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# Some physiological comparisons between the resurrection grass, *Eragrostis nindensis*, and the related desiccation-sensitive species, *E. curvula*

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**Abstract**

Both the poikilochlorophyllous resurrection grass, *Eragrostis nindensis*, and the desiccation sensitive species, *E. curvula*, dehydrate to a relative water content (RWC) of less than 5% in two weeks. On rewatering, most *E. nindensis* leaves (except the older, outer ones) rehydrate and resume normal metabolic activity within a few days, whereas *E. curvula* does not recover. There is a controlled loss of photosynthetic pigments, paralleled with a gradual shutdown in gas exchange during dehydration of *E. nindensis.* On rehydration respiration resumes almost immediately but photosynthesis only restarts at 70% RWC by which time chlorophyll has been resynthesised and anthocyanin content reduced. In contrast, photosynthetic activity in *E. curvula* is maintained down to 40% RWC, after which further drying results in a sudden breakdown of the photosynthetic system and its pigments. At this point, electrolyte leakage and increases $F_V/F_M$ decreases such that below *ca.* 40% RWC, metabolism is irreparably damaged. Interestingly, the older outer leaf in most tillers of *E. nindensis* does not rehydrate. These leaves show signs of membrane damage and curl in an irregular manner similar to those of *E. curvula* during dehydration.

## Introduction

Desiccation tolerance is observed in many prokaryotes, lower eukaryotes, and orthodox angiosperm seeds but is uncommon in vegetative tissues of higher plants (Gaff 1977). Of the approximately 60 desiccation-tolerant (resurrection) angiosperms, more than two thirds are monocotyledonous and nearly half of these are from the family Poaceae (Bewley and Krochko 1982). There have been numerous studies on *Sporobolus stapfianus* [Ghasempour et al. (1998a); Kuang et al. (1995); Quartacci et al. (1997) *inter alia*], but it is suprising that apart from a few isolated studies (Ghasempour et al. 1998b; Sutaryono and Gaff 1992), little is known of the other resurrection grasses.

*Eragrostis nindensis* Ficalho & Hiern (previously *E. denudata*), one of the 11 resurrection grasses from the subfamily Eragrostoideae, is unusual in that it is poikilochlorophyllous (Gaff and Ellis 1974). It is a perennial growing in shallow soils across a broad region of southern Africa (Gibbs Russel et al. 1990). As in a few other resurrection angiosperms, the oldest leaves usually cannot rehydrate and hence do not recover metabolic activity (Gaff 1989).

*E. curvula* (Schrad.) Nees, a popular South African pasture grass (Puliga et al. 1996), which is possibly closely related to *E. nindensis*, is desiccation-sensitive (Gaff and Ellis 1974). In this study the physiology and ultrastructure of the leaves of the desiccation-tolerant *E. nindensis* are characterised and compared with those of the older desiccation-sensitive leaves, and also those of *E. curvula*, during both drying and rehydration.

## Materials and methods

*Plant material*

Seeds of *Eragrostis nindensis* and *E. curvula* were sown in seedling flats and individual seedlings transplanted into 1l bags (1:1 potting soil:river sand mix) and maintained in a glasshouse for six months. Two weeks prior to experimentation plants were transferred to a controlled environment chamber (16 h light (2 000 $\mu$mol $m^{-2}$ $s^{-1}$) 26 °C; 8 h dark, 15 °C, 50–65% RH).

The physiological parameters detailed below were recorded at regular intervals on more than five separate plants during both dehydration (watering withheld) and rehydration (daily watering resumed). Plants remained in a dry state for at least two weeks prior to rewatering. All measurements detailed below (excluding the microscopy studies) were taken on the same piece of leaf tissue from a region *ca*. 2 cm distal to the leaf sheath. Both the oldest leaf on a tiller (referred to as "outer leaf") and the next leaf on a sheath (referred to as "inner leaf") were analysed in *E. nindensis*. Only inner leaves of *E. curvula* were assessed. After rehydration, it was confirmed that the remaining proximal part of inner and outer leaves of *E. nindensis* used in the study did and did not recover, respectively.

*Relative water content (RWC)*

RWC was calculated as the water content divided by that estimated at full turgor. Mean moisture content at full turgor was calculated separately for outer and inner leaves in *E. nindensis* as well as inner leaves of *E. curvula.* This was determined using more than 30 representative leaf samples from hydrated plants which had been covered overnight with plastic bags (most leaves did not rehydrate detached from the plant). Water contents were determined gravimetrically by oven drying at 70 °C for 48 h.

