Desiccation-induced ultrastructural and biochemical changes in the leaves of the resurrection plant *Myrothamnus flabellifolia*

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**Abstract.** Light microscopy and low-temperature scanning electron microscopy were used to systematically compare the surface and internal ultrastructures of hydrated and desiccated leaves of the resurrection plant *Myrothamnus flabellifolia* (Welw.). This revealed that leaf tissue underwent considerable shrinkage and collapse on desiccation but was supported by a framework of vascular and sclerenchymous tissue, which is responsible for the fan-like shape of the leaves. In addition, the leaf ribs were covered with wax and an internal wax cuticle was observed. Biochemical analysis showed that the cyanidin 3-glucoside content increased on desiccation as did the trehalose and sucrose contents. Salt deposits were observed at the apices of desiccated leaves in the proximity of hydathode-like structures. We propose that this might regulate the leaf salt content since decreased intracellular cation concentration was observed in desiccated leaves. We believe that these unique adaptations contribute to the remarkable desiccation-tolerance properties of this plant.

**Introduction**

Resurrection plants are unique in that their vegetative tissue possesses the ability to survive reversible dehydration (desiccation) to an air-dry state (>5% the relative water content, RWC) (Gaff 1971, 1977). These interesting plants are predominately located in arid and semi-arid regions of the world (Porembski and Barthlott 2000). A high number of resurrection plant species are found in Africa, Australia and South America (Gaff 1971, 1977). In Africa, *Myrothamnus flabellifolia*, a woody shrub, is considered to be one of the largest (0.5–1.5 m high) and most widely distributed resurrection plant species (Sherwin and Farrand 1998; Glen et al. 1999; Moore et al. 2007). Its distribution is restricted to Africa, south of the Sahara Desert, although a related species, *Myrothamnus moschatus*, is reported to grow in Madagascar (Glen et al. 1999). The African distribution of *M. flabellifolia* is disjunct, with populations found in western Namibia and Angola separated by the Kalahari Desert from populations in South Africa and Zimbabwe (Wiemarck 1936; Puff 1978; Glen et al. 1999). Although phenotypically very similar, these populations have recently been shown to be genetically different and to produce different polyphenols (Moore et al. 2005a, 2005b). A recent phylogenetic analysis has placed the *Gunnera* as the closest relative to *Myrothamnus* (Wanntorp et al. 2001). On the basis of a combination of molecular data and fossil evidence, it has been proposed that the divergence between the *Gunnera* and the *Myrothamnaceae* occurred 110 million years ago (Wanntorp et al. 2001). The survival of *Myrothamnus* from the Cretaceous period to the present may have been brought about by both its desiccation tolerance as well as its adaptation to a niche environment, namely the rock inselbergs (Porembski and Barthlott 2000; Wanntorp et al. 2001). This plant may therefore display novel anatomical, ultrastructural and biochemical adaptations to desiccation tolerance. As an example, *M. flabellifolia* is the only resurrection plant with fan-like (*flabellifolia*) leaves, which fold upon desiccation.

In this paper, we have investigated the anatomical adaptations and ultrastructural changes that occur on desiccation of these fan-like leaves. We have used low-temperature scanning electron microscopy to systematically compare hydrated and desiccated leaves with respect to their surface and internal ultrastructures. We also performed a qualitative and quantitative biochemical analysis of pigments, saccharides and metal ions in both hydrated and desiccated *M. flabellifolia* leaf tissue.

**Materials and methods**

**Plant material**

*Myrothamnus flabellifolia* plants, collected from the Buffelskloof Nature Reserve and the Magaliesberg Mountains, Mpumalanga Province, South Africa, were maintained in a glasshouse at the Department of Botany, University of Cape Town. Desiccation of whole hydrated (~90% RWC) plants was performed by withholding water and allowing the plants to dry naturally under ambient environmental conditions until the plants reached an air-dry state (~12% RWC) after approximately 5–7 days, similar to that observed previously (Goldsworthy 1992). RWC was calculated as described previously (Cooper and Farrand 2002).

