Leaf tensile properties of resurrection plants differ among species in their response to drying

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Abstract

Previous studies report that leaf tensile strength (TS) of the desiccation tolerant (resurrection) grass Eragrostis nindensis does not change on drying, but increases in dried desiccation sensitive Eragrostis species. In this paper we tested whether unchanging TS on dehydration is a common feature among 4 resurrection species, Craterostigma wilmsii, Sporobolus stapfianus, Xerophyta humilis and Xerophyta schlecteri, and how this might relate to leaf structure and mechanisms of protection against mechanical stress of drying. Desiccation sensitive controls were Zea mays and Arabidopsis thaliana. Light and transmission electron microscopy of leaves was performed to determine lignification and the nature of subcellular mechanical stabilization. There was a positive correlation between % lignin/unit cross-sectional area and TS of hydrated leaves. Only the grass, S. stapfianus, did not change TS when naturally dried. All others increased in TS when naturally dried, but there was variation among them when flash dried. In S. stapfianus, mechanical stabilization was by both wall folding (mesophyll) and vacuole packaging (bundle sheath) as reported for E. nindensis. This combination may account, in part, for unchanging TS during drying and may be a feature of resurrection grasses. We conclude that leaf tensile properties differ among resurrection plants and are not necessarily affected by protection mechanisms associated with mechanical stress.

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1. Introduction

Drought tolerance refers to the ability of plants to tolerate the partial loss of cellular water for short periods, whereas desiccation tolerant (resurrection) species tolerate loss of virtually all free cellular water. Plants become quiescent but retain viability in this air-dry state for prolonged periods, resuming metabolism upon rehydration (Farrant, 2000; Walters et al., 2002). Drought tolerance (or resistance) is related to inherent features of the plant, such as having mechanically stiff cell walls (higher tensile strength and modulus of elasticity) (Balsamo et al., 2003a,b, 2006), but may also involve the upregulation of “housekeeping” protection systems (such as antioxidants and, in the more tolerant species, the induction of some Late Embryo Abundant [LEA] proteins), these usually being detected at high relative water contents (RWC) in the range 100–60% (Illing et al., 2005). Desiccation tolerance, on the other hand, includes the induction of a large number of protection mechanisms at RWC below 60% (Farrant, 2000; Mundree et al., 2002; Illing et al., 2005). Among the latter are protection against the mechanical stress of wall collapse and plasmalemma tearing caused by the massive loss of cell volume during drying. Such mechanisms include regulated wall folding (maintaining wall suppleness) and organelle packing that minimize the shear forces associated with such water loss (reviewed in Farrant, 2000; Vander Willigen et al., 2004; Vicre et al., 2004).

The tensile properties of leaves may be a useful tool in studying the response of plants to water-deficit stress (Balsamo...
et al., 2003b, 2005, 2006). In dicotyledonous species it appears as if leaf architecture may play an important role in tensile strength and resistance to damage caused by wilting during periods of water deficit (Balsamo et al., 2003a). In a recent study on three species of grasses from the genus Eragrostis, each with a different drought tolerance, Balsamo et al. (2006) demonstrated that leaf tensile strength increased with degree of drought tolerance. Vascular bundle size and degree of lignification correlated with increased mechanical properties and extent of water loss tolerated before loss of viability. However, in the desiccation tolerant (resurrection) grass, Eragrostis nindensis, there was no change in tensile properties upon natural drying of the plants (a two to four week process when water is withheld from mature plants), despite the structural features of leaves of this species being similar to those of the significantly drought tolerant E. curvula. If leaves are artificially dried extremely rapidly (within 24 h using a flash dry apparatus), then tensile properties of E. nindensis increased on drying, as was observed in the drought tolerant relatives of this species (Balsamo et al., 2005, 2006). Since flash drying is so rapid, these changes in tensile properties could not be due to changes in lignification or bundle sheath size.

However, the lack of changes in tensile properties observed in naturally dried E. nindensis (Balsamo et al., 2005) may be related to the unique desiccation protection mechanisms acquired in this species to survive cellular dehydration to 5% relative water content. Vander Willigen et al. (2004) have shown that mechanical stress is avoided by regulated wall folding in the mesophyll cells, while in bundle sheath cells volume is maintained by the accumulation of small vacuoles filled with proline and protein. It is possible that these changes in the different leaf cell types balance each other out, resulting in the apparent lack of change in tensile properties in E. nindensis. Alternatively, leaves from plants that are ‘flash dried’ do not accumulate protection mechanisms (particularly the vacuole filling) in time but may experience desiccation stress related wall collapse, this possibly resulting in measured increases (artificial) in tensile properties typical of the drought tolerant varieties.