*Soil water content*

Whole potted plants were weighed regularly and soil dry weight estimated from subsamples from each bag at the completion of the experiments. Soil water potential was measured on subsamples in C52 sample chambers using a HR-33T thermocouple psychrometer in the dew point mode (Wescor, Logan, Utah, USA).

*Quantum efficiency of photosystem II*

Quantum efficiency ($F_V/F_M$) was measured on dark adapted leaves using an OS-500 Modulated Fluorometer (Opsi-Sciences, Haverhill, USA) using a saturating light intensity of *ca*. 4 mmol photons $m^{-2}$ $s^{-1}$ and a duration of 1 s.

*Gas exchange*

Net $CO_2$ assimilation was measured across a range of light intensities from dark to an intensity of 2 000 $\mu$mol $m^{-2}s^{-1}$ using a Ciras-1 infrared gas analyser with a Parkinson's Leaf Cuvette and in built illumination unit (PP Systems, Hertfordshire, UK) operated in the differential mode at an ambient $CO_2$ concentration of 350 ppm and 22 °C (50% RH). Stomatal conductance and transpiration rates were also recorded and gas exchange parameters calculated (von Caemmerer and Farquhar 1981).

*Electrolyte leakage*

Membrane integrity was assessed as a percentage of maximum electrolyte leakage. Initial leakage was measured as the change in conductivity during a 40 min period using a CM100 conductivity meter (Reid and Associates, Durban, South Africa). Maximum electrolyte leakage was measured after repeated snap freezing of the leaf samples in liquid $N_2$.

*Pigment content*

The absorbance of leaf extracts in 100% acetone was measured at 470, 644.8 and 661.6 nm on a Beckman DU-64 spectrophotometer (Fullerton, California, USA). Total chlorophyll ($a$+$b$) and carotenoid ($x$+$c$) contents were then calculated (Lichtenthaler 1987) and expressed on a dry weight basis. Anthocyanin concentration was determined from the absorbance of leaf extracts in acidified methanol (methanol:water:HCl (79:20:1)) at 530 and 657 nm (Mancinelli et al. 1975).

*Electron microscopy*

Leaf segments were fixed and embedded for transmission electron microscopy according to the methods used for other resurrection plant material (Sherwin and Farrant 1996). Sections were cut using a Reichert Ultracut-S ultramicrotome (Leica, Austria), post

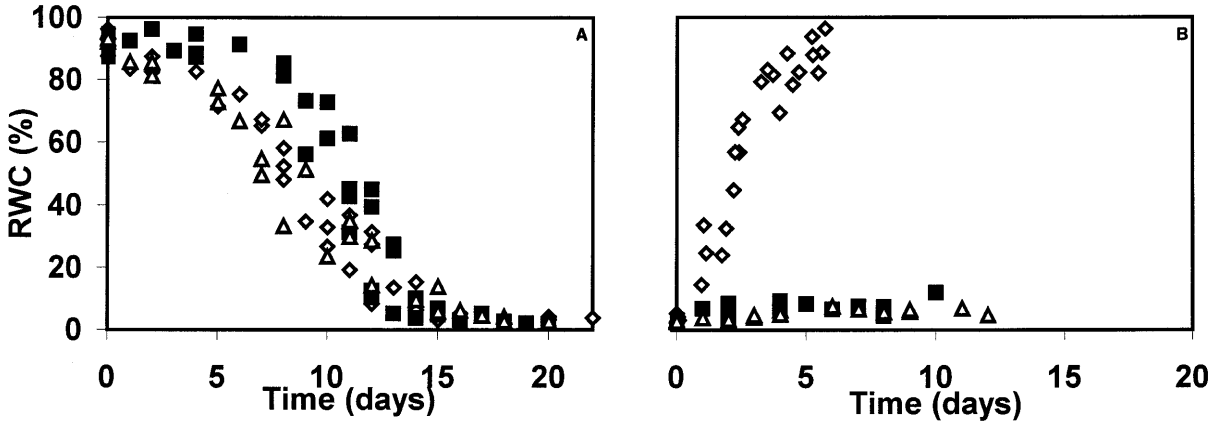


*Figure 1.* Time course of the changes in relative water content during dehydration (a) and rehydration (b) of inner (◇) and outer (△) leaves of *E. nindensis* and inner leaves of *E. curvula* (■).

stained with uranyl acetate and lead citrate and viewed using a Jeol 200CX transmission electron microscope (Akishma, Japan).