**Light microscopy**

Whole leaves and leaf segments were viewed and photographed with a WILD W400 photomakroskop microscope fitted with...
an AxiosCam digital camera; images were captured by using Zeiss software. Leaf segments (1–2 mm²) were excised from the mid-blade of hydrated or desiccated leaves and fixed overnight at 4°C with 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.4 supplemented with 0.5% caffeine. Fixed samples were dehydrated in ethanol and embedded in epoxy resin (Spurr 1969). Semi-thin sections (0.25 µm) were cut with a Reichert Ultracut-S ultramicrotome. Sections were then transferred onto glass slides and stained with 1% w/v solution of toluidine blue. The sections were then rinsed with H2O and viewed with a Leitz Diaplan optical microscope fitted with an AxiosCam digital camera.

Scanning electron microscopy (SEM)

Scanning electron microscopy was performed with a fully analytical Leica Stereoscan 440 digital scanning electron microscope equipped with a Fisons LT7400 Cryo Transfer System. Leaves from hydrated and desiccated plants were flash-frozen in liquid nitrogen and viewed directly or after freeze-fracturing. Fixation and embedding of leaf segments was as described for light microscopy. Resin blocks were polished with 0.5 µm alumina slurry and viewed in the SEM with a four quadrant backscatter detector. For elemental analysis, certain specimens were coated with gold–palladium or carbon before viewing.

Pigment analysis

Anthocyanins were extracted from lyophilised leaf material with 0.1% (v/v) aqueous HCl (Harborne 1998). The extract was loaded from hydrated and desiccated plant leaves with 0.1% (v/v) aqueous methanol and analysed by using a Dionex HPLC system (Harborne 1998; Salvador et al. 2000). Cytochrome c with 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.4 was added to the extract to confirm the presence of anthocyanins. The extract was then rinsed with H2O and viewed with a Leitz Diaplan optical microscope fitted with an AxiosCam digital camera.

Leaf ultrastructure of M. flabellifolia

Leaf ultrastructure of desiccated and hydrated plants was examined under a dissecting light microscope. The hydrated adaxial leaf surface (not shown) appeared green and reflective and consisted of alternating lighter ridges and darker furrows. The arrangement of ridges and furrows caused the leaf to resemble an open fan (flabellifolia = fan-like). The hydrated abaxial leaf surface (Fig. 1A) viewed under identical conditions looked remarkably similar but with a more corrugated appearance. Examination of desiccated leaves revealed that the structures of the adaxial (not shown) and abaxial (Fig. 1D) surfaces were very similar to one another but distinctly different from that of hydrated leaves (Fig. 1A). Essentially, the furrows that were visible in hydrated leaves were narrower in desiccated leaves, resulting in the ridges being brought closer together concomitant with a reduced leaf surface area (Fig. 1B). Whereas the abaxial ridge surfaces (Fig. 1B) were dark reddish-brown in colour, uneven and highly reflective, the adaxial leaf surface had light green reflective striations (not shown). Transverse sections through hydrated and desiccated leaves are shown in Fig. 1C. D. Hydrated leaves were of a uniform green colour (Fig. 1C), with a wax-like coating on the abaxial surface. In contrast, desiccated leaves appeared as a reddish-brown and light green rosette-like cluster of several leaves (Fig. 1D), with the reddish-brown pigment present only along the abaxial surfaces. As with hydrated leaves, a wax-like coating was present on the abaxial surfaces (Fig. 1D).

We considered that the reddish-brown pigment observed might be due to the presence of anthocyanins. The presence of these pigments was therefore investigated spectrophotometrically at 529 nm in an extract from both desiccated and hydrated leaves (Harborne 1998; Rivas-Gonzalo 2003). Chlorophyll and carotenoids were extracted from leaf material with 80% acetone (Lichtenthaler 1987; Harborne 1998). The chlorophyll and carotenoid contents were determined (Lichtenthaler 1987) from the absorbance of the extracts at 470, 644.8 and 661.6 nm.

