American Journal of Botany 94(8): 1282–1288. 2007.

GIANT CUTICULAR PORES IN *EIDOTHEA ZOEXYLOCARYA* (PROTEACEAE) LEAVES[1]

RAYMOND J. CARPENTER,[2] GREGORY J. JORDAN,[3,5] ANDREA LEIGH,[4] AND TIMOTHY J. BRODRIBB[3]

[2]School of Earth and Environmental Sciences, University of Adelaide, Adelaide, South Australia 5005, Australia; [3]School of Plant Science, University of Tasmania, Private Bag 55, Hobart, Tasmania 7001, Australia; and [4]School of Botany and Zoology, The Australian National University, Canberra, Australian Capital Territory 0200, Australia

Ubiquitous, large diameter pores have not previously been adequately demonstrated to occur in leaf cuticles. Here we show conclusively that such structures occur in *Eidothea zoexylocarya*, a rainforest tree species of Proteaceae restricted to the Australian Wet Tropics. The pores are abundant, large-diameter apertures (~1 μm), that extend perpendicularly most of the way through the cuticle from the inside. They occur on both sides of the leaf, but are absent from the cuticle associated with stomatal complexes on the abaxial side. No such pores were found in any other species, including the only other species of *Eidothea*, *E. hardeniana* from New South Wales, and other species that have previously been purported to possess cuticular pores. To determine whether these pores made the cuticles more leaky to water vapor, we measured astomatous cuticular conductances to water vapor for *E. zoexylocarya* and seven other Proteaceae species of the Wet Tropics. Cuticular conductance for *E. zoexylocarya* was relatively low, indicating that the prominent pores do not increase conductance. The function of the pores is currently obscure, but the presence of both pores and an adaxial hypodermis in *E. zoexylocarya* but not *E. hardeniana* suggests evolution in response to greater environmental stresses in the tropics.

Key words: cuticular conductance; cuticular morphology; cuticular pores; *Eidothea*; Proteaceae; stomatal complex.

Cuticles of leaves need to be both permeable and impermeable. They protect internal leaf tissues by providing a physical barrier against pathogens, herbivores, and the conductance of ultraviolet light and water (Riederer and Müller, 2006 and references therein). Waxes and other substances, however, must still be able to pass through the cuticle. There is even evidence that some plants absorb water through their cuticles in conditions of abundant fog, mist, or dew (Stone, 1957; Yates and Hutley, 1995; Burgess and Dawson, 2004).

One suggested solution to these contradictory requirements would be the presence of open or wax-filled canals. Thus, Miller (1985, 1986a, b) was adamant that isolated and dewaxed leaf cuticles of a wide range of species have pores (in surface view) leading to transcuticular canals (in transverse view) of approximately 1 μm in diameter. Also, Šantrůček et al. (2004) presented evidence suggesting that large, wax-filled canals traverse the cuticle of *Hedera helix* (Araliaceae). However, recent reviews dispute the existence of cuticular canals and show untreated and gently processed cuticles that are amorphous, lamellate, and/or reticulate in transverse section with no evidence of either open or wax-filled canals (e.g., Jeffree 1996, 2006). It is also currently understood that the small but physiologically significant amounts of liquid water that are lost through the cuticle mostly diffuse as single molecules at the ultrastructural level (reviewed by Schönherr, 2006).

Proteaceae are a relatively large southern hemisphere family well known for having a range of anatomical and physiological features that are explicable as adaptations to nutrient-deficient soils and periodic drought (Johnson and Briggs, 1975). Carpenter (1994) reported the presence of pore-like structures in the leaf cuticle of ''Proteaceae sp. code #771,'' a North Queensland rainforest species now described as *Eidothea zoexylocarya* (Douglas and Hyland, 1995). Here we refer to these entities simply as pores, although they are obviously not open to the outside surface of the leaf. The aims of this paper are to present a more detailed study of the cuticle of *E. zoexylocarya* using a variety of microscopic techniques and determine whether pores also occur in the only other member of the genus, the recently described *E. hardeniana* (Weston and Kooyman, 2002). We also reinvestigate *Hedera helix*, *Hoya carnosa* (Asclepiadaceae) and *Cycas revoluta* (Cycadaceae) to clarify whether the occurrence of cuticular pores is universal in plants, or extraordinary. Miller (1986a) focused on *H. carnosa* as a species exemplifying the presence of pores, and *Cycas* species have also been reported to show prominent pits in their cuticles (Thomas and Bancroft, 1913; Pant and Nautiyal, 1963; Greguss, 1968). Astomatous cuticular conductances for *E. zoexylocarya* leaves and a range of similarly distributed rainforest Proteaceae were also calculated because the pores in *E. zoexylocarya* might be expected to result in unusually high conductance. Finally, we discuss a possible adaptive role for the pores in leaf hydration following water stress.

MATERIALS AND METHODS

Eidothea species—*Eidothea zoexylocarya* A. W. Douglas & B. Hyland is known only from 600–1460 m a.s.l. in the Wet Tropics region of North Queensland, between 16° and 18° S (Douglas and Hyland, 1995; Weston and Kooyman, 2002). Mean annual temperature is ~20°C, with only small seasonal variation, and mean annual rainfall is probably well in excess of 3000 mm (Tracey, 1982; Nix, 1991). Most rainfall is in the summer months, but there is frequent supplementary input from low cloud, mist, or fog in all seasons. The 20–40 m high trees of *E. zoexylocarya* form part of the canopy of complex mesophyll to simple notophyll vine forest on metamorphic or mostly granitic substrates (Douglas and Hyland, 1995; Weston and Kooyman, 2002). *Eidothea hardeniana* Weston & Kooyman is known only from simple notophyll vine forest on acid volcanic rhyolite-derived soils in a single catchment in

[1] Manuscript received 30 October 2006; revision accepted 3 June 2007.