## Results

At full turgor absolute water contents were $3.54 \pm 0.42$ and $3.15 \pm 0.56$ g $g^{-1}$ dry mass for inner and outer leaves of *E. nindensis* respectively. The turgid inner leaves of *E. curvula* had an absolute water content of $3.28 \pm 0.35$ g $g^{-1}$ dry mass.

### *Dehydration*

Once water was withheld, soil water content dropped to less than 0.2 g $g^{-1}$ (−1 MPa) in approximately 5 d (data not shown). Concomitant with a precipitous decrease in soil water potential at this point, RWC of all leaves from both species started to decline. After 16 d, RWC for all leaves was less than 5%. There was a slightly more gradual decline in RWC for leaves of *E. nindensis* compared with those of *E. curvula* (Figure 1a).

During dehydration most electrolyte leakage occurred from the leaves of *E. curvula* (Figure 2a). It was found that these leaves were able to recover from a loss of water down to a RWC of *ca.* 40% (data not shown). This critical point, below which further dehydration was lethal, corresponded to a 50% increase in electrolyte leakage. At this time, both edges of the leaves started rolling inwards. There was also an increase in electrolyte leakage in the outer leaves of *E. nindensis* upon drying below 40% RWC, indicating some loss of membrane integrity in those leaves. Similar irregular leaf inrolling was also observed. There was no apparent membrane damage to inner leaves of this species (Figure 2a). These leaves rolled inwards from one edge to form a tight cylinder on drying.

There were no differences in quantum efficiency of photosystem II between the species or with leaf insertion level (Figure 2c). However, differences in pigment composition were observed (Figure 3a, c, e). Chlorophyll and carotenoid contents decreased gradually in all leaves of *E. nindensis* (Figure 3a, c). In *E. curvula* carotenoid content declined gradually but chlorophyll was retained until the critical 40% RWC, at which point there was a sudden loss in pigmentation. Anthocyanin was synthesised only in the inner leaves of *E. nindensis* (Figure 3e).

At high RWCs only was there an immediate stomatal response to changes in light intensity in all species (data not shown). However, as leaf water content dropped, the sequence of gas exchange shutdown differed both between species and with leaf age (Figure 4a, c, e). When soil water became limited, the greatest restriction in transpiration was observed in *E. curvula*, as seen by the large decline in stomatal conductance between 90 and 80% RWC (Figure 4a). These leaves continued to assimilate $CO_2$ until leaf death occurred at *ca.* 40% RWC, although respiration declined more gradually from higher RWCs (Figure 4c, e). At full turgor both net $CO_2$ assimilation and stomatal conductance of the outer leaves of *E. nindensis* were considerably lower than the other leaf types (Figure 4a, e). By 40% RWC, stomatal conductance had declined to low levels in all leaves from both species (Figure 4a). Net $CO_2$ assimilation de-