Sugar and cation analysis

Sugars were extracted from lyophilised leaf material with 70% aqueous methanol and analysed by using a Dionex HPLC system (Harborne 1998; Salvador et al. 2000). Saccharides were identified by comparison with the retention time of commercial standards and quantitated relative to mannitol as the internal standard. The metal-ion content of lyophilised leaf material was determined (Le Roex et al. 2001) after tissue digestion with HF and HNO3 in teflon beakers before analysis using a Perkin Elmer/Spectra Elan 6000 inductively coupled plasma mass spectrometer (ICP-MS, Perkin Elmer, Waltham, Massachusetts, USA). Calibration was achieved by using a set of standard metal-ion solutions. Internal standardisation against rhodium was used to correct for instrument drift. The analytical procedure involved 300 individual intensity measurements with dwell times of 35 ms at each of the analyte masses.

Results

The general appearance of the adaxial and abaxial leaf surfaces of desiccated and hydrated plants was examined under a dissecting light microscope. The hydrated adaxial leaf surface (not shown) appeared green and reflective and consisted of alternating lighter ridges and darker furrows. The arrangement of ridges and furrows caused the leaf to resemble an open fan (flabellifolia = fan-like). The hydrated abaxial leaf surface (Fig. 1A) viewed under identical conditions looked remarkably similar but with a more corrugated appearance. Examination of desiccated leaves revealed that the structures of the adaxial (not shown) and abaxial (Fig. 1D) surfaces were very similar to one another but distinctly different from that of hydrated leaves (Fig. 1A). Essentially, the furrows that were visible in hydrated leaves were narrower in desiccated leaves, resulting in the ridges being brought closer together concomitant with a reduced leaf surface area (Fig. 1B). Whereas the abaxial ridge surfaces (Fig. 1B) were dark reddish-brown in colour, uneven and highly reflective, the adaxial leaf surface had light green reflective striations (not shown). Transverse sections through hydrated and desiccated leaves are shown in Fig. 1C. D. Hydrated leaves were of a uniform green colour (Fig. 1C), with a wax-like coating on the abaxial surface. In contrast, desiccated leaves appeared as a reddish-brown and light green rosette-like cluster of several leaves (Fig. 1D), with the reddish-brown pigment present only along the abaxial surfaces. As with hydrated leaves, a wax-like coating was present on the abaxial surfaces (Fig. 1D).

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Fig. 1. Light micrographs of surface and transverse sections of hydrated and desiccated Myrothamnus flabellifolia leaves. (A) Abaxial surface of hydrated leaf, (B) abaxial surface of desiccated leaf; (C) transverse sections of hydrated leaves; (D) transverse sections of desiccated leaves. Key: chl, chlorophyll; Anth, anthocyanin; wax, wax covering. Scale bars: 1 mm (A); 0.5 mm (B); 1 mm (C, D).

Table 1. Pigments content in the leaves of desiccated and hydrated Myrothamnus flabellifolia plants. Data represent the means and standard deviations for two independent experiments, with at least two replicates per experiment.

<table>
<thead>
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<th>Compound</th>
<th>Hydrated</th>
<th>Desiccated</th>
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<tr>
<td>Anthocyanins (µg g⁻¹ dw)</td>
<td>11.95 ± 1.91</td>
<td>32.26 ± 1.03</td>
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<td>Chlorophyll a (mg g⁻¹ dw)</td>
<td>2.74 ± 0.40</td>
<td>2.28 ± 0.47</td>
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<tr>
<td>Chlorophyll b (mg g⁻¹ dw)</td>
<td>1.58 ± 0.19</td>
<td>1.26 ± 0.24</td>
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<tr>
<td>Chlorophyll a + b (mg g⁻¹ dw)</td>
<td>4.05 ± 0.36</td>
<td>3.52 ± 0.72</td>
</tr>
<tr>
<td>Carotenoids (mg g⁻¹ dw)</td>
<td>0.56 ± 0.06</td>
<td>0.61 ± 0.12</td>
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mass difference of m/z 162 was shown to be glucose by hydrolysis of the anthocyanin extract and subsequent DIONEX HPLC saccharide analysis of the soluble hydrolysate (not shown). We therefore propose that the main leaf anthocyanin is cyanidin 3-glucoside (Fig. 2). Cyanidin 3-glucoside has been reported to be the most common anthocyanin present in the leaves of many angiosperms (Harborne 1998).