In this paper we test the tensile properties of leaves of a number of resurrection plants during natural and flash drying to see if the trends observed for E. nindensis are universal among them, and if they can be related to structural features (degree of lignification) and mechanisms of protection against mechanical stress. As controls, the tensile properties and structural features of desiccation sensitive plants during drying were also tested. The resurrection plants examined included another grass, Sporobolus stapfianus Gandoger, and two other monocot species, Xerophyta humilis (Baker) T. Durand & Schinz and Xerophyta schlechteri (Baker) N.L. Menezes. Since the vein architecture of monocots and dicots are different, and may play an important role in mechanical properties of leaves (Vincent, 1982; Niklas, 1992), the dicot resurrection plant Craterostigma wilmsii Engl. was included. As desiccation sensitive controls we used juvenile Zea mays L. (monocot) that shares similar leaf morphology with the two Xerophyta sp. tested and Arabidopsis thaliana (L.) Heynh. (dicot) the latter also being of similar size and rosette growth form to C. wilmsii.

2. Materials and methods

2.1. Plant collection and maintenance

C. wilmsii Engl, X. schlechteri (Baker) N.L. Menezes, X. humilis (Baker) T. Durand & Schinz and S. stapfianus Gandoger were collected from the field and maintained in a glasshouse at the University of Cape Town (UCT) as previously described (Sherwin and Farrant, 1996). During the experimental period, plants were maintained under controlled environmental conditions of 16 h light (1000 µmol m⁻² s⁻¹)/8 h dark, 25 °C, 40–60% RH. Z. mays L. and A. thaliana (L.) Heynh. (ecotype Columbia) were grown from seed in potting soil. Z. mays was grown under the same conditions as the resurrection plants above. A. thaliana plants were grown at 16 h light (150 µmol m⁻² s⁻¹)/8 h dark cycle; 22 °C; 80–90% RH. Mature, fully expanded adult leaves from all species were used for all experiments detailed below with the exception of Z. mays where juvenile, fully expanded leaves (leaves 2 and 3 from the base) were used (See Orkwiszewski and Poethig (2000) for details on juvenile vs. adult leaves in this species). The work was repeated over two years, 2004 and 2005 and thus the biological repeats are both among individual plants and across years.

2.2. Dehydration treatments and water content determination

For all species, plants were watered to field capacity approximately 12 h prior to all measurements taken representing that of fully hydrated plants. Natural drying of the plants occurred by withholding watering for up to 1 month, during which time leaf tissue had reached an air-dry state of <10% RWC. For the flash drying experiments, fully hydrated leaves of all species were detached and dried in flash drying apparatus (Farrant et al., 1985) for 24 h until the RWC of leaves had reached <10%. Relative water content of leaf tissue was determined as previously described (Sherwin and Farrant, 1996). Five leaf sections from each of three different plants (2004) or five different plants (2005) were used for water content determination.

2.3. Tensile strength measurements

Leaf tensile strength measurements were recorded using tensometers as previously described (Balsamo et al., 2005, 2006). Four centimeter leaf pieces from the mid section of leaves were used, since Balsamo et al. (2005) have shown that leaf tensile strength, elastic modulus and toughness decrease from leaf base to tip in monocots. The whole leaf of C. wilmsii and A. thaliana were used intact (leaf lengths were approximately 4 cm in total). The failure load values were recorded in Newtons (N). The thickness and width at the point of fracture for all leaves, with the exception of S. stapfianus, were measured using a Promax digital caliper (Fowler instruments, Boston, MA; USA). The cross-sectional areas of the naturally dried and flash-dried S. stapfianus leaves were measured using AxioVision 2.05 software (Carl
Zeiss Vision GmbH, Hallbergmoos, Germany) from digital images captured with an AxioCam digital camera (Zeiss, Hallbergmoos, Germany) of the fractured ends of this grass on a dissecting microscope (Wild Photomikroskop M400, Heerbrug, Germany). These leaves were too curled to measure with calipers. Tensile strength was calculated by dividing the failure load by the cross-sectional area at the fracture (N/mm² = MPa). Between five and 10 replicate leaves were measured for each species and treatment from at least two different plants in 2004 or five different plants in 2005.

