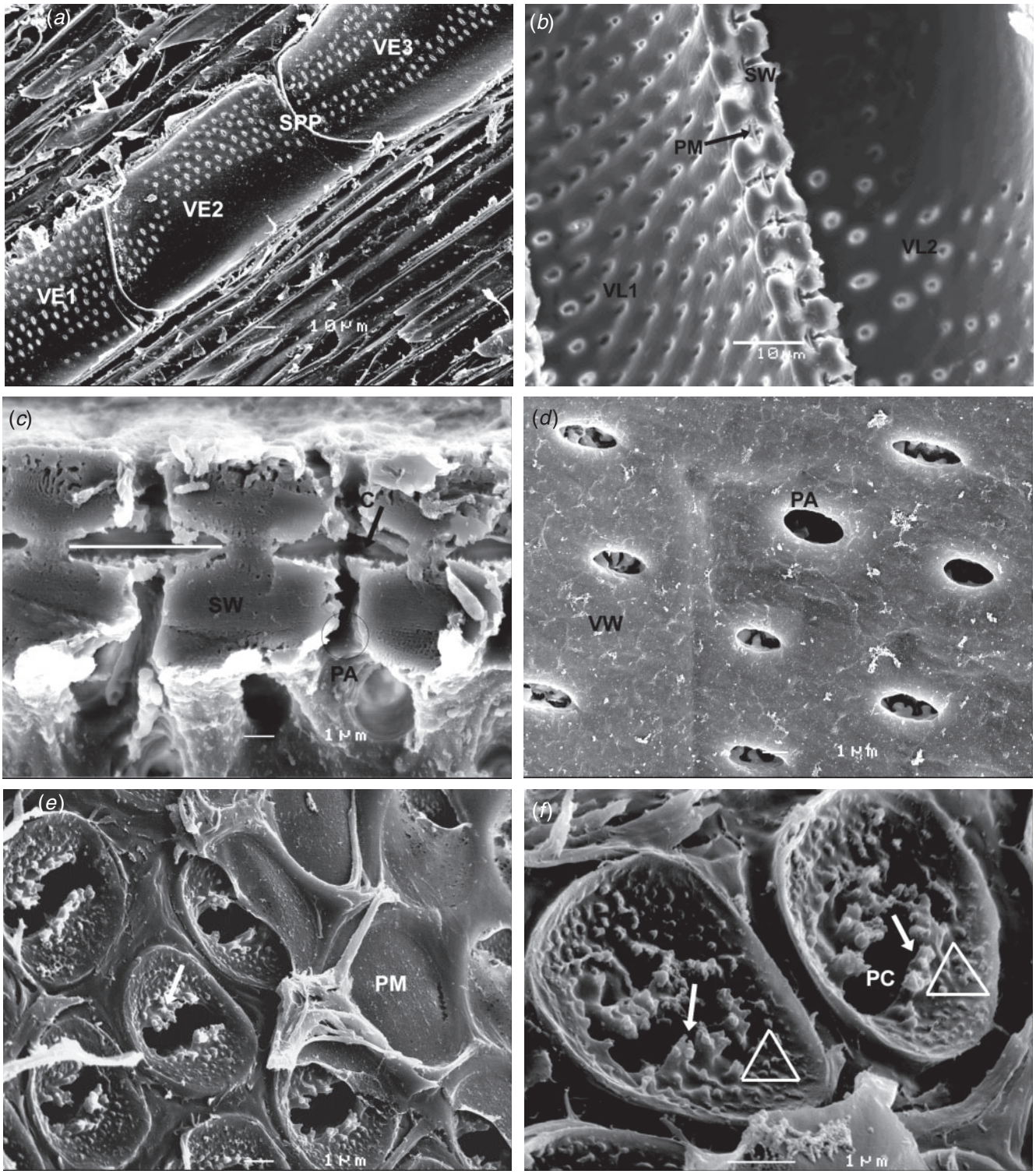

Fig. 3. Scanning electron micrographs of *Acacia holosericea* xylem vessel walls. (*a*) A single vessel. Several vessel elements are vertically aligned and connected via simple perforation plates to form a single vessel. (*b*, *c*) Longitudinal view of inter-vessel pits connecting adjacent vessel lumens. In (*c*), the position of pit membrane is indicated by a white line and the pit aperture is marked with a black circle. (*d*) Surface view of pits on vessel wall from the vessel side. (*e*) The 'homogenous' pit membrane has been dislodged from some pits to reveal the vestures (arrow) from the secondary wall over and into the pit cavity. (*f*) Branched vestures (arrows) and simple vestures or bumps (triangular areas) line the pit chamber on the secondary cell wall face. PA, pit aperture; PC, pit chamber; PM, pit membrane; SW, secondary wall; VE, vessel element; VL, vessel lumen; VW, vessel wall.