We then investigated the general leaf anatomy by light microscopy; to view stained resin-embedded sections of hydrated and desiccated leaves (Fig. 3A, B). Examination of hydrated leaves (Fig. 3A) revealed relatively small (~20µm diameter) cells, many of which were darkly stained, and primitive vasculature (tracheids). The epidermis was uniseriate and the epidermal cells appeared devoid of the darkly stained material (Fig. 3A). Large round cells were observed adjacent to the epidermis in the mesophyll (Fig. 3A). These were spaced at regular intervals along the epidermal mesophyll leaf
Leaf ultrastructure of *M. flabellifolia*

**Fig. 3.** Light microscope (A, B) and scanning electron microscope (C, F) images of fixed and resin-infiltrated leaf tissue from hydrated and desiccated *Myrothamnus flabellifolia* plants. Light micrographs of transverse sections through hydrated (A) and desiccated (B) leaves. SEM image (with backscatter detector) of transverse section through a hydrated (C) and desiccated (D) leaf. SEM micrographs of freeze-fractured surfaces of hydrated leaves (E, F).

Key: V, vascular tissue; P, polyphenol body; E, epidermis; G, gland cell; C, calcium oxalate crystal; S, sclerenchyma tissue; T, tracheids.

Scale bars: 180 µm (A, B); 30 µm (C, D); 20 µm (E); 8 µm (F).

The darkly stained material observed in the majority of the leaf mesophyll cells is possibly phenolic in nature and resembled phenolic-containing cells found in other plant species (Schneider 1976; Mueller and Greenwood 1978; Hayat 1981). In addition, crystalline deposits, possibly calcium oxalate crystals (Grundell 1933), were also visible interspersed among the spongy and palisade mesophyll cells (Fig. 3A). The anatomical features described above for hydrated leaves were also present in desiccated leaves (Fig. 3B). In addition, many of the cells displayed irregular morphology and partial cell-wall folding (Fig. 3B).

The electron-microscopy stain osmium tetroxide is known to fix cellular structures such as membrane lipids, unsaturated macromolecules and phenolics (Hayat 1981; Hall and Hawes 1991). We post-fixed certain aldehyde-fixed specimens with osmium tetroxide to ascertain the cellular location of various cytoplasmic, organelar and vascular substances in hydrated and desiccated leaves (Fig. 3C, D). Alumina-polished resin-embedded samples were imaged by SEM with a four quadrant backscatter detector. This detector allowed the image of regions containing heavy metals to appear as lighter areas in the micrographs against the darker background. These
regions, present as spherical intra-vacuolar deposits in the bulk of the mesophyll cells from both hydrated and desiccated leaves (Fig. 3C, D), were considered to be oxidised phenolic compounds cross-linked to the osmium tetroxide. Phenolic compounds are known to oxidise and cross-link in the presence of osmium tetroxide into hard complexes (Schneider 1976; Hayat 1981). Analysis of the elemental composition of these spherical deposits (Fig. 3C, D) revealed a high relative concentration (>95%) of osmium present. Additional features evident were vascular tissue supported by thick-walled sclerenchyma cells (Fig. 3C) and xylem tracheids (Fig. 3D). Irregular crystalline deposits, corresponding to those observed by light microscopy, were also detected as bright regions in the micrographs (Fig. 3C). Elemental analysis of these irregular deposits revealed a high relative concentration (>98%) of calcium ions, suggesting that these irregular structures might be calcium oxalate crystal deposits (Franceschi and Horner 1980; Webb 1999). These crystal deposits have been reported previously (Grundell 1933) to be present in M. flabellifolia leaf tissue. Further characterisation of leaf vascular tissue was performed by imaging freeze-fractured surfaces of frozen hydrated leaves by cryo-scanning electron microscopy (Fig. 3E, F). The micrographs revealed small sclerenchyma and vascular cells surrounded by larger turgid mesophyll cells (Fig. 3E, F). The cell walls of both these cells were considerably thickened compared with the adjacent mesophyll cell walls (Fig. 3E, F). The sclerenchyma tissue which occurs in the leaf as vascular strands (Grundell 1933) likely acts to reinforce the general leaf shape, and we suggest this is responsible for the fan-like ridges (Fig. 1A–D).