The authors thank P. Weston, Sydney (NSW), R. Elick, Atherton (QRS), and G. Sankowsky for provision of leaf material, and they especially thank L. Waterhouse (Adelaide Microscopy) for assistance with electron microscopy. Funding from the Australian Research Council to G.J.J. and Prof. R. Hill assisted this research.

[5] Author for correspondence (e-mail: greg.jordan@utas.edu.au)

northeastern New South Wales (approximate latitude 28°33′) (Weston and Kooyman, 2002). In general terms the climates of both habitats are probably similar, but with higher total precipitation and evapotranspiration in North Queensland (Webb and Tracey, 1981). The leaves of both species are evergreen, relatively sclerophyllous, glabrous, and of similar size and shape (Weston and Kooyman, 2002; our observations). Herbarium specimens (*J. G. Tracey 14994*, *B. Gray 6042*, *B. G. Briggs 7416*; QRS and NSW) of mature leaves of *E. zoexylocarya* were studied, as well as leaves from cultivated trees grown by G. Sankowsky at Tolga, North Queensland, and at the CSIRO Atherton Arboretum, North Queensland. We also studied herbarium (*P. H. Weston 2469*; NSW) and cultivated (Sydney Royal Botanic Gardens) specimens of *E. hardeniana*.

Other species—Cuticles from cultivated *Cycas revoluta* L., *Hedera helix* L. and *Hoya carnosa* R. Br. were examined in some detail. We also searched the collection of leaf cuticles of gymnosperm and angiosperm species in the School of Earth and Environmental Sciences, University of Adelaide, for evidence of cuticular pores. This collection comprises species from over 170 families and includes numerous species that occur with *E. zoexylocarya* in upland rainforests of North Queensland. Astomatous cuticular conductances were measured from *E. zoexylocarya* and other rainforest Proteaceae trees native to the same region. These were *Lomatia fraxinifolia* F. Muell. (a common forest associate of *E. zoexylocarya*), *Carnarvonia araliifolia* F. Muell., *Helicia australasica* F. Muell., *Hollandaea sayeriana* (F. Muell.) L. S. Smith, *Megahertzia amplexicaulis* A. S. George & B. Hyland, *Neorites kevediana* L. S. Smith, and *Stenocarpus sinuatus* Endl. These plants were all growing at the CSIRO Atherton Arboretum, North Queensland.

Light microscopy (LM)—Transverse sections approximately 20 μm thick of leaves of *Eidothea*, the other rainforest Proteaceae, and *C. revoluta*, *H. helix* and *H. carnosa* were cut using a freezing microtome, then stained with a saturated ethanolic solution of the cuticle specific stain, Sudan III. Cuticle thicknesses for *E. zoexylocarya* and the other Proteaceae were measured at 1000× magnification using these sections. Small areas of lamina were also cut from the margins of *Eidothea* species, *C. revoluta*, *H. helix* and *H. carnosa* at approximately half the leaf length. These sections were placed in 10% aqueous chromium trioxide to clear their inner tissues from the cuticle (Alvin and Boulter, 1974), using gentle heating. Apart from its extensive use in paleobotany, chromium trioxide is widely used for exposing the micromorphology of the inner cuticular surface in comparative studies of extant taxa (e.g., Carpenter, 1994; Stockey et al., 1998) and in studies exploring the ultrastructure of the cuticle in transverse section (Osborn and Taylor, 1990). Any possibility that the cuticular pores of *E. zoexylocarya* are the result of acid-induced degradation was readily dismissed by the observation that identical pores were clearly visible in transverse sections of intact leaves. *H. carnosa* leaves were also subjected to stronger acid and dewaxing treatment following Miller (1986b) to check whether this might reveal cryptic poral structures in the cuticle. Thus, leaf sections were soaked in 70% nitric acid for several hours before being placed in 10% aqueous chromium trioxide. The resultant cleared cuticles were then washed in water followed by three changes (10 min each) in absolute ethanol and three changes (10 min each) in absolute chloroform.

The isolated cuticles of both adaxial and abaxial (stomatiferous) surfaces from each species were rinsed in water, stained in safranin O, and mounted on glass slides in glycerin jelly. Sections of fresh leaf of *E. zoexylocarya* from the Atherton Arboretum and of cuticle portions isolated using chromium trioxide were also embedded in resin, using a Procure-Araldite Embedding Kit (ProSciTech, Brisbane, Australia). Leaf sections were fixed overnight in 4% paraformaldehyde–1.25% glutaraldehyde in PBS + 4% sucrose at pH 7.2. These were then twice washed in washing buffer (PBS + 4% sucrose) for 10 min each, and postfixed in 2% osmium tetroxide for 1 h. The sections were then dehydrated: 70% ethanol, three changes of 20 min each; 90% ethanol, three changes of 20 min each; 100% ethanol, three changes of 20 min each plus one change of 1 h; then propylene oxide, 20 min. Resin infiltration was undertaken by placement in a 1 : 1 mixture of propylene oxide and epoxy resin overnight, followed by three changes of 100% resin at approximately 8 h intervals. Sections were then finally embedded in fresh resin and polymerized in an oven at 70°C overnight. Ultrathin (75 nm) transverse sections were obtained by microtomy and stained with 4% aqueous uranyl acetate and Reynold's lead citrate. Thicker (~5 μm) resin-embedded sections of leaf and isolated cuticles were also mounted on glass slides for LM. Photographs for all LM work were taken with either an Olympus DP11 (Tokyo, Japan) or Nikon DS-5M (Tokyo, Japan) digital camera attached to a Zeiss Axioskop (Jena, Germany) microscope.