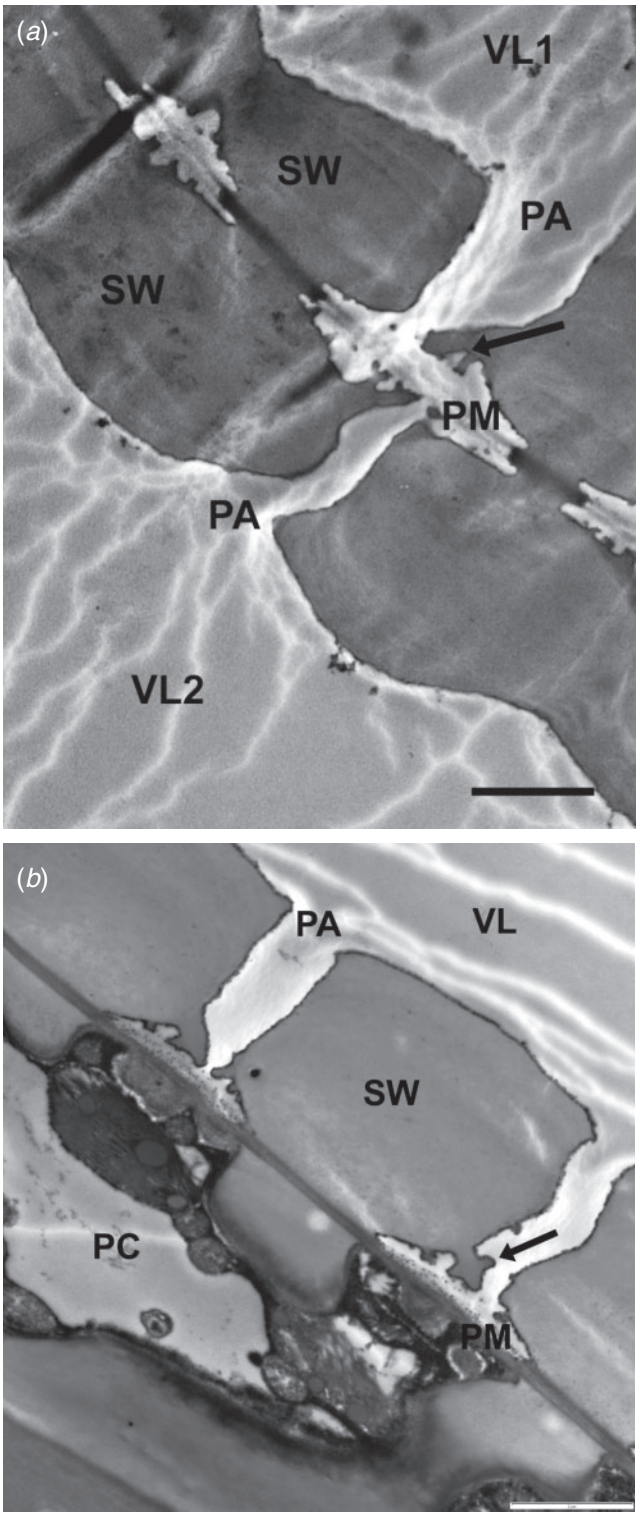

Fig. 4. Transmission electron micrographs of transverse sections through pits showing vestures in the pit structure. (*a*) Inter-vessel pit. (*b*) Vessel–parenchyma pit. PA, pit aperture; PC, parenchyma cell; PM, pit membrane; SW, secondary wall; VL, vessel lumen; arrow, branched vestures. White reticulate patterns are an artefact on the Formvar (ProSciTech, Thuringowa Central, Qld, Australia) film. Scale bars = 2 µm.

Both surfaces of the phyllode had stomata at equal densities of 232 ± 2 mm^{-2} (mean ± s.d.; $n = 6$). The average guard cell length (stomatal length) was 25.2 ± 2 µm (mean ± s.d.; $n = 40$). Unicellular erect trichomes were also present on both surfaces.