Further characterisation of the adaxial leaf surface was undertaken by cryo-scanning electron microscopy to determine the effect of desiccation on leaf morphology. The adaxial surfaces of flash-frozen hydrated (Fig. 4A, C) and desiccated leaves (Fig. 4B, D) were viewed at increasing magnification. The cuticular ridges and furrows on the adaxial surface of a hydrated leaf appeared as alternating light and dark regions, with the ridges covered in a layer of wax (Fig. 4A, C). Both the epidermal tissue of the ridge surfaces as well as the tissules surrounding the furrows of the hydrated stomata, gland cells and epidermal cells (Fig. 4C). The stomata, which were flush with the leaf surface, consisted of two crescent-shaped guard cells forming the stomatal pore, the lips of which appeared wax-like (Fig. 4C). The stomata appeared to be more prevalent within the furrows, in agreement with a previous study (Goldsworthy 1992) which revealed stomatal indices of 6.2 ± 0.2 for the leaf furrows and 1.3 ± 0.6 for the cuticular ridges. Leaf trichomes and hairs were not present on the adaxial leaf surface (Fig. 4C). Desiccation of the adaxial leaf surface resulted in the cuticular ridges approaching one another, thereby reducing leaf face area (Fig. 4B, D). Wax striations running parallel to the leaf longitudinal axis were clearly visible along the ridges (not shown). Epidermal tissue, particularly in the furrows, underwent extensive cell folding, with only the stomata and gland cells remaining identifiable (Fig. 4D). Desiccated gland cells appeared enlarged (Fig. 4D), which we propose is due to shrinkage of the surrounding epidermal tissue, exposing more of the seemingly rigid gland cells. Desiccation resulted in considerable shrinkage of the stomatal guard cells, leaving only an open stomatal pore and the associated wax lips visible (Fig. 4D). The desiccation-induced folding of the epidermal tissue around the stomata resulted in the stomata appearing sunken beneath the epidermal folds (Fig. 4D). The presence of open stomatal pores in both hydrated and desiccated plants supports previous observations that severe dehydration of resurrection plants results in passive re-opening of their stomata (Gaff 1989; Schwab et al. 1989; Vicré 2001; Vicrè et al. 2004).

A similar cryo-scanning electron microscopic characterisation of the abaxial leaf surface was also undertaken. The abaxial surfaces of flash-frozen hydrated (Fig. 4E, G) and desiccated (Fig. 4F, H) leaves were viewed at increasing magnification as before. The cuticular ridges present on the abaxial surface of hydrated leaves (Fig. 4E) were considerably broader and more defined than those observed on the corresponding adaxial surface (Fig. 4F). These ridges consisted of only epidermal and gland cells and were also covered in a thick layer of wax (Fig. 4G). Leaf furrows consisting of epidermal tissue were also evident on the abaxial surface (Fig. 4G). The adjacent epidermal tissue in the furrows consisted of numerous stomata and gland cells, interspersed among epidermal cells (Fig. 4G, and inset). Previous analysis (Goldsworthy 1992) revealed a stomatal index of 9.0 ± 0.9 for the leaf furrows, with no stomata detected on the cuticular ridges. Leaf trichomes and hairs were not present on the abaxial leaf surface (Fig. 4E, G). On desiccation, the epidermal tissue of the leaf furrows underwent considerable shrinkage and folding, resulting in the cuticular ridges coming together, thereby causing a reduction in leaf face area (Fig. 4F). The cuticular ridges appeared buckled and consisted of lines of wax-covered cells which retained their hydrated size and shape (Fig. 4F, H). The striated wax coating is clearly visible under higher magnification (Fig. 4H).