2.4. Microscopy: light microscopy and lignin analysis

After fracturing, leaf sections (minimum of 10 samples of 5 mm² pieces, per leaf) in the lower clamp (proximal end of leaf) were fixed and wax embedded as described in Balsamo et al. (2006). Thin sections (12 µm thick) were cut on a Leica Reichert rotary microtome (Vienna, Austria) and mounted on slides coated with Haupt’s adhesive (Johansen, 1940). The sections were de-waxed using xylene and rehydrated though a decreasing ethanol gradient and stained with 1% aqueous toluidine blue solution, staining lignified tissue blue and non-lignified tissue purple (Orkwiszewski and Poethig, 2000). Stained sections were viewed with an inverted light microscope (Nikon, Tokyo, Japan) and images were captured with an AxioCam digital camera (Carl Zeiss Vision GmbH, Hallbergmoos, Germany). Axiovision software was used to measure the total cross-sectional area of the photographed leaf section and the cross-sectional area of lignin per leaf section. The percentage of lignin per unit area was then calculated for each species (wet and dry). There were up to five replicates for each species (both naturally dried and hydrated) per year of analysis.

2.5. Transmission electron microscopy (TEM)

The means by which leaf tissues of the resurrection plants achieved mechanical stabilization was investigated by ultrastructural examination of naturally dried leaves. For comparative purposes, flash-dried leaves were also examined, as were naturally dried leaves of the desiccation sensitive species. 15 leaf segments (approximately 5 mm²) of dry leaf tissue from each of 3 different plants were processed for TEM as described in Cooper and Farrant (2002) and Vander Willigen et al. (2003). Tissues were sectioned using a Reichert Ultracut-S microtome (Vienna, Austria), stained with uranyl acetate and lead citrate (Reynolds, 1963) and viewed with a Jeol CX TEM (Jeol, Tokyo, Japan).

2.6. Statistical analyses

Results were analyzed using STATISTICA version 6.1 ANOVA, Tukey’s HSD and Students t-tests where appropriate. A regression analysis between tensile strength and % lignin/unit area of wet and dry leaf cross sections was determined.

3. Results

3.1. Tensile strength

Fully hydrated leaves of the monocotyledonous species had higher tensile strength values when compared to the leaves of the dicotyledonous species. Mean tensile strength values of desiccation tolerant leaves were higher than desiccation sensitive leaves of the same architectural type. The trend in mean tensile strengths when either fully hydrated or naturally dried was S. stapfianus > X. humilis > X. schlecteri > Z. mays > C. wilmsii > A. thaliana (Table 1). The mean tensile strength of the leaves of the desiccation tolerant monocotyledonous species behaved in two different ways. The mean tensile strength of the leaves of X. humilis and X. schlecteri increased from fully hydrated to both naturally dried and flash dried. Conversely, the mean tensile strength of S. stapfianus leaves remained the same when naturally dried but increased when flash dried. The mean tensile strength of the desiccation sensitive Z. mays did not change when RWC decreased (both naturally dried and flash dried). The mean tensile strength of the leaves of the desiccation tolerant dicotyledonous species C. wilmsii increased from fully hydrated to naturally dried but decreased from fully hydrated to flash dried. The mean tensile strength of the leaves of the desiccation sensitive A. thaliana decreased with reduced RWC (both naturally dried and flash dried) (Table 1).

3.2. Lignin analyses

Leaves from all the species in this study exhibit some degree of lignification as indicated with Toluidine blue staining (Fig. 1). Toluidine blue stains non-lignified tissue/cell walls purple and lignified tissue/cell walls blue (Lawson and Poethig, 1995; Orkwiszewski and Poethig, 2000). The two species with the lowest tensile strength (A. thaliana and C. wilmsii) only exhibited lignification within the vascular bundles. However, dense areas of lignified tissue were observed in the tips of leaves of the desiccation tolerant monocotyledonous plants from the Xerophyta species but not the desiccation tolerant grass S. stapfianus. S. stapfianus had a high degree of lignification in the epidermal cells as well as in bundle sheath extensions.

There was a positive correlation between % lignin/unit cross-sectional area leaf and tensile strength at full hydration (Fig. 2).