Discussion

Vessels with simple perforation plates and bordered vestured pits are the major conductive elements in *A. holosericea* secondary xylem. Secondary xylem in plants typically consists of axial tracheids, vessels, axial parenchyma and fibres, and radial ray systems. The presence of these cell types, including ray cells, in LM images (Fig. 2*a*, *b*) indicates that the sections of *A. holosericea* stem were of secondary xylem. The presence of storied ray cells is characteristic of the Fabaceae, which is allied to the Mimosaceae as they were formerly grouped together under Leguminosae (Cutler *et al*. 2008; Fig. 2*a*, *b*).

Bordered intervessel pits in *A. holosericea* have protuberances (vestures) that arise from the secondary cell wall inner surface to the pit chamber. Pits are essential components of xylem conduits and bordered pits are cavities in the lignified secondary cell wall of xylem conduits (Evert 2006). Pits allow lateral water transport between xylem conduits, and serve to limit the spread of air emboli and vascular pathogens in the xylem (Choat *et al*. 2008). In bordered pits, structural variations in vestures are seen in many plant species, including *A. melanoxylon* (Scurfield *et al*. 1970). Jansen *et al*. (2001) reviewed the occurrence of vestured pits in eudicots. Various forms of vesturing in pit chambers appeared bead-like or filamentous. Compared with *A. melanoxylon*, which has prominently bead-like vestures (Scurfield *et al*. 1970), *A. holosericea* has coralloid-like vestures (Fig. 3*e*, *f*). Jansen *et al*. (2004) described a systematic relationship between plant species distribution in different climatic zones and the structure of their xylem vessels and suggested that vestured pits increased hydraulic resistance and minimised vulnerability to air seeding. Their literature survey of 6428 genera covering 11 843 species from diverse climatic regions indicated that the highest frequencies of vestured pits occurred in desert and tropical seasonal woodland plants. Moreover, branched vestures were mainly restricted to warm habitats in both mesic and dry subtropical lowlands. If indeed bordered vestured intervessel pits evolved for protection against spread of air emboli, then the present observations imply that *A. holosericea* xylem is vulnerable to embolism. Moreover, in the absence of root pressure, as is the case in cut stems, the consequences for K_h could be significant. In this context, research into the occurrence of embolism and cavitation in cut *A. holosericea* stems and their effect on water relations is warranted.

There are relationships between structure and function of different anatomical structures, and ecological conditions correlate with xylem features (Jansen *et al*. 2000, 2001, 2004; Sano and Jansen 2006; Choat *et al*. 2008). Xylem K_h is mainly a function of conduit length and diameter, and also the nature of the interconnections between adjacent (pits) and adjoining (perforation plates) elements (Zimmermann 1983). Vessel elements are considered to have less resistance to water flow than tracheids due to the perforated nature of vessel element walls; i.e. wide vessel lumens and partial loss of end walls (Schulte and Castle 1993; Ellerby and Ennos 1998). Wider