We next investigated the effect of desiccation on the morphology of the leaf apices by cryo-scanning electron microscopy. Micrographs at increasing magnification of hydrated (Fig. 5A, C) and desiccated (Fig. 5B, D) leaf apices are shown. Numerous gland cells and stomata interspersed between regular epidermal cells in a pattern similar to that found on the abaxial leaf surface were also present at the apex of the hydrated leaf (not shown). A ridge, possibly an extension of a cuticular ridge on the leaf abaxial surface (Fig. 4E–G), was found to extend to the extreme apex of the abaxial leaf tip (not shown). These cuticular ridges were covered in wax striations (not shown) similar to what was observed at the abaxial surface (Fig. 4H). In contrast, furrows rather than cuticular ridges were visible at the adaxial tip surface (Fig. 5A, D). Higher magnification revealed numerous open stomata protruding above the level of the leaf surface (Fig. 5C). These unusual raised stomata possessed fully open stomatal pores (Fig. 5C, inset), with an opening seemingly wider than observed in the other stomata (Fig. 4C, D, G, and inset), and resembled leaf hydathodes (Fahn 1982). It has been proposed that, in certain plant lineages, stomata have become modified to function as leaf hydathodes in guttation and the secretion of salts (Fahn 1992). Amorphous deposits of unknown composition were observed adjacent to many of these hydathode-like stomata (not shown). Elemental...
analysis of these deposits revealed high relative concentrations (>98%) of calcium ions, suggesting that these deposits are calcium salt secretions from these stomata.

Desiccation caused similar effects at the abaxial surface of the leaf apex as described previously for the general abaxial surface (Fig. 4F, H). Extensive cell-wall folding of epidermal cells occurred, whereas the cuticular ridge cells, gland cells and stomata remained less affected (not shown). A cuticular ridge, consisting of wax striations running parallel to the leaf longitudinal axis, was observed to extend to the leaf apex.
Fig. 5. Scanning electron micrographs of the hydrated (A, C) and desiccated (B, D) adaxial leaf apices as well as freeze-fractured transverse surfaces of hydrated (E, G) and desiccated (F, H) *Myrothamnus flabellifolia* leaves. Key: G, gland cell; st, stomata; D, deposit (calcium salts); sm, spongy mesophyll; pm, palisade mesophyll; ep, epidermis; pd, plasmodesmata; c, calcium oxalate crystal; cs, cytosolic salts; wf, wax filaments. Scale bars: 300 µm (A, B); 80 µm (C, D); 10 µm (E, F); 2 µm (G); 1 µm (H).

(not shown). In contrast, the adaxial surface showed extensive folding of the epidermal tissue, resulting in the formation of distinct furrows extending to the leaf apices (Fig. 5B). In addition, large amorphous deposits resembling desiccated liquid droplets were observed on the adaxial surfaces of the leaf apices of many desiccated leaves (Fig. 5B, D). Elemental analysis showed that these deposits (Fig. 5B, D) also contained a relatively high concentration (>98%) of calcium ions.
Hydathode-like stomata similar to those observed on hydrated leaves were found on the adaxial surfaces adjacent to many of the calcium-rich deposits and surrounded by extensively folded cells (Fig. 5D).