Table 1

<table>
<thead>
<tr>
<th>Species</th>
<th>Fully hydrated (MPa)</th>
<th>Naturally dried (MPa)</th>
<th>Flash dried (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sporobolus stapfianus</td>
<td>32.5±1.6</td>
<td>33.7±1.5</td>
<td>51±5.0</td>
</tr>
<tr>
<td>Xerophyta humilis</td>
<td>10.5±1.6</td>
<td>35.2±3.5</td>
<td>25.1±1.8</td>
</tr>
<tr>
<td>Xerophyta schlecteri</td>
<td>13.0±1.3</td>
<td>&gt;25</td>
<td>&gt;25</td>
</tr>
<tr>
<td>Zea mays</td>
<td>5.5±0.7</td>
<td>7.5±1.2</td>
<td>6.8±1.0</td>
</tr>
<tr>
<td>Craterostigma wilmsii</td>
<td>0.56±0.03</td>
<td>2.0±0.2</td>
<td>0.1±0.01</td>
</tr>
<tr>
<td>Arabidopsis thaliana</td>
<td>0.24±0.1</td>
<td>0.1±0.02</td>
<td>0.2±0.02</td>
</tr>
</tbody>
</table>

* Leaves of X. schlecteri had to be notched to facilitate tearing.
However, the results for dried laminas were less definitive (Fig. 2). The desiccation sensitive *Z. mays* had the lowest lignin content and tensile strength of the four monocots tested. The two dicot species had lower tensile strength values than the monocots. However, the trend was the same as the monocots in that the desiccation tolerant *C. wilmsii* had higher tensile strength values than the desiccation sensitive *A. thaliana* (Table 1).

### 3.3. Ultrastructural studies

The subcellular organization of naturally dried mesophyll of dry leaves of the *Xerophyta* species was typical of species in which organelle packing is used for mechanical stabilization (Fig. 3A). The central vacuole had either split into a number of smaller ones, or was replaced by numerous small vesicles that were filled with relatively electron dense material. Chloroplasts had become dedifferentiated and filled much of the remaining cytoplasmic space. In *S. stapfianus*, on the other hand, organelle packing occurred only in the bundle sheath cells (Fig. 3B) of naturally dried leaves but mesophyll cells showed wall folding (Fig. 3C). A similar pattern of mechanical stabilization as reported for the desiccation tolerant grass *E. nindensis* (Vander Willigen et al., 2004). Naturally dried mesophyll cells of *C. wilmsii* showed the classical wall folding previously reported (Vicre et al., 1999, 2004) for this species (not shown).
Flash drying resulted in few of the protection mechanisms associated with mechanical stress to be observed in leaves of any of the resurrection plants. With the exception of *C. wilmsii*, in which at least some survival was observed (Cooper and Farrant, 2002) such drying will kill all species used in this study. The subcellular organization of mesophyll cells of flash-dried *S. stapfianus* and *X. humilis* (Fig. 4A and B respectively) showed plasmalemma withdrawal and rupture typical of mechanical damage associated with severe water loss (Farrant et al., 1985, 1997, 1999, Walters et al., 2002). Bundle sheath cells of *S. stapfianus* had similar loss of subcellular integrity (not shown). In *C. wilmsii*, some wall folding was observed as previously reported for such tissue (Cooper and Farrant, 2002) but subcellular damage was also evident (Fig. 4C).

Natural drying of the desiccation sensitive controls resulted in substantial subcellular damage, akin to that evidenced in flash-dried material of resurrection plants (Fig. 5). Membrane rupture and general cytoplasmic dissolution was evident in mesophyll tissues from both *Z. mays* and *A. thaliana*.

4. Discussion

Tensile properties varied among the leaves of desiccation tolerant and sensitive species and in their respective responses to drying. Our study supports previous observations (De Sousa et al., 1982; O’Reagain, 1993; Vincent, 1982; Balsamo et al., 2003a, 2006; Read and Sanson, 2003; inter alia) that reported...
tensile strength values are consistently higher for most monocotyledonous than dicotyledonous leaves. Within these classes, our results suggest that desiccation tolerant species have higher tensile strengths than desiccation sensitive ones that exhibit similar vein architecture (Table 1; Fig. 2).

One hypothesis of this study was that tensile strength in leaves is positively correlated to the percentage of lignin per unit cross-sectional area of leaf. Fig. 2 shows a positive relationship in fully hydrated leaves where an increase in the amount of lignin correlates with increased tensile strength as has been previously suggested (De Sousa et al., 1982; Balsamo et al., 2003b, 2006). Furthermore, the monocots had higher lignin content per cross-sectional area than dicots supporting most of the available literature in this field. Differences in leaf architecture between monocotyledonous and dicotyledonous plants (parallel vs. reticulate venation) have been proposed as a potential explanation for measured differences in leaf tensile strengths (Cutler, 1971; Niklas, 1992) and this hypothesis was supported by this study (Fig. 1). However, the results for dried tissue were less definitive. One possibility is that the test species responded differently to chemical fixation, or that dry tissues may have suffered varying degrees of swelling and shrinkage during chemical fixing and embedding. Another possibility is that overall gross morphological characteristics that hold for hydrated tissues may not be relevant once tissues are in the dry state for desiccation tolerant laminas as the degree of wall folding and cellular packing of organelles likely varies amongst these species. Further investigations in this area appear warranted.