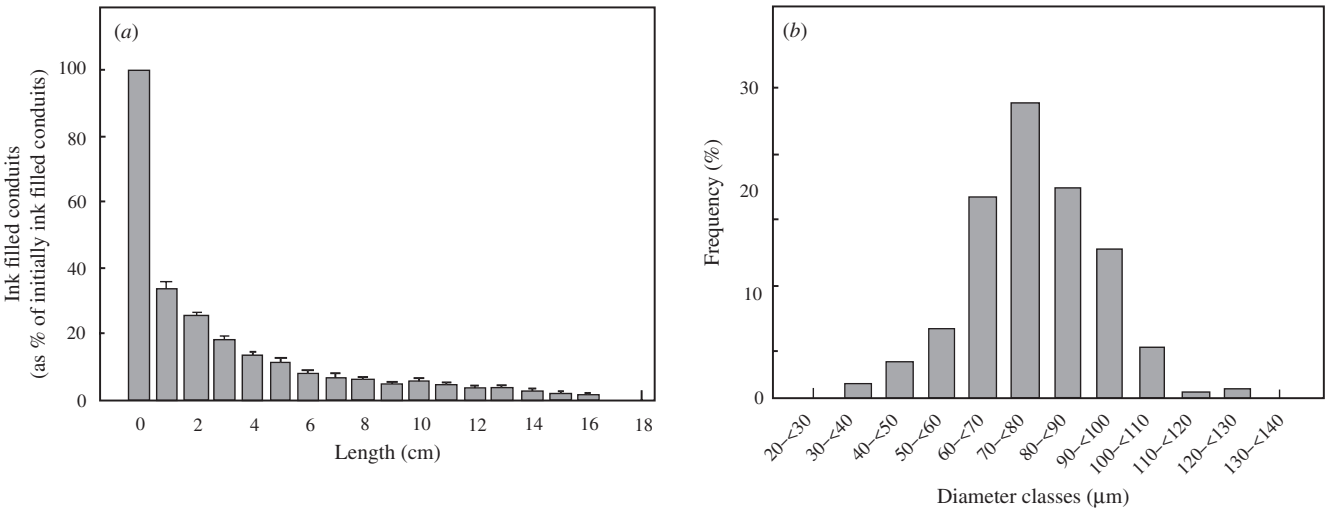

Fig. 5. Xylem conduit dimensions of *Acacia holosericea* current season stems. (*a*) Conduit length distribution. The number of ink-filled conduits per centimetre as a proportion (%) of the number of initial ink-filled conduits at the basal end. Bars = ± s.e., $n = 5$. (*b*) Xylem conduit diameter profile as 10 µm incremental diameter classes versus frequency. Data are means of three replicates with 100 conduits counted per replicate.

Fig. 6. A scanning electron micrograph showing the distribution of stomata and trichomes on unifacial phyllodes of *Acacia holosericea*. S, stomata; T, trichome.

vessels are more efficient because the resistance to flow through a cylindrical vessel is inversely proportional to the fourth power of the vessel's diameter (see the Introduction and Milburn 1979). Nonetheless, Zimmermann (1983) emphasised that plant xylem vessels do not resemble ideal pipes due to finite lengths of vessels, irregularities in the vessel lumen wall (e.g. presence of ridges and warts) and perforation plates, and because lateral water movement from one vessel to another occurs via pit membranes.

Conduit length counts of *A. holosericea* revealed (Fig. 5*a*) that the majority were very short at 1–5 cm, with only a small proportion being long at 15–16 cm. This result is similar to that obtained for *A. subulata*, where the maximum conduit length was 12–13 cm (Ritman and Milburn 1988). Compared with ring porous trees, such as *Q. rubra* and *F. americana* (Zimmermann and Jeje 1981), these lengths are relatively short. The longest conduits in those two species were ~10 m long. The presence of a high proportion of short conduits in *A. holosericea* could constitute hydraulic 'insurance' against large negative water potentials in dry environments. However, they impose a restriction on water volume flow. Moreover, the prevalence of short conduits could cause an increased pressure drop across the water conduction pathway, thus increasing the incidence of embolism (Canny *et al*. 2007). It remains to be ascertained whether these structural features have a significant impact on the K_h of cut *Acacia* stems standing in vase water.

Vessel diameters vary from 4 to 500 µm in various different plant species (Comstock and Sperry 2000). Reported mean diameters of early wood vessels in *Elaeagnus argentea* Pursh, *Eleagnus umbellata* Thunb. and *Shepherdia argentea* (Pursh) Nutt. were 127, 68 and 52 µm, respectively (Jansen *et al*. 2000), overlapping with 70 to 80 µm for *A. holosericea*. There is a large variation in vessel diameters in different plant species and growth forms, and across environmental conditions (Ewers and Fisher 1989; Ewers *et al*. 1990). Thus, a comprehensive assessment of xylem diameter data for *A. holosericea* from a range of environments may merit investigation. Nevertheless, a low proportion of long and wide conduits is meaningful in the trade-off between safety and efficiency (Zimmermann 1983). Long and wide conduits transport water more efficiently than do narrow and short conduits, but are more prone to cavitation. Some reports suggest that the width of xylem vessels has an impact on bacteria-induced xylem blockage in cut flower stems (Put and Clerkx 1988). However, this is yet to be investigated with regard to cut *Acacia*. It has also been suggested that large pit membrane micropores rather than wide conduit diameters determines vulnerability to cavitation and subsequent embolism (Choat *et al*. 2008). Thus far, there are no data on the size of pit membrane micropores in *A. holosericea*.