The effect of desiccation on the internal ultrastructure of the leaf was also investigated by cryo-scanning electron microscopy after freeze fracture (Fig. 5E–F). Cross-sections of hydrated leaves (not shown) revealed the expected epidermal and mesophyll cell layers found in other plant species (Fahn 1982). The upper and lower epidermal cell layers consisted of a single layer of cells (uniseriate) (Moore et al. 2006). The mesophyll layer could be subdivided into palisade mesophyll cells adjacent to the adaxial surface (1–2 cell layers) and spongy mesophyll cells adjacent to the abaxial surface (4 or 5 cell layers) (Moore et al. 2006). Calcium oxalate crystals in the form of ‘druses’ (Franceschi and Horner 1980; Webb 1999) were observed in cross-sections of hydrated leaves (not shown). Higher-magnification images of the cell surface of hydrated spongy mesophyll cells (Fig. 5E) revealed an unusual filamentous network of ~100–200-nm-diameter fibres covering the surface (Fig. 5G). These filaments resembled external wax deposits reported to occur in other species (Juniper and Jeffree 1983). In contrast, cross-sections of desiccated leaves revealed that considerable shrinkage and folding of epidermal and mesophyll cells had occurred, resulting in no clearly defined cell layers remaining visible (not shown). Calcium oxalate crystals (Fig. 5F) and the filamentous network (Fig. 5H) were also detected in cross-sections of desiccated leaves. Amorphous deposits, possible cytosolic constituents emanating from cells damaged during the freeze-fracture procedure, were observed in the vicinity of foliar cells (Fig. 5F).

Finally, we investigated whether a major change in leaf cations and saccharides occurred on desiccation since the soluble cation ratio has been proposed to be an important adaptation to desiccation (Schwab and Gaff 1986). HPLC analysis of the soluble saccharides present in hydrated leaves showed that the predominant saccharides present in the order of prevalence, were trehalose, fructose, sucrose, glucose, stachyose and raffinose (Table 2). Whereas the concentration of fructose was found to decrease by approximately two-thirds on desiccation, the concentrations of sucrose, trehalose and raffinose were found to be elevated on desiccation, with the latter concentration approximately doubling (Table 2). ICP-MS determination of the common leaf cations K, Ca, Na, Mg and Fe showed that the monovalent cations Na and K decreased in concentration and that the divalent cation Mg increased in concentration upon desiccation.

Discussion

In general, desiccation causes the following three main types of stress: mechanical, resulting in tension developing on the cell wall and plasma membrane; and photo-oxidative, resulting in the production of free radicals; and metabolic (Dalton 1995; Walters et al. 2002; Farrant et al. 2003). M. flabellifolia leaves appear to have evolved several adaptations to counteract these stresses.

The combination of unique structural and ultra-structural features appears to prevent mechanical damage associated with desiccation in M. flabellifolia leaves. The general morphology of these leaves consists of a rigid network of vascular and sclerenchymous tissue responsible for the fan-like leaf shape and supporting the surrounding mesophyll and epidermal tissue. We propose that the retention of leaf shape during desiccation was due to this rigid arrangement, which further allows the morphology to be rapidly regained on ‘resurrection’. In addition, these gland cells, possibly responsible for the production of known essential oils (Fahn 1982; Viljoen 2000; Canny 2000). Recent NMR studies of the refilling process have shown that two different lipid layers occur in the stem, namely a central core of water-dispersible lipids (Wagner et al. 2000; Zimmermann et al. 2001; Schneider et al. 2003). It was therefore suggested that lipids define the spatial pattern and temporal rate of water rise in refilling branches and thus are vital for the integrity of the water-conducting elements and cells of this species during desiccation (Schneider et al. 2003). It was also proposed that these lipid layers limit the rate of water loss from transpiration during rehydration (Schneider et al. 2003). We believe these properties are equally important in the vascular and mesophyll tissue of the leaves and propose that the lipid lining is an extension of that of the stem into the leaf and might function as a wax drawing water from the xylem into the mesophyll tissue during rehydration. The wax-like lipid filaments in