Fig. 5. Subcellular organization of mesophyll cells from naturally dried leaf of Z. mays. Note total rupture of plasmalemma (arrow) and lack of cytoplasmic detail. C, chloroplast; cw, cell wall; v, vacuole. The scale bar represents 2 µm.
Studies on the tensile properties of forage grasses have found that the amount of fibers and the degree of lignification (De Sousa et al., 1982; Vincent, 1982) in leaf tissues play a major role in the mechanical strength of the lamina. The internal structure of *S. stapfianus* has lignified sclerenchyma, the main fibrous tissue, occurring in bundles of fibers and associated with the vascular tissues (Fig. 3C). Typically in grasses, the vascular tissue accounts for 90–95% of the longitudinal stiffness of hydrated leaves (Vincent, 1982). This could explain the higher tensile strength measurements in *S. stapfianus* when compared to *X. humilis* and *X. schlechteri*, the other desiccation tolerant monocotyledonous plants in this study as *S. stapfianus* had a higher percentage of lignin in leaf cross sections. Kneebone (1960) suggested that lignin content and different structural arrangements explains differences in tensile properties of leaves. The latter half of this hypothesis was supported by Balsamo et al. (2003a) who demonstrated that leaf architecture plays a significant role in tensile strength and drought tolerance in two related species of dicots. Of interest is our observation that *S. stapfianus*, a monocot, had the highest degree of lignification of species tested in this study and had both a different leaf architecture and a lignin deposition pattern when compared with *X. humilis* and *X. schlechteri*, with the lack of a central midrib but extensive lignification of the epidermal cells and bundle sheath extensions evident in leaf cross-section of *S. stapfianus* (Fig. 1). Thus, leaf architecture, even amongst monocotyledonous species, also correlates with tensile strength. Additionally, the non-vascular components may play a role in leaf tensile properties (Greenberg et al., 1989) especially when tissues are partially dehydrated to fully dehydrated (Vincent, 1983; Balsamo et al., 2006) as the mechanical properties of hygroscopic material such as primary cell walls are markedly impacted by overall tissue RWC (Vincent, 1983; Balsamo et al., 2006).

A second hypothesis stated that the tensile strength values of leaves of desiccation tolerant plants would not change upon natural drying. This was observed only in the resurrection grass *S. stapfianus*, in which our data agreed with studies by Balsamo et al. (2005) who found that leaf tensile strength of the desiccation tolerant grass, *E. nindensis*, did not change with decreases in RWC, but there was an increase in tensile strength when leaves were flash dried (and thus not allowed to physiologically ameliorate or lay down protection mechanisms). Usually, it takes two to four weeks for plants to naturally dry down to below 20% RWC when water is withheld. However, flash drying involves placing excised leaves in a closed chamber while dry air is blown over the samples (Farrant et al., 1985). Using this technique tissue typically will reach <20% RWC within 24 h.

*S. stapfianus* was the only species to have both organelle packing (bundle sheath cells Fig. 3B) and wall folding (mesophyll tissue, Fig. 3C) as mechanisms of mechanical stabilization — a feature also present in *E. nindensis* (Vander Willigen et al., 2004), which might account for the observed similarities in tensile properties with drying in these grasses. Furthermore, *E. nindensis* was found to retain mobile water, even when naturally dried to below 20% RWC (Balsamo et al., 2005). As *S. stapfianus* is also a member of the subfamily Eragrostoideae and hence a C4 species (Proctor and Pence, 2002), and the similarity in mechanical behavior between *S. stapfianus* and *E. nindensis* in terms of tensile strength is apparent, it is possible that *S. stapfianus* may behave in a similar fashion to *E. nindensis* with respect to retention of mobile water during desiccation.

