Anomalous Pressure Volume Curves of Resurrection Plants Do Not Suggest Negative Turgor

CLARE VANDER WILLIGEN*† , JILL M. FARRANT‡ and N. W. PAMMENTER§

†Botany Department, University of Cape Town, Private Bag, Rondebosch, 7701, South Africa, ‡Department of Molecular and Cellular Biology, University of Cape Town, Private Bag, Rondebosch, 7701, South Africa and §School of Life and Environmental Sciences, George Campbell Building, University of Natal, Durban, 4041, South Africa

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Pressure-volume (PV) curves of the desiccation-tolerant angiosperms, Eragrostis nindensis, Craterostigma wilmsii and Xerophyta humilis, and the desiccation-sensitive species, E. curvula, were compared. The shape of curves for E. nindensis and C. wilmsii differed from the usual curvilinear form. Over the relative water content (RWC) range of approx. 70 to 25%, PV curves indicated water potentials higher than directly measured water activity on frozen-thawed tissue. Anatomical studies showed considerable cell wall folding and a consequent reduction in cell volume in these two species; this was not seen in X. humilis or E. curvula which showed normal PV curves. It is suggested that this wall folding may have prevented the development of negative turgor and physical stress in the cells, and contributed to desiccation tolerance.

Key words: Negative turgor, Craterostigma, Eragrostis, Xerophyta, psychrometry, water relations, water activity, PV isotherm, resurrection plants, desiccation tolerance.

INTRODUCTION

Mechanical damage associated with loss of turgor is proposed to be one of the major causes of irreversible desiccation-induced damage in plants (Iljin, 1957; Vertucci and Farrant, 1995). However, a few plants are able to survive dehydration to an air-dry state (Gaff, 1971; Bewley and Krochko, 1982 inter alia). Desiccation tolerance must involve repair processes during rehydration (observed in many lower order desiccation-tolerant plants) and/or protection mechanisms during dehydration (reviewed by Gaff, 1989, 1997; Oliver and Bewley, 1997; Farrant, 2000), including protection against mechanical damage.

Pressure-volume (PV) curves are a valuable tool in describing plant water relations; the isotherms characterize the relationship between water potential (measured as the negative of the applied pressure) and relative water content (RWC) (Tyree and Hammel, 1972). Previous studies have revealed that some desiccation-tolerant plants have atypical PV curves as illustrated in Fig. 1 (Sherwin, 1995; Beckett, 1997). Beckett (1997) proposed that the unusual isotherms indicate that negative turgor develops during dehydration of these plants. However, no direct evidence or functional significance of the phenomenon was given.

In this study the water relations of three desiccation-tolerant angiosperms (resurrection plants) and one desiccation-sensitive plant were investigated. Anatomical evidence and direct measurements of water activity during dehydration might offer an alternative explanation for the anomalous PV curves.

MATERIALS AND METHODS

Plant material

Eragrostis nindensis Ficalho & Hiern and E. curvula (Schrad.) Nees plants were grown from seed. Whole plants of Xerophyta humilis (Bak.) Dur. and Schinz and Craterostigma wilmsii Engl. were collected from the field as described previously (Sherwin and Farrant, 1996). All
plants were maintained in a glasshouse without additional lighting.

**Pressure-volume curves**

Well-watered potted plants were fully hydrated overnight in plastic bags to bring leaves to full turgor. Leaf discs from mature leaves [second outermost whorl (C. wilmsii, X. humilis) or segments from the second oldest leaf on a tiller (E. curvula, E. nindensis)] were used. Water potentials were measured using CS2 sample chambers and an HR-33T microvoltmeter (Wescor, Logan, Utah, USA) in the dew point mode. Leaf material and instruments were kept at 25 °C for the duration of the experiment. The following procedure was used to construct PV curves. Leaf tissue was weighed at full turgor, sealed in the sample chambers, and water potential was measured after an appropriate equilibration period. The sample holder was then removed from the chamber and the tissue was allowed to dry slightly, before being resealed in the chamber; the water potential was remeasured after equilibration. Tissue was weighed immediately after measuring water potential (in case slight water loss occurred during the equilibration period). This process was repeated until the tissue lost no more weight. Generally, only short times of bench drying between measurements were necessary, although this increased as the tissue dried. The time required for equilibration varied among species and increased (up to 6 h) as tissue water content decreased. When there was no further decrease in weight, leaf explants were dried for 48 h at 70 °C to determine dry weight. Between seven and ten measurements were recorded for each tissue piece, and tissue was taken from at least four different plants for each species. All data were combined to draw a single PV curve. To verify the unusual shape of the curves in E. nindensis and C. wilmsii, water potentials and RWC of leaves that were excised from both dehydrating and rehydrating plants were also measured. These data were combined with the previous measurements from the bench-dried samples for the PV curves presented for these two species.