Transmission electron microscopy (TEM)—A Philips CM100 (Eindhoven, Netherlands) transmission electron microscope operated at 80 kV was used to examine transverse sections of leaf and isolated cuticles from both sides of the *E. zoexylocarya* leaf, obtained as described earlier. Brief descriptions of cuticular ultrastructure follow Osborn and Taylor (1990) and the review of Jeffree (2006).

Scanning electron microscopy (SEM)—A Philips XL 30 (Eindhoven, Netherlands) field emission gun scanning electron microscope operated at 10 kV was used to examine the inner and outer surfaces of cuticle from both sides of *E. zoexylocarya*, *E. hardeniana*, and *H. carnosa* leaves. Cuticles were prepared as for LM and mounted on double-sided adhesive tape on aluminum stubs and gold/carbon coated. Transverse faces of *E. zoexylocarya* and *H. carnosa* leaves obtained by fracturing the leaf under liquid nitrogen were also examined using SEM.

The size, abundance, and area of cuticular pores on the leaves of *E. zoexylocarya* were estimated. Average diameter was calculated by measuring a random sample of pores from epidermal cell cuticles on both inner surfaces using the x component of the SEM's measurement function. The density of cuticular pores (expressed as number per mm^2) associated with normal epidermal cells (i.e., not guard cells, subsidiary cells, or cells immediately lateral to subsidiary cells) was calculated by counting the number of pores in random areas of view under SEM. The surface area of each pore was estimated by considering each pore as an open cylinder. This area is thus equal to $2\pi rh + \pi r^2$, where r is the radius of the pore and h is its depth.

Cuticular conductance—Astomatous cuticular conductances were estimated from gravimetric measurement of water loss from isolated leaves. Leaf samples were collected and quickly processed early in the morning, so that leaf water potential was high. The leaves were young, fully expanded, growing in direct sunlight for at least part of the day, and devoid of obvious trichomes, surface irregularities, or epiphytes. The stomatiferous surface and petiole of the leaf was sealed with petroleum jelly, the leaves were laid out on trays in a room with warm (22–30°C), still, dry air (22–31% relative humidity), then weighed at ~1 h intervals for ~6 h. Total water loss per leaf in this period ranged from 2–4% of wet mass. Leaf areas were measured using image analysis of digital photographs of the leaves. Conductances were then calculated following von Caemmerer and Farquhar (1981). Three replicates of each species were measured on each of two successive days, giving six replicate samples for each species. Environmental conditions were similar on each day, with temperature increasing from 22–23°C to 29–30°C and relative humidity decreasing from 30–31% to 22–23% on each day.

For estimating water loss through the petroleum jelly, water loss was measured from leaves of a wide range of species. The leaves were entirely covered in a layer of similar thickness to that described earlier. They showed conductances in the range of 0.04–0.16 mmol·m^{-2}·s^{-1} (G. J. Jordan, unpublished data), which can be attributed to water loss from cuticle and petroleum jelly. These conductances were less than 10% of those for leaves with only the stomatiferous surface sealed. The measured rates for cuticular conductance were therefore reduced by the average value of 0.1 mmol·m^{-2}·s^{-1}.

Astomatous cuticular conductance can vary with leaf water potential, temperature, and relative humidity (Boyer et al., 1997; Riederer and Schreiber, 2001; Schreiber et al., 2001). Although comparisons among the species are valid because the species were measured simultaneously, conductances tended to decrease through the experiment. To make the results comparable to other studies, we standardized the rates to constant conditions as follows.

For each leaf, average conductance was calculated between successive measurements. These data produced approximately normally distributed residuals and linear regressions. The data were modeled with a restricted maximum likelihood general linear model (implemented with the MIXED procedure of SAS 9.1 [SAS Institute, Cary, North Carolina, USA]):

$$g = \text{time} + \text{species} + \text{time} \times \text{species} + \textit{replicate}\ (\textit{species}) + \textit{time} \times \textit{replicate}\ (\textit{species}) + \text{residual},$$

where g is the astomatous cuticular conductance for time interval, time is the average slope of the regression of conductance with time, species is the effect of species, time × species represents differences in slopes among species, *replicate*(*species*) is the effect of replicates nested within species, and *time* ×

replicate(*species*) represents differences in slopes among replicates of the species. The last two effects were treated as random.

The zero intercept of a fixed slope regression was calculated for each replicate of each species (implemented with the REG procedure of SAS 9.1). The slope was fixed to the average slope (-2.374×10^{-5} mmol·m^{-2}·s^{-1}) because the average slope was highly significant ($F_{1,39} = 48.8$, $P < 0.0001$) and did not vary significantly either among species or among replicates within species ($P > 0.05$). These zero intercepts therefore represent unbiased estimates of cuticular conductance at the starting conditions on each day of 22–23°C, 29–30% relative humidity, and approximately zero water potential.

RESULTS

Obvious pores on both the abaxial (Fig. 1) and adaxial (Fig. 2) inner cuticular surfaces were present on all specimens of *E. zoexylocarya*, although their positions could not be discerned on the outer cuticle surface using SEM (Fig. 3). The pores were abundant (1.2×10^5 mm^{-2}) in the cuticle associated with the normal epidermal cells (Figs. 1, 2, 4). On the abaxial surface they were absent from the cuticle associated with the guard cells and narrow subsidiary cells and absent or much less frequent in the cuticle overlying each neighboring cell immediately lateral to the subsidiary cells (Figs. 1, 4). In surface view using LM, the pores gave the impression of canals that penetrate across the cuticle (Fig. 4). However, transverse view showed that the pores reached nearly all the way through the 4–8 μm thick cuticle from the inside (Fig. 5). This view also showed that pore diameters were more or less uniform and that the pores were mostly perpendicular to the leaf surface. The mean diameter of the pores as viewed from the inner cuticle surface under SEM was 0.955 μm (SE = 0.029, $N = 55$). If each cuticular pore is considered an open cylinder 5 μm long with a diameter of 1 μm, the extra surface area of epidermis in contact with each pore would be approximately 16.5 μm^2. Given a pore density of 1.2×10^5 mm^{-2}, this translates to an additional epidermal area of 1.98×10^6 μm^2·mm^{-2}, or 1.98 mm^2·mm^{-2}. TEM (Figs. 6–8) confirmed that the cuticular pores extended across most of the width of the cuticle but did not reach into the narrow (~0.15 μm) cuticle proper. The pores were obviously formed during leaf development because the ultrastructural lamellae of the cuticular layer and cutinized cell walls followed the outlines of the pores (Figs. 6, 7). Removal of noncutinized cell and cell wall material with chromium trioxide allowed the pores to be viewed as open canals extending into the cuticle (Fig. 8). The SEM mount of the edge of freeze-fractured *E. zoexylocarya* leaf also indicated the presence of the cuticular pores (Fig. 9). This preparation is interpreted as showing the cuticle to have fractured in the regions of the pores where the cuticle was weakest, thus giving an irregular edge to the cuticle, where it would be expected to be smooth if the cuticle were of uniform density, or nearly so.