In the other resurrection species tested here, natural drying resulted in an increase in tensile properties, but responses to flash drying varied among them. In the *Xerophyta* spp, flash drying also resulted in an increase in tensile properties, but in *C. wilmsii* there was a four fold decline in tensile strength (Table 1). Since the desiccation sensitive control species behaved differently from the resurrection species in response to natural (dicots) and flash (monocots and dicots) drying, we pose the question: Is it possible that the changes in tensile properties observed in the desiccation tolerant species is related to the nature of the protection mechanisms against mechanical stress that are induced upon natural, but not flash drying?

These studies, in tandem with previous work (Balsamo et al., 2005) demonstrate that there is a correlation in resurrection plants between leaf tensile properties and anatomical behaviour (vacular packing and/or wall folding) during drying. For *C. wilmsii* (wall folding) and the two species of *Xerophyta* (vascular packing), tensile strength increased with plants allowed to dry naturally. However, there was no increase in tensile strength for *Sporobolus staphianus* (wall folding for mesophyll tissues, vascular packing in bundle sheath cells) which agrees with previous data collected for the closely related *E. nindensis* (Balsamo et al., 2005). The desiccation sensitive monocot *Z. mays* showed no difference in tensile strength when dry (and dead) while the desiccation sensitive dicot *A. thaliana* decreased in tensile strength when dry (also dead). Due to the small number of available species to test it is difficult to draw any concrete conclusions from these results. All species exhibited a strong correlation between tensile strength and lignin content in the hydrated state. It is possible that mechanical stability may differ markedly between monocots and dicots in the dry state due to vastly different vein architecture. However, it is clear from this study and supported by previous work by others (Sun and Liddle, 1993) that leaf tissues with little lignification become fragile when dried as exemplified by *A. thaliana* when either flash dried or naturally dried to death or with *C. wilmsii* when flash dried (Table 1). What is less clear is the contrasting behaviour of *C. wilmsii* and *S. staphianus* when naturally dried and flash dried. Due to the paucity of recognized dicot desiccation tolerant species it will prove difficult to sort this out. One possibility for future studies is to investigate the architecture and mechanical behaviour of desiccation tolerant ferns, whose vein architecture tends to more closely resemble dicots over grasses and other monocots.

In *C. wilmsii*, mechanical stabilization during natural drying is achieved by regulated wall folding and the “locking” of these folds in place in the dry tissue to minimize mechanical stress of rehydration (Viere et al., 1999, 2004). This mechanism could explain the increased tensile strength of dry leaves, as intricately folded and locked walls might be more difficult to rupture when
pulled due to the increased surface area per unit volume of solids (i.e. — wall material) vs. intercellular space. Flash drying prevented extensive wall folding (Fig. 4C) and possibly also the stabilization of folded walls (Cooper and Farrant, 2002), which could explain the observed decline in tensile strength of leaves dried in this manner. On the other hand, in the *Xerophyta* spp., mechanical stabilization is achieved by increased numbers of small vacuoles and organelle packing (Fig. 3A) and there are no apparent changes in wall architecture (Farrant, 2000; Mundree and Farrant, 2000). It is feasible that cellular packaging could enhance tensile strength of cells, as any solid material that attracts water typically tends to exhibit enhanced tensile properties when dehydrated. However, while flash drying prevented induction of vacuolation (Fig. 4B), the tensile strength of dried leaves was still greater than that of hydrated ones, suggesting an alternative (or additional) factor(s) facilitates the increased tensile strength in dry leaves of these species. It has been shown for several desiccation sensitive monocotyledons, that loss of free water in the cellulose of the mesophyll results in an increase in tensile strength during dehydration (Vincent, 1983; Balsamo et al., 2005, 2006) and we propose that this is simply the case too of the *Xerophyta* spp. tested here.

Our study has shown that there is no consistent trend among resurrection plants with respect to changes in tensile properties of leaves on dehydration. This could be due, in part, to the fact that there are differences among them in their mechanisms of mechanical stabilization (shown in this paper) and other protection upregulated in response to desiccation (reviewed by Farrant, 2000; Mundree et al., 2002; Vicre et al., 2003; *inter alia*). This is unlike the situation in drought tolerant species, where a marked increase in leaf tensile strength in response to water loss is a common and possibly required feature (Vincent, 1983; Balsamo et al., 2006). This study supports the contention that drought and desiccation tolerance require different mechanisms (Alpert and Oliver, 2002; Walters et al., 2002; Balsamo et al., 2006), and demonstrates the necessity of coupling biomechanical measurements to anatomical, ultrastructural and histochemical investigations of plant response to water loss, in order to fully understand the survival strategies of drought tolerant and desiccation sensitive species vs. desiccation tolerant species.

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