High transpiration rates and large negative xylem pressures occur in plants in warmer and drier environments (Sperry and Tyree 1988), and can frequently give rise to embolism. When an embolised vessel lies adjacent to a functional vessel under pressure, a substantial pressure difference develops across any pit membranes that connect these vessels. Gas will penetrate the pit membrane at a critical pressure difference (i.e. air seeding pressure) and lead to cavitation of the previously water-filled vessel (Choat *et al*. 2008). Vestures have been suggested to play a functional role in preventing pit membrane rupture by providing mechanical support of the pit membrane (Choat *et al*. 2004). These authors showed, through moderating pit membrane porosity by limiting deflection and stretching, that vestures may act to reduce the probability of air seeding and thereby decrease vulnerability to water stress-induced embolism. Chattaway (1949) suggested that the size of the pit aperture (opening) on the xylem vessel wall was a determinant of gum and tylose development into the vessel lumen in response to wounding or cavitation. So far, tylose or gum formation in *A. holosericea* xylem has not been demonstrated. A large number of solitary vessels, compared with the number of paired vessels in *A. holosericea* xylem transverse section, suggests that vessel overlap areas could be very short. This may be an important structural adaptation to minimise air seeding from an embolised vessel to a functional vessel. However, it could considerably increase the resistance to water flow, particularly in an event of lumen blockage (Zimmermann 1983).

The unifacial structure of more or less scleromorphic phyllodes in *Acacia* is attended by the lack of any distinct anatomical differences between the two faces (Lange and Ullmann 1987). The equal stomatal density (~232 mm^{-2}) observed on the two sides of *A. holosericea* phyllodes is in general agreement with the observations of Lange and Ullmann (1987). They reported similar densities of stomata on the 'east' and 'west' faces of *Acacia longifolia* (Andr.)Willd. (~106 mm^{-2}) and *A. melanoxylon* (~221 mm^{-2}) phyllodes. They further noted that stomatal opening and closing on the two faces was synchronous despite the differences in irradiation. The stomatal density of *A. holosericea* unifacial phyllodes is close to that of some other *Acacia* species (e.g. *A. melanoxylon*). However, it is moderate when compared with the lower (155 mm^{-2}) and higher (548 mm^{-2}) densities obtained with *Gossypium hirsutum* L. and *Eucalyptus citrodora* Hook, respectively (El-Sharkawy *et al*. 1985). The stomatal size of *A. holosericea* phyllodes (~25 µm) is comparable to that of other tree species, such as *Tilia americana* L. and *Tilia europa* L., which have average stomatal lengths of 26 and 25 µm, respectively, but only on the abaxial surface (Willmer and Fricker 1996). As stomatal distribution and size are influenced by genetic and environmental factors, such as temperature, humidity and irrigation regime, accurate direct comparisons across the species are difficult (Beaulieu *et al*. 2008). Nevertheless, as increased stomatal density is associated with greater stomatal conductance and transpiration rates (Beaulieu *et al*. 2008), the presence of a high density of stomata on both surfaces of phyllodes could result in increased water loss from cut foliage. However, research is needed into the sensitivity of *A. holosericea* stomatal conductance in association with changing water potential.

Acacias occur naturally in semi arid regions of the world (Gómez-Acevedo *et al*. 2007) and *A. holosericea* occurs in the northern part of Australia, which has a warm and seasonally dry climate. It is likely that the presence of xylem vessels with simple perforation plates and bordered vestured pits plus a large proportion of shorter comparatively narrow conduits in *A. holosericea* are adaptive for survival in warm, dry climates. However, a high proportion of shorter conduits could impose a restriction on water volume flow. Nonetheless, simple perforation plates confer a relatively low resistance to water flow in individual vessels. However, bordered vestured pits could provide substantial resistance to lateral flow between individual vessel elements comprising whole vessels. Research focussed on changes in xylem K_h of cut *A. holosericea* stems over time is warranted to relate these anatomical restrictions with the observed decline in postharvest water uptake. In the event of lumen blockage by bacteria, embolism, and tyloses and gum (van Doorn 1997), high resistances to water flow between adjacent vessels could develop. This, in association with a high proportion of short xylem conduits, may at least partially account for the characteristically short vase life of *A. holosericea* cut stems. Further studies will investigate the potential for microbial or physiological plugging of the xylem conduits, and also changes in transpiration and water potential of cut stems in terms of their implications for K_h.

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