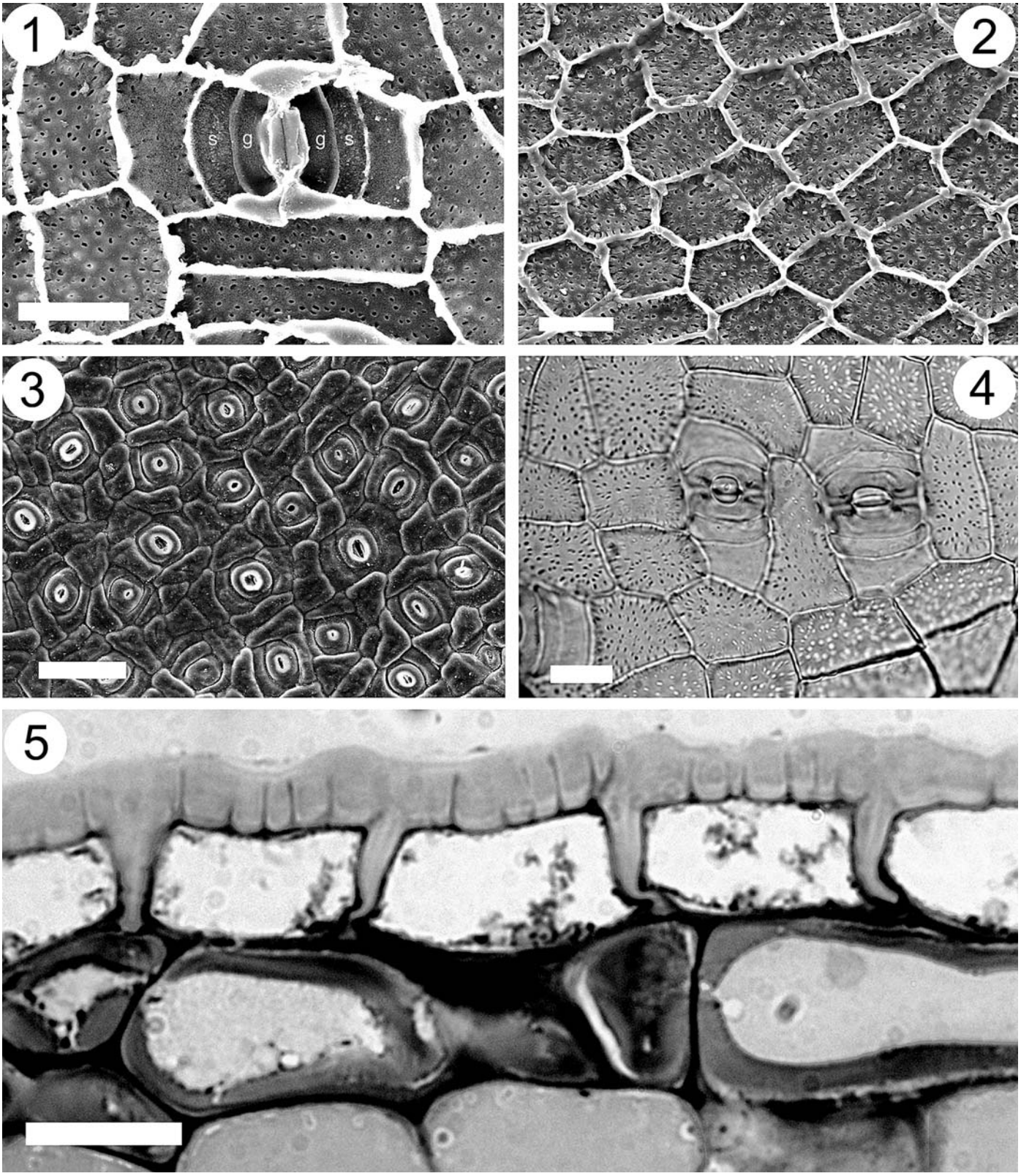
The cuticle of *E. hardeniana* lacks pores (Fig. 10) but otherwise was similar to that of *E. zoexylocarya*. Similarly, no cuticular pores were observed in any other species. In transverse section under LM, the cuticles of *C. revoluta, H. helix* and *H. carnosa* appeared entirely homogenous, even after strong acid and dewaxing treatments in the latter. However, obvious pits did occur in the outer epidermal cell walls of *C. revoluta*, which often remain adhered to the cuticle following chromium trioxide treatment. The edges of cuticle of freeze-fractured *H. carnosa* leaves observed under SEM were entirely smooth (Fig. 11), as would have been expected for a cuticle lacking large pores. Tiny pits were also evident in the outer epidermal cell walls of *H carnosa* in this preparation. The cuticles of *H. carnosa* had a granular appearance under LM, and granulations could be observed on the inner cuticular surface using SEM (Fig. 12).