**Direct measurement of ‘osmotic’ potential (water activity)**

Fully hydrated plants were allowed to dry naturally by withholding water. Leaf samples were taken at regular intervals during dehydration. Leaf water potential and sample weight were recorded as described above. Thereafter, the leaf sample and sample holder, wrapped in at least four layers of ParafilmTM (American National Can) and covered with aluminium foil, were plunged five times into liquid N2 for approx. 30 s over a period of 10 min. After the sample and cup had warmed to 25 °C, they were uncovered and replaced in CS2 sample chambers. After an equilibration period, water potential and weight were recorded again. Dry weight of the leaf sample was measured after oven drying at 70 °C for 48 h. Measurements were taken from at least 15 different leaf segments from each of at least four different plants for each species. The water potential measured after freezing and thawing of tissue is generally considered to be osmotic potential if apoplastic water is negligible (Jones and Rawson, 1979). However, at the low RWCs achieved in this experiment, it is highly likely that the system deviated from ideal behaviour. Thus the term ‘water activity’ (measured by vapour phase equilibration) is used.

**Anatomical studies**

Leaves were sampled at regular intervals from plants which were allowed to dry naturally. For each leaf sectioned RWC was determined on a portion of the leaf immediately distal to that sampled for microscopy. Water content and dry weight (oven-dried for 48 h at 70 °C) were determined gravimetrically and RWC calculated from this and the mean water content at full turgor. Mean water content at full turgor was determined from at least 30 replicates (leaves hydrated on well watered plants sealed in plastic bags overnight).

**Light microscopy**

Transverse hand sections of leaf tissue were viewed with a light microscope (Ascoscope; Zeiss, Hallbergmoos, Germany) and photographed. Cell wall perimeter and area of the region enclosed by the walls were measured from electronic images using an image analysis program (AnalySIS, Soft Imaging Software). At least ten cells were measured on each of approx. 20 images from four replicate leaves from two different plants for each species. Vascular tissue was not measured.

Care was taken to maintain the leaf tissue at the RWC to which it had dried. Preliminary experiments were undertaken to ensure that the RWC of the tissue did not change during sectioning and viewing. Fully hydrated tissues were cut and viewed in deionized water. Tissues of RWC from between 80 and 50 % were hand cut and viewed in a solution of sucrose of equal osmotic potential (measured psychrometrically) to that of the tissue. Hand sections of tissues of 40 % RWC and lower were cut dry and viewed in a solution of PEG 6000 (polyethylene glycol) of comparable osmotic potential (measured psychrometrically). The osmotic potential of the sucrose and PEG 6000 solutions was remeasured after viewing to confirm that there had been no change.

**RESULTS**

Pressure-volume curves of the desiccation-sensitive species, E. curvula, and the three desiccation-tolerant species, E. nindensis, C. wilmsii and X. humilis are illustrated in Figs 2–5 respectively. Apoplastic water, assessed as the intercept on the RWC axes, showed negative values. Similar observations have been made previously on woody tissue using both the pressure chamber and thermocouple psychrometry. E. curvula (Fig. 2) and X. humilis (Fig. 5) had typical curvilinear isotherms, with turgor loss points of −1.25 and −1.05 MPa respectively. However, there was a clear deviation in the shape of the PV isotherms of E. nindensis (Fig. 3) and C. wilmsii (Fig. 4) such that it was not possible to determine a point at which turgor was...
lost in these species. After approaching loss of turgor there was a period during which tissue water content decreased with little change in water potential (70 to 45 % RWC, *E. nindensis* and 65 to 25 %, *C. wilmsii*). Thereafter a linear relationship between tissue volume and the inverse of water potential was observed (Figs 3 and 4).

There was a linear relationship between the negative inverse of the direct measurements of water activity and RWC in all species (Table 1, Figs 2–5). These data correlated well with extrapolations of the linear portion of the PV curves at RWCs below the range where deviation from the norm was observed. Direct measurements of water activity were consistently slightly more negative than values predicted from the respective PV curves (Figs 2–5, Table 1); this result is not due to dilution effects consequent on membrane rupture as that effect would be in the opposite direction. Ultrastructural examination of tissue samples on which water activity had been measured confirmed that in these samples cellular membranes were ruptured (data not shown).