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<th>Compound</th>
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<th>Desiccated</th>
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<tr>
<td>Trehalose</td>
<td>26.9 ± 19.8</td>
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<td>Fructose</td>
<td>22.7 ± 4.7</td>
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<td>Sucrose</td>
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<tr>
<td>Glucose</td>
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<tr>
<td>Stachyose</td>
<td>4.9 ± 2.7</td>
<td>1.8 ± 1.5</td>
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<tr>
<td>Raffinose</td>
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<tr>
<td>Potassium</td>
<td>12261.1 ± 4677.6</td>
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<td>Calcium</td>
<td>4920.4 ± 1649.5</td>
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<tr>
<td>Iron</td>
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Table 2. Content of various saccharides and metal ions present in the leaves of hydrated and desiccated Myrothamnus flabellifolia plants

Data represents the means and standard deviations of the mean from two independent experiments, with at least two replicates per assay.
the mesophyll might also function to maintain cellular and tissue integrity when desiccated, reduce transpirational loss on rehydration and maintain the pattern and rate of water movement in rehydrating leaves. 

Myrothamnus flabellifolia is regularly exposed to high light intensities (Glen et al. 1999). We propose that this plant uses three lines of defence against photo-oxidation. These are leaf-folding to minimise the area exposed to light, the presence of longitudinal striations of wax coating the sclerenchyma ribs to reflect light (Juniper and Jeffree 1983) and the presence of the pigments cyanidin 3-glucoside and 3,4,5-tri-O-galloylgliconic acid (Moor et al. 2004, 2005) to protect the underlying chlorophyll and carotenoid pigments (Hroullard and Mazza 1990; Bobbio et al. 1994, Chalker-Scott 1999). These two pigments co-occur in the epidermal cells of the abaxial leaf surface. We have previously shown (Moore et al. 2004) that these pigments act synergistically to increase the range of light absorption, thereby minimising the exposure of the underlying chlorophyll and carotenoid pigments to light. It has been proposed that metabolic stress in desiccation-tolerant organisms is ameliorated by an increased soluble sugar:cation ratio (Schwab and Gaff 1986, Golowin and Dirr 1992). This response is believed to protect membranes and cell walls from damage caused by severe water loss (Walter et al. 2002). Our data concur with this proposal as we found an increased non-reducing disaccharide together with a decreased monosaccharide content, which would diminish the possibility of Maillard reactions. In addition, we have previously demonstrated that 3,4,5-tri-O-galloylgliconic acid can protect membrane systems against desiccation and oxidative stress (Moor et al. 2005b). The reduced monovalent cation observed might be brought about by the hydathode-like stomata observed at the leaf apices since hydathodes have been reported to secrete amino acids and cations from Arabidopsis leaves via guttation (Pilot et al. 2004).

Although M. flabellifolia has adapted to desiccation in its own unique manner, there are remarkable similarities in the mechanisms by which it responds to desiccation when compared with other resurrection plants such as Craterostigma spp. Although both M. flabellifolia and Craterostigma accumulate anthocyanins and fold their cell walls on desiccation (Vicrê 2001; Vicrê et al. 2004; Moore et al. 2006), the mechanisms governing these processes are different. M. flabellifolia uses wax to reflect light whereas, C. wilmsii possesses leaf hairs to perform this task (Vicrê 2001; Vicrê et al. 2004). In addition, there are differences in both the quantity and types of anthocyanins present (Dzobo 2005). Wall-folding in M. flabellifolia occurs between the ribs present in the leaf involving no de novo changes in architecture and is facilitated by high levels of arabinose polymers (Moor et al. 2006). In contrast, all mesophyll cell walls fold in C. wilmsii, with the folding involving decreased length of xyloglucan units and an increase in acid pectin and wall-associated calcium ions, proposed to minimise mechanical stress during rehydration (Vicrê 2001; Vicrê et al. 2004). The lipid material currently appears to be unique to M. flabellifolia. However, this requires substantiation as lipid linings and filaments may represent a more widespread adaptation to desiccation than is currently appreciated. Understanding how resurrection plants adapt their ultrastructure and biochemistry to desiccation tolerance has applications in molecular studies aimed at improving the drought tolerance of crop species (Mundree et al. 2002).

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References


Leaf ultrastructure of Myrothamnus flabellifolius

Australian Journal of Botany

491