Cuticular conductances varied significantly among Proteaceae species ($F_{7,39} = 9.14$, $P < 0.0001$). The cuticular conductance of *E. zoexylocarya* at high (near zero) water potential and approximately 22°C and 30% relative humidity was 1.06 mmol·m^{-2}·s^{-1}, which was toward the lower end of the range of the other tropical rainforest Proteaceae measured (Fig. 13). There was no relationship between cuticular conductance and cuticle thickness, and in particular, the conductance of *E. zoexylocarya* was lower than that of species with comparable cuticle thicknesses (Fig. 13).

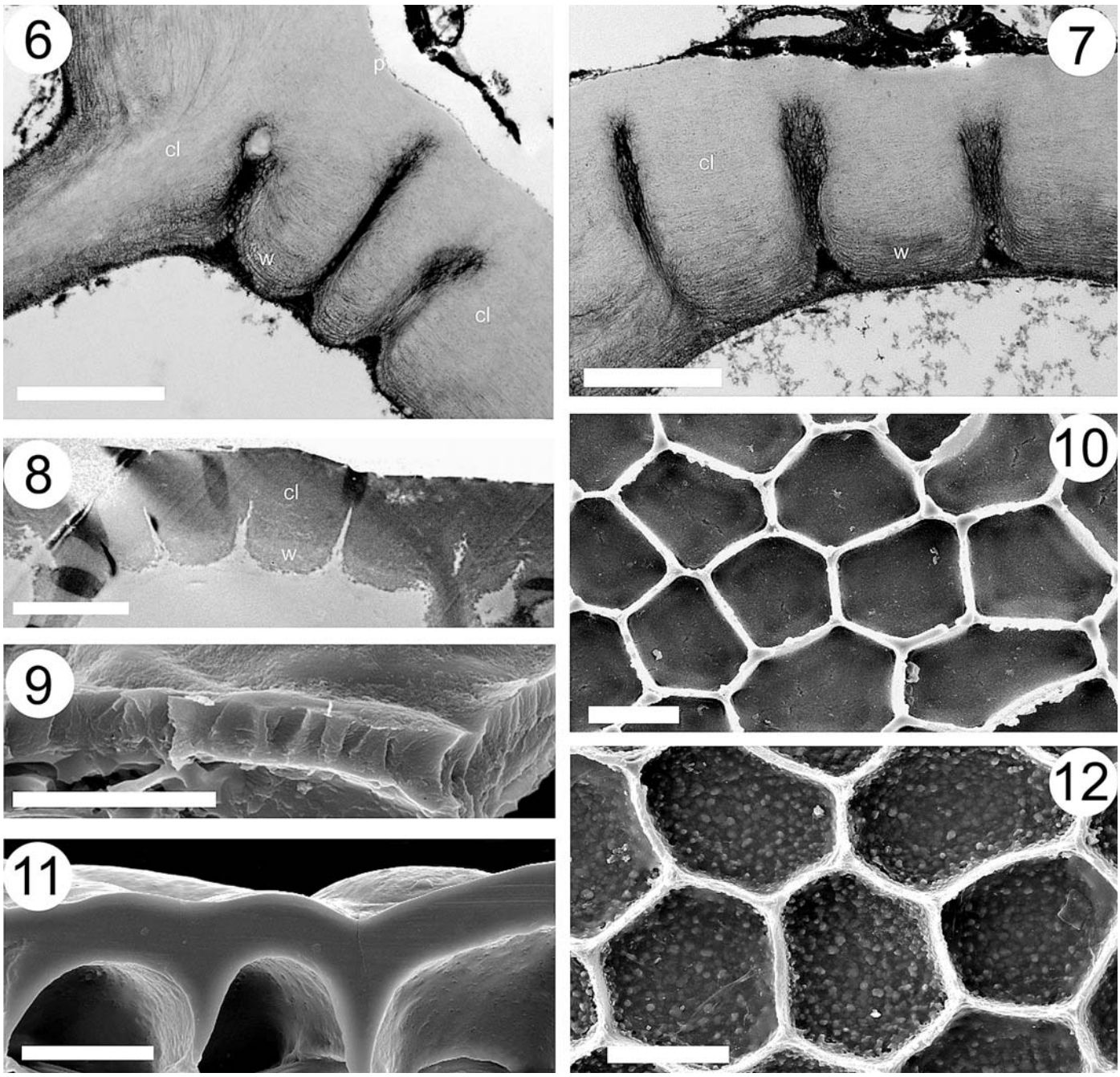
DISCUSSION

To our knowledge, the abundant, readily visible cuticular pores in *E. zoexylocarya* are a unique and remarkable exception to the commonly held belief (see Jeffree, 2006) that plant cuticles do not possess large diameter pores. The leaves of many plants have poral structures that facilitate uptake or removal of water or solutes, but these are associated with specialized trichomes or glands (e.g., Naidoo and Naidoo, 1998; Martin and von Willert, 2000). Our observations indicate that the large pits in *Cycas* epidermes, illustrated and discussed in detail by Greguss (1968), occur in the epidermal cell wall, not in the overlying cuticular layer. The canal-like features shown in Miller's (1982, 1985, 1986b) LM illustrations of transverse cuticle sections remain enigmatic. We could not replicate Miller's (1986a) observation of abundant cuticular pores and canals between 0.5 and 0.75 μm in diameter in dewaxed cuticles of *H. carnosa*. Such canals would be almost as large as the very obvious pores in *E. zoexylocarya*, so that if present they should be clearly apparent under both LM and SEM. We also note that Miller mostly relied on a method of air-mounting the cuticles, did not show images of fresh material sections, and only presented SEM images of his apparent pores in an earlier paper on apple fruit cuticles (Miller, 1982). These SEM images are very unconvincing because the "pores" are infrequent and could be attributed to the cuticle being degraded. For all of the preceding reasons, the comparatively abundant "pores" visible under LM in this and his subsequent papers may be artifacts. At least in the case of *H. carnosa*, inner surface cuticular granulations could also lead to misinterpretation. Finally, we could find no evidence for pores in the cuticles of any other species, including *Hedera helix*. Šantrůček et al. (2004) suggested that large, wax-filled pores traverse the cuticle of this species, although they could not demonstrate pores in dewaxed cuticle using SEM.