Changes in cell volume during dehydration, measured as a proportion of cell area (area enclosed within the cell wall) at full turgor, are also illustrated for each species in Figs 2–5. Examples of sections illustrating shrinkage from the hydrated to the dehydrated state in the four species are illustrated in Fig. 6. There were no differences in mean cell wall perimeter at any point during dehydration in any species (*P* > 0.05, *n* > 170, data not shown). There was a slight decrease in cell area in the desiccation-sensitive species, *E. curvula* (Fig. 2). In the three resurrection plants,
there was either no change (X. humilis, Figs 5 and 6D) or a
dramatic reduction in the cell area (by 53% in E. nindensis,
Fig. 3, and by 74% in C. wilmsii, Fig. 4) on drying. The
change in cell volume in the latter two species occurred over
the range of RWCs during which no or minimal change in
water potential was recorded—the region of the PV curves
which deviated from the typical shape. Although there are
numerous errors associated with measuring cell volumes
(Zimmermann et al., 1981; Malone and Tomos, 1990), these
data were used for comparative purposes only.
Transmission electron microscopical images of
E. nindensis leaves prepared by freeze substitution (i.e. no
aqueous fixation) showed that the thinner walled mesophyll
cells and bulliform cells (associated with leaf curling) folded
extensively on drying, but the thicker walled bundle sheath
and epidermal cells did not fold (data not shown). Limited
cell wall collapse was observed in the mesophyll and
bulliform cells of E. curvula (data not shown). The
ultrastructure of X. humilis (no cell wall folding but
extensively vacuolated) and C. wilmsii (considerable cell
wall folding in all cell types) is reported elsewhere (Vicré
et al., 1999; Farrant, 2000).

**DISCUSSION**

Unusual PV curves similar to those found in this study
(Figs 3 and 4) have been reported previously, using both
pressure chambers (Oertli, 1993) and thermocouple
psychrometry (Sherwin, 1995; Beckett, 1997). Two possible
interpretations of curves of this type are illustrated in Fig. 1.
The first interpretation is that removal of water beyond the
turgor loss point leads to the development of negative

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**Fig. 4.** Pressure-volume curve of C. wilmsii (●). Open circles indicate
water activity measured on freeze-thawed tissues. Areas enclosed by the
cell walls indicated by open squares. Error bars indicate standard
deviations. B, Enlargement of A, between 0 and 1 MPa⁻¹ (A). Dotted
lines indicate the linear regression and 95% confidence limits for the
water activity data. Solid lines indicate the linear regression and 95%
confidence limits for the water potential data (closed circles marked
with a white dot).

**Fig. 5.** Pressure-volume curve of X. humilis (●). Open circles indicate
water activity measured on freeze-thawed tissues. Areas enclosed by the
cell walls indicated by open squares. Error bars indicate standard
deviations. B, Enlargement of A, between 0 and 1 MPa⁻¹ (A). Dotted
lines indicate the linear regression and 95% confidence limits for the
water activity data. Solid lines indicate the linear regression and 95%
confidence limits for the water potential data (closed circles marked
with a white dot).
turgor. Cavitation or cytorrhysis (cell wall collapse) then releases water which increases the turgor of neighbouring cells (Oertli, 1989, 1993; Beckett, 1997). Although Tyree (1976) disputed early studies predicting negative turgor from osmotic and water potential measurements, this author did suggest that if negative turgor was possible in living plant cells, the PV curve would indeed have to deviate from the usual shape described by Tyree and Hammel (1972). However, even with advances in the pressure probe technique (reviewed by Tomos and Leigh, 1999), negative turgor has yet to be measured in living tissues. The second interpretation is that negative turgor does not develop, and there are two possible mechanisms by which this could occur. Firstly, there could be more than one population of cell types, each having a different turgor loss point, such that the resultant PV curve is in fact a compilation of numerous PV curves. Secondly, close to the turgor loss point there is a reduction in the volume enclosed by the cell wall (wall folding), such that the tissue is maintained at zero to slightly positive turgor, and negative turgor does not develop even when considerable water is lost.

Beckett (1997) proposed that resurrection plants with unusual PV curves had more rigid cell walls, that negative turgor developed in the tissues, and that the cells initially resisted collapse until after a period of negative turgor. However, unlike the irreparable damage that can be caused by extensive cytorrhysis, tissues in resurrection plants do recover from cell wall folding, the regulated process associated with changes in the biochemical properties of the cell walls at specific stages of desiccation (Hallam and Luff, 1980; Goldsworthy and Drennan, 1991; Vicré et al., 1999; Farrant, 2000). Beckett (1997) did not present actual data points, only the results of a spline fit to the data, and so direct comparison of the data presented here and those of Beckett (1997) is not possible.