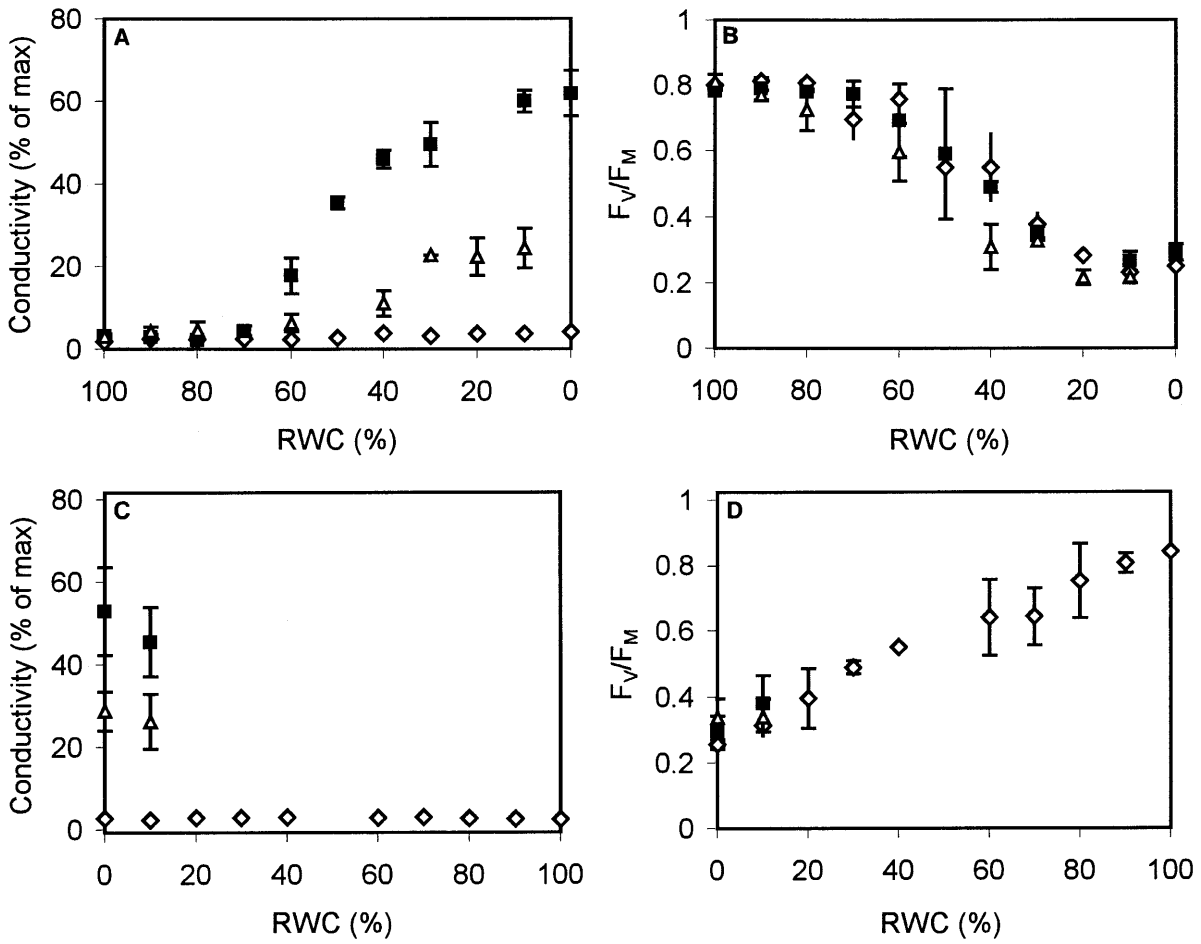


*Figure 2.* Electrolyte leakage and quantum efficiency of photosystem II during dehydration (a and c, respectively) and rehydration (b and d, respectively) of inner (◇) and outer (△) leaves of *E. nindensis* and inner leaves of *E. curvula* (■). Error bars represent standard errors for each 10% RWC interval.

clined gradually in the inner leaves of *E. nindensis* (Figure 4c) but respiration continued until 40% RWC, after which there was a gradual shutdown in this activity (Figure 4e).

Hydrated bundle sheath cells of both species contained a single large vacuole and centrifugally arranged chloroplasts (Figure 5), this Kranz anatomy being indicative of NAD-ME type $C_4$ photosynthesis. Numerous active mitochondria were interspersed among the chloroplasts (Figure 5a). Drying of inner leaves of *E. nindensis* resulted in plasmalemma withdrawal from the cell wall in these cells, but integrity appeared to have been maintained (Figure 6a). Chloroplasts and mitochondria were dispersed in the cytoplasm and numerous small vacuoles were present. Thylakoids had been dismantled. Drying resulted in considerable subcellular damage to the outer leaves of *E. nindensis* (not shown) and those of *E. curvula* (Figure 6b), such that organelles were indistinguishable.

*Rehydration*

Soil water content and water potential rose to previous maximum levels within hours of rewatering (data not shown). Approximately 75% of the leaves of *E. nindensis* recovered to at least 95% RWC after 6 d (Figure 1b) and subcellular organisation was typical of hydrated (control) material (not shown). These leaves rehydrated in a front; the tips often remained dry. All leaves of *E. curvula* and outer leaves of *E. nindensis* did not rehydrate.

There was no change in electrolyte leakage during rehydration in either species (Figure 2b). Cells from