The relatively low leaf cuticular conductance of *E. zoexylocarya* compared to the other rainforest Proteaceae indicates that the pores do not increase conductance of water vapor from inside to outside the leaf. The conductances measured here all fall well within the published range for angiosperms (Kerstiens, 1996; Riederer and Schreiber, 2001) and specifically within the published range of a group of temperate, deciduous trees with relatively high conductances compared with tropical epiphytes and Mediterranean xeromorphic plants (Riederer and Schreiber, 2001). Published conductances for other species have been measured under different conditions (either at 0% or 100% relative humidity, and



Figs. 1–5. *Eidothea zoexylocarya* cuticle. **1.** SEM image of inner abaxial surface showing a stomatal complex. Note the absence of pores in guard (g) and subsidiary (s) cell cuticle. Note also the absence (right) or lower density (left) of pores associated with the epidermal cells immediately lateral to the subsidiary cells. Bar = 20 μm. **2.** SEM image of inner adaxial surface showing abundant pores associated with each epidermal cell. Bar = 20 μm. **3.** SEM image of outer abaxial surface. Bar = 50 μm. **4.** LM image of abaxial cuticle. Note the abundant pores that appear to traverse the cuticle but which are absent in cuticle associated with guard and subsidiary cells. Bar = 20 μm. **5.** LM image of stained, adaxial transverse leaf section. Note the numerous pores that extend perpendicularly most of the way through the cuticle, cuticular extensions or pegs marking epidermal cell wall positions, and thick hypodermal layer beneath the epidermis. Bar = 10 μm.



Figs. 6–12. *Eidothea zoexylocarya* (6–9), *Eidothea hardeniana* (10), and *Hoya carnosa* (11, 12) leaf sections and cuticle. **6, 7.** TEM images of stained cuticle from transverse leaf sections showing part of a cuticular peg at left in Fig. 6 and three pores extending into the cuticle in each image. Variably electron-dense lamellae in the cuticular layer (cl) and cutinized cell wall (w) follow the outlines of the pores. The pores do not reach into the narrow amorphous cuticle proper (p in Fig. 6). Bars = 2 μm. **8.** TEM image of transverse section of unstained isolated cuticle showing two cuticular extensions marking cell wall positions and three open pores (arrowed) extending into the cuticular layer (cl) that overlies the paler band of cutinized cell wall (w). Dark shadows are artifacts. Bar = 5 μm. **9.** SEM image showing irregular cuticle edge following freeze-fracturing of leaf. Bar = 10 μm. **10.** SEM image of inner adaxial surface showing absence of pores. Bar = 20 μm. **11.** SEM image showing smooth cuticle edge following freeze-fracturing of leaf. Bar = 10 μm. **12.** SEM image of inner adaxial surface showing absence of pores but abundance of granulations. Bar = 20 μm.

typically at 25°C) than those measured here (30% relative humidity and ~22°C). Although both vapor pressure and temperature can affect cuticular conductance (Boyer et al., 1997; Riederer and Schreiber, 2001), these effects are small compared to published variation among species. The combination of low conductances and large, abundant pores transgressing most of the width of the cuticle in *E. zoexylocarya* dramatically confirms the effectiveness of the hydrophobic epicuticular wax layer in controlling cuticular transpiration (Schönherr, 1976).

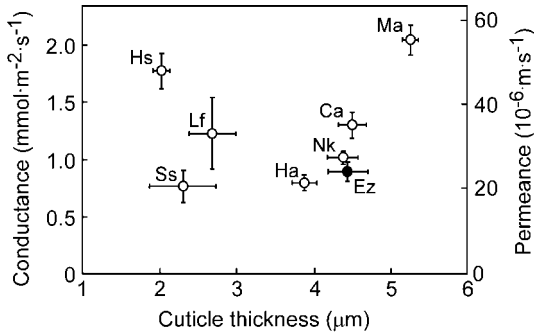


Fig. 13. Mean cuticular conductances and cuticle thicknesses (± SE) of the astomatous (adaxial) surface of *Eidothea zoexylocarya* (filled circle) and seven other tropical rainforest Proteaceae tree species, *Lomatia fraxinifolia*, *Carnarvonia araliifolia*, *Helicia australasica*, *Hollandaea sayeriana*, *Megahertzia amplexicaulis*, *Neorites kevediana* and *Stenocarpus sinuatus* (open circles). The species are labeled with their initials. The conductances are estimates at near zero water potential and approximately 22°C and 30% relative humidity. Equivalent values as permeances assuming a temperature of 22°C and atmospheric pressure of 920 hPa are given on the other axis.

The size, abundance, and apparent uniqueness of the pores in *E. zoexylocarya* raise the obvious question of whether the pores are functional. In our opinion, the lack of cuticular pores over the closely connected guard and subsidiary cells is strongly suggestive of some water-related function. It also argues against alternatives such as pathways for the extrusion of cuticular and epicuticular waxes or means of protecting underlying tissues from UV light through diffraction.