We propose that the unusual PV curves found in many resurrection plants are not a consequence of cavitation and water release, i.e. negative turgor does not develop. There

<table>
<thead>
<tr>
<th>Species</th>
<th>Data source</th>
<th>$m$</th>
<th>$C$</th>
<th>$R^2$</th>
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<tbody>
<tr>
<td><em>E. curvula</em></td>
<td>PV curve (70 %–0 %)</td>
<td>7.27</td>
<td>0.11</td>
<td>0.90</td>
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<td>Direct measurement</td>
<td>5.40</td>
<td>0.08</td>
<td>0.90</td>
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<td><em>E. nindensis</em></td>
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<td>4.20</td>
<td>0.19</td>
<td>0.78</td>
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<td>Direct measurement</td>
<td>2.11</td>
<td>0.20</td>
<td>0.62</td>
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<tr>
<td><em>C. wilmsii</em></td>
<td>PV curve (25 %–0 %)</td>
<td>0.33</td>
<td>0.01</td>
<td>0.81</td>
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<td>0.29</td>
<td>0.38</td>
<td>0.82</td>
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<tr>
<td><em>X. humilis</em></td>
<td>PV curve (70 %–0 %)</td>
<td>0.01</td>
<td>0.10</td>
<td>0.94</td>
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<tr>
<td></td>
<td>Direct measurement</td>
<td>0.01</td>
<td>0.08</td>
<td>0.92</td>
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There were no significant differences between the slopes of the regressions within each species (ANCOVA, $P < 0.05$, $n > 30$).

![Fig. 6](image_url) Light micrographs of mesophyll tissue of *E. curvula* (A), *E. nindensis* (B), *X. humilis* (C) and (D) *C. wilmsii* photographed in isosmotic PEG 6000 solutions at 5 % RWC ($\times$1380 for all images). Note the extensive cell wall folding in *E. nindensis* (B) and *C. wilmsii* (D). Some cell shrinkage is visible in *E. curvula* (A) but not in *X. humilis* (C).
are a number of lines of evidence to support this hypothesis: (1) Even though the solute concentration would be high and possibly non-ideal at low RWCs, direct measurements of water activity from frozen-thawed tissue were always below the measured water potential values. This indicates that negative turgor does not develop; (2) Extrapolating a straight line from the linear portion of the PV curve data (indirect estimate of ‘osmotic potential’) at low relative water contents (below the RWCs corresponding to the deviation) yields a line statistically indistinguishable from that determined by direct measurement of water activity (Table 1). This indirect estimate of water activity also suggests that negative turgor does not occur; (3) If the deviation from the normal PV curve is a consequence of cavitation or cytorrhysis, extensive membrane damage and hence electrolyte leakage would be expected. There is, in fact, very little leakage of electrolytes from dehydrated leaves of either species with unusual curves (C. wilmsii, Farrant et al., 1999; E. nindensis, Vander Willigen et al., 2001), whereas considerable leakage occurs from E. curvula (Vander Willigen et al., 2001).

Consequently, there has to be an alternative explanation for the unusual curves which does not suggest negative turgor. Unfortunately, it was not possible to identify clearly the point of turgor loss during the anatomical analysis of the tissue at the various water contents, and thus the possibility that numerous PV curves have been superimposed to give the anomalous shape cannot be excluded. However, this would be unlikely as the cells of C. wilmsii, one of the species with an unusual curve, are fairly uniform in their appearance and physical properties. We thus propose that the unusual curves are the result of a reduction in the volume enclosed by the walls. Firstly, the extensive cell wall folding and hence reduction in cell volume measured in this study occurred over the range of RWCs in which the PV isotherms deviate from the norm (Figs 3 and 4). Secondly, the shape of the PV curves could imply considerable changes in the elastic properties of the tissue. No attempt was made to measure elastic moduli, however, changes in cell wall chemistry during dehydration of C. wilmsii have been observed. Such changes, which are likely to affect physical properties of cell walls, are coincident with wall folding (Vicrè et al., 1999).

Tissues that are tolerant of desiccation must prevent mechanical damage associated with the shrinkage consequent upon the removal of water. One way by which this might be achieved is via accumulation of insoluble material that effectively replaces the lost water. This phenomenon has been shown to occur in X. humilis: during drying the vacuoles become packed with insoluble material (Farrant, 2000). This species yields normal PV curves (Fig. 5). An alternative possibility is a reduction in cell volume by cell wall folding. We suggest that the wall folding observed in a number of resurrection plants (which is associated with atypical PV curves) is a phenomenon that reduces physical damage on drying and contributes to the desiccation tolerance of these plants. The fact that there are changes in cell wall chemistry in C. wilmsii (Vicrè et al., 1999) suggests that this is a regulated phenomenon.

The development of single cell sampling and direct measurements of turgor using the pressure probe (reviewed by Tomos and Leigh, 1999) provide an opportunity to analyse the unusual water relations of resurrection plants in more detail. As yet these techniques are limited to peripheral cell layers, and since individual cell volume changes cannot be quantified accurately during dehydration, measurements of elastic properties and PV curves of individual cells within complex tissues are not yet possible. However, measurements of turgor from both pressure probes and psychrometric techniques do agree (Nonami et al., 1987), thus whole tissue investigations will continue to provide holistic interpretations of a system.

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LITERATURE CITED