One possibility is that the pores could play a role in absorbing water from mist after periods of water stress or otherwise rehydrating the leaf internally. The low conductance shown in Fig. 13 would argue against a water uptake capacity. However, Beyer et al. (2005) showed that, for isolated leaf cuticular membranes of a range of species, the cuticular permeability for water uptake was much greater than the cuticular permeability for transpiration. Also, absorption of water through leaves has been shown in other species in seasonally water-stressed but frequently misty environments, including *Sequoia sempervirens* (D. Don) (Burgess and Dawson, 2004) and the Australian rainforest tree *Sloanea woollsii* F. Muell. (Yates and Hutley, 1995). However, more research is required to explain this uptake because no cuticular pathways were demonstrated in these studies (see also Kerstiens, 1996). Water stress in the Wet Tropics occurs not only during the winter dry season but also in the wet season when rainfall events are erratic and high radiation levels and temperatures at the canopy level can result in large vapor pressure deficits (Cunningham, 2004). Such stress may be particularly significant physiologically because the wet season is the growing season for most plants in this region. Because the cuticular pores of *E. zoexylocarya* effectively add 200% more epidermal area, they may be a unique means of increasing leaf water content without expensive trade-offs such as increasing outside leaf surface area. *Eidothea zoexylocarya* leaves also have at least one other unusual anatomical feature: Jordan et al. (2005) found that, unlike almost all other closed-forest Proteaceae, including *E. hardeniana*, *E. zoexylocarya* has an adaxial hypodermis (see Fig. 5). This trait was shown to have had multiple derivations in Proteaceae and to be strongly associated with exposure to excess solar radiation. A possible additional stress for *E. zoexylocarya* is that granite-derived soils such as those that support *E. zoexylocarya* are usually acknowledged as being nutrient poor and are more nutrient deficient than the rhyolite-derived soils (Mackey, 1993) that support *E. hardeniana*.

The absence of obvious cuticular pores over the guard and subsidiary cells in *E. zoexylocarya* suggests that the function of the cuticle in these regions is different from elsewhere on the epidermis. One option for this apparent independence is that fluctuations in pore water volume (see earlier Discussion) might compromise guard cell control. Differences in stomatal vs. normal epidermal cell cuticle function occur in other plants and are related to the heterogeneous distribution of aqueous vs. lipophilic pathways for water molecules (Schlegel et al., 2005; Schreiber, 2005; Schönherr, 2006; Schreiber et al., 2006). It has also been proposed that stomatal regulation in plants may be mediated via ''peristomatal'' transpiration, in which the guard and/or subsidiary cell cuticle has a greater permeability to water than the surrounding epidermal cuticle (Kerstiens, 1997; Maier-Maercker, 1999; Eamus and Shanahan, 2002; Šantrůček et al., 2004; but see Buckley, 2005).

The hypothesis that the cuticular pores in *E. zoexylocarya* are one of a suite of anatomical and physiological traits associated with evolution in stressful environments requires further testing. On present evidence, this type of cuticle may be seen as yet another of the many unusual, or indeed bizarre, anatomical features possessed by Proteaceae (Johnson and Briggs, 1975).

LITERATURE CITED

ALVIN, K. L., AND M. C. BOULTER. 1974. A controlled method of comparative study of taxodiaceous cuticles. *Botanical Journal of the Linnean Society* 69: 277–286.

BEYER, B., S. LAU, AND M. KNOCHE. 2005. Studies on water transport through the sweet cherry fruit surface. IX. Comparing permeability in water uptake and transpiration. *Planta* 220: 474–485.

BOYER, J. S., S. C. WONG, AND G. D. FARQUHAR. 1997. CO_2 and water vapor exchange across leaf cuticle (epidermis) at various water potentials. *Plant Physiology* 114: 185–191.

BUCKLEY, T. N. 2005. The control of stomata by water balance. *New Phytologist* 168: 275–292.

BURGESS, S. S. O., AND T. E. DAWSON. 2004. The contribution of fog to the water relations of *Sequoia sempervirens* (D. Don): foliar uptake and prevention of dehydration. *Plant, Cell & Environment* 27: 1023–1034.

CARPENTER, R. J. 1994. Cuticular morphology and aspects of the ecology and fossil history of North Queensland Proteaceae. *Botanical Journal of the Linnean Society* 116: 249–303.

CUNNINGHAM, S. C. 2004. Photosynthetic responses to vapour pressure deficit in temperate and tropical evergreen rainforest trees of Australia. *Oecologia* 142: 521–528.

DOUGLAS, A. W., AND B. P. M. HYLAND. 1995. Subfamily 3. Eidotheoideae. *In* P. M. McCarthy [ed.], Flora of Australia, vol. 16, Proteaceae, 127–129. ABRS/CSIRO Publishing, Melbourne, Australia.

EAMUS, D., AND S. T. SHANAHAN. 2002. A rate equation model of stomatal responses to vapour pressure deficit and drought. *BMC Ecology* 2: 8.

GREGUSS, P. 1968. Xylotomy of the living cycads. Akademiai Kiado, Budapest, Hungary.

JEFFREE, C. E. 1996. Structure and ontogeny of plant cuticles. *In* G. Kerstiens [ed.], Plant cuticle: an integrated functional approach, 33–82. Environmental plant biology series. BIOS Scientific Publishers, Oxford, UK.

JEFFREE, C. E. 2006. The fine structure of the plant cuticle. *In* M. Riederer and C. Müller [eds.], Biology of the plant cuticle, 11–125. Blackwell Publishing, Oxford, UK.

JOHNSON, L. A. S., AND B. G. BRIGGS. 1975. On the Proteaceae—the

evolution and classification of a southern family. *Botanical Journal of the Linnean Society* 70: 83–182.

JORDAN, G. J., R. A. DILLON, AND P. H. WESTON. 2005. Solar radiation as a factor in the evolution of scleromorphic leaf anatomy in Proteaceae. *American Journal of Botany* 92: 789–796.

KERSTIENS, G. 1996. Cuticular water permeability and its physiological significance. *Journal of Experimental Botany* 47: 1813–1832.

KERSTIENS, G. 1997. In vivo manipulation of cuticular water permeance and its effect on stomatal response to air humidity. *New Phytologist* 137: 473–480.

MACKEY, B. G. 1993. A spatial analysis of the environmental relations of rainforest structural types. *Journal of Biogeography* 20: 303–336.

MAIER-MAERCKER, U. 1999. New light on the importance of peristomatal transpiration. *Australian Journal of Plant Physiology* 26: 9–16.

MARTIN, C. E., AND D. J. VAN WILLERT. 2000. Leaf epidermal hydathodes and the ecophysiological consequences of foliar water uptake in species of *Crassula* from the Namib Desert in southern Africa. *Plant Biology* 2: 229–242.

MILLER, R. H. 1982. Apple fruit cuticles and the occurrence of pores and transcuticular canals. *Annals of Botany* 50: 355–371.

MILLER, R. H. 1985. The prevalence of pores and canals in leaf cuticular membranes. *Annals of Botany* 55: 459–471.

MILLER, R. H. 1986a. The morphology and permeability of isolated cuticular membranes of *Hoya carnosa* R. Br. (Asclepiadaceae). *Annals of Botany* 58: 407–416.

MILLER, R. H. 1986b. The prevalence of pores and canals in leaf cuticular membranes. 2. Supplemental studies. *Annals of Botany* 57: 419–434.

NAIDOO, Y., AND G. NAIDOO. 1998. *Sporobolus virginicus* leaf salt glands: morphology and ultrastructure. *South African Journal of Botany* 64: 198–204.

NIX, H. A. 1991. Biogeography: pattern and process. *In* H. A. Nix and M. A. Switzer [eds.], Rainforest animals: atlas of vertebrates endemic to Australia's Wet Tropics, 11–40. Australian National Parks and Wildlife Service, Canberra, Australia.

OSBORN, J. M., AND T. N. TAYLOR. 1990. Morphological and ultrastructural studies of plant cuticular membranes. I. Sun and shade leaves of *Quercus velutina* (Fagaceae). *Botanical Gazette* 161: 465–476.

PANT, D. D., AND D. D. NAUTIYAL. 1963. Cuticle and epidermis of recent Cycadales. Leaves, sporangia and seeds. *Senckenbergiana Biologica* 44: 257–347.

RIEDERER, M., AND C. MÜLLER [EDS.]. 2006. Biology of the plant cuticle. Blackwell Publishing, Oxford, UK.

RIEDERER, M., AND L. SCHREIBER. 2001. Protecting against water loss: analysis of the barrier properties of plant cuticles. *Journal of Experimental Botany* 52: 2023–2032.

ŠANTRŮČEK, J., E. ŠIMÁŇOVÁ, J. KARBULKOVÁ, M. ŠIMKOVÁ, AND L. SCHREIBER. 2004. A new technique for measurement of water permeability of stomatous cuticular membranes isolated from *Hedera helix* leaves. *Journal of Experimental Botany* 55: 1411–1422.

SCHLEGEL, T. K., J. SCHÖNHERR, AND L. SCHREIBER. 2005. Size selectivity of aqueous pores in stomatous cuticles of *Vicia faba* leaves. *Planta* 221: 648–655.

SCHÖNHERR, J. 1976. Water permeability of isolated cuticular membranes: the effect of cuticular waxes on diffusion of water. *Planta* 131: 159–164.

SCHÖNHERR, J. 2006. Characterization of aqueous pores in plant cuticles and permeation of ionic solutes. *Journal of Experimental Botany* 57: 2471–2491.

SCHREIBER, L. 2005. Polar paths of diffusion across plant cuticles: new evidence for an old hypothesis. *Annals of Botany* 95: 1069–1073.

SCHREIBER, L., S. ELSHATSHAT, K. KOCH, J. LIN, AND J. SANTRUCEK. 2006. AgCl precipitates in isolated cuticular membranes reduce rates of cuticular transpiration. *Planta* 223: 283–290.

SCHREIBER, L., M. SKRABS, K. D. HARTMANN, P. DIAMANTOPOLOUS, E. SIMANOVA, AND J. SANTRUCEK. 2001. Effect of humidity on cuticular water permeability of isolated cuticular membranes and leaf disks. *Planta* 214: 274–282.

STOCKEY, R. A., B. J. FREVEL, AND P. WOLTZ. 1998. Cuticle micromorphology of *Podocarpus*, subgenus *Podocarpus*, section *Scytopodium* (Podocarpaceae) of Madagascar and South Africa. *International Journal of Plant Sciences* 159: 923–940.

STONE, E. C. 1957. Dew as an ecological factor. 1. A review of the literature. *Ecology* 38: 407–413.

THOMAS, H. H., AND N. BANCROFT. 1913. On the cuticles of some recent and fossil cycadean fronds. *Transactions of the Linnean Society of London, B* 8: 105–204.

TRACEY, J. G. 1982. The vegetation of the humid tropical region of North Queensland. CSIRO, Melbourne, Australia.

VON CAEMMERER, S., AND G. D. FARQUHAR. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* 153: 376–387.

WEBB, L. J., AND J. G. TRACEY. 1981. Australian rainforests: patterns and change. *In* A. Keast [ed.], Ecological biogeography of Australia, 607–694. Dr. W. Junk, The Hague, Netherlands.

WESTON, P. H., AND R. M. KOOYMAN. 2002. Systematics of *Eidothea* (Proteaceae), with the description of a new species, *E. hardeniana*, from the Nightcap Range, north-eastern New South Wales. *Telopea* 9: 821–832.

YATES, D. J., AND L. B. HUTLEY. 1995. Foliar uptake of water by wet leaves of *Sloanea woollsii*, and Australian subtropical rainforest tree. *Australian Journal of Botany* 43: 157–167.