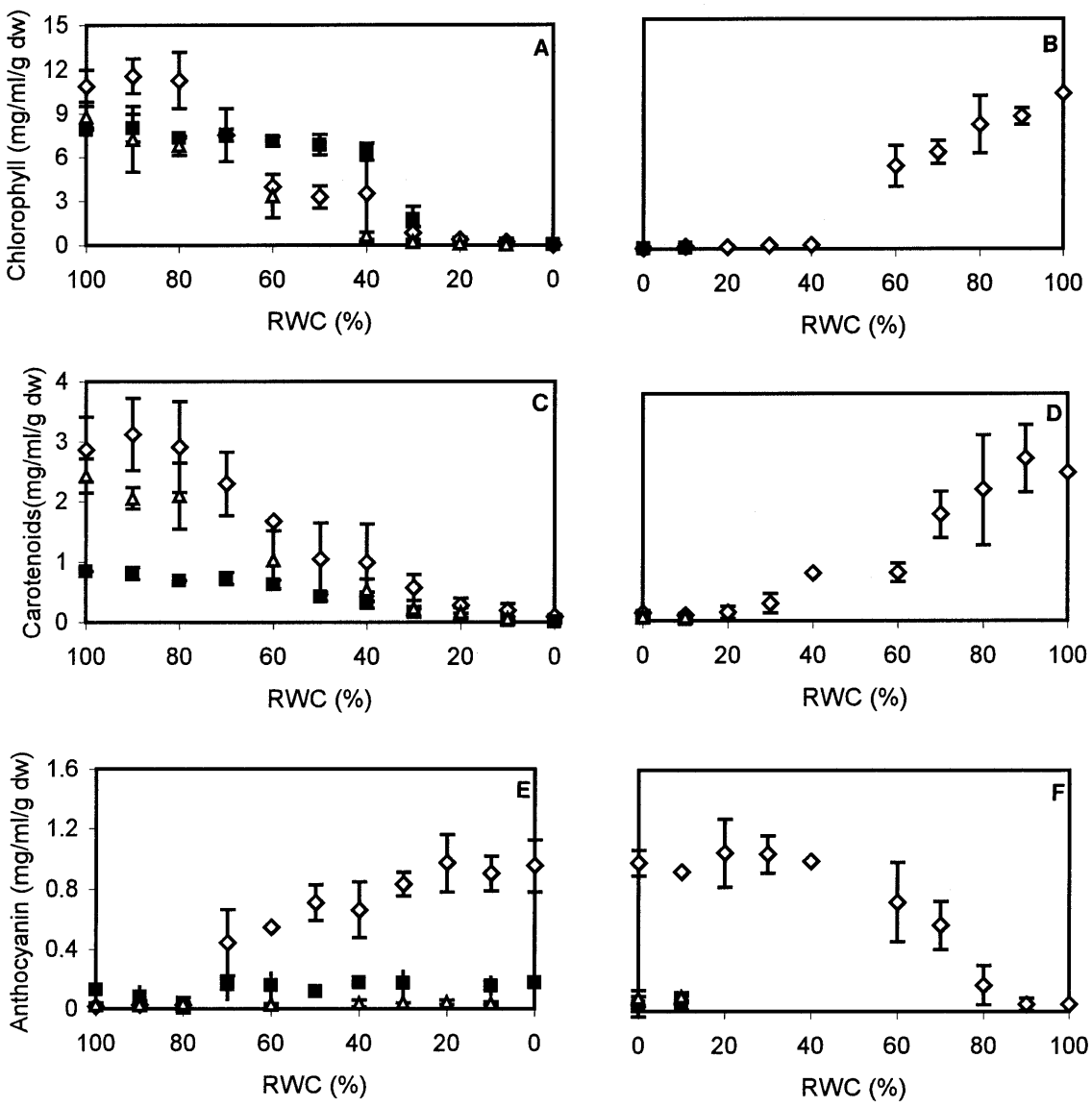


*Figure 3.* Pigment composition of inner (◇) and outer (△) leaves of *E. nindensis* and inner leaves of *E. curvula* (■). Total chlorophyll (a and b), carotenoids (c and d) and anthocyanin (e and f) are plotted against RWC during dehydration and rehydration respectively. Error bars represent standard errors for each 10% RWC interval.

inner leaves of *E. nindensis* appear to have maintained membrane integrity but outer leaves which had incurred membrane damage during dehydration did not recover. Inner leaves of *E. nindensis* showed a gradual restoration of the quantum efficiency of photosystem II after 40% RWC had been attained (Figure 2d). This corresponded with the resynthesis of chlorophyll and carotenoids (Figure 3b, d) and the breakdown of anthocyanin (Figure 3f) in these leaves. There was no change in pigment composition or $F_V/F_M$ in outer leaves of *E. nindensis* or those of *E. curvula* (Figure 3b, d, f).

Gas exchange resumed as soon as rehydration began in the inner leaves of *E. nindensis* (Figure 4b, d). At 40% RWC, respiration peaked. Thereafter stomatal conductance and respiration stabilised at previous rates (Figure 4b, d). Photosynthesis resumed only at 70% RWC (Figure 4f) and reached maximal rates more than 5 d after full turgor had been reached (data not shown). No gas exchange was recorded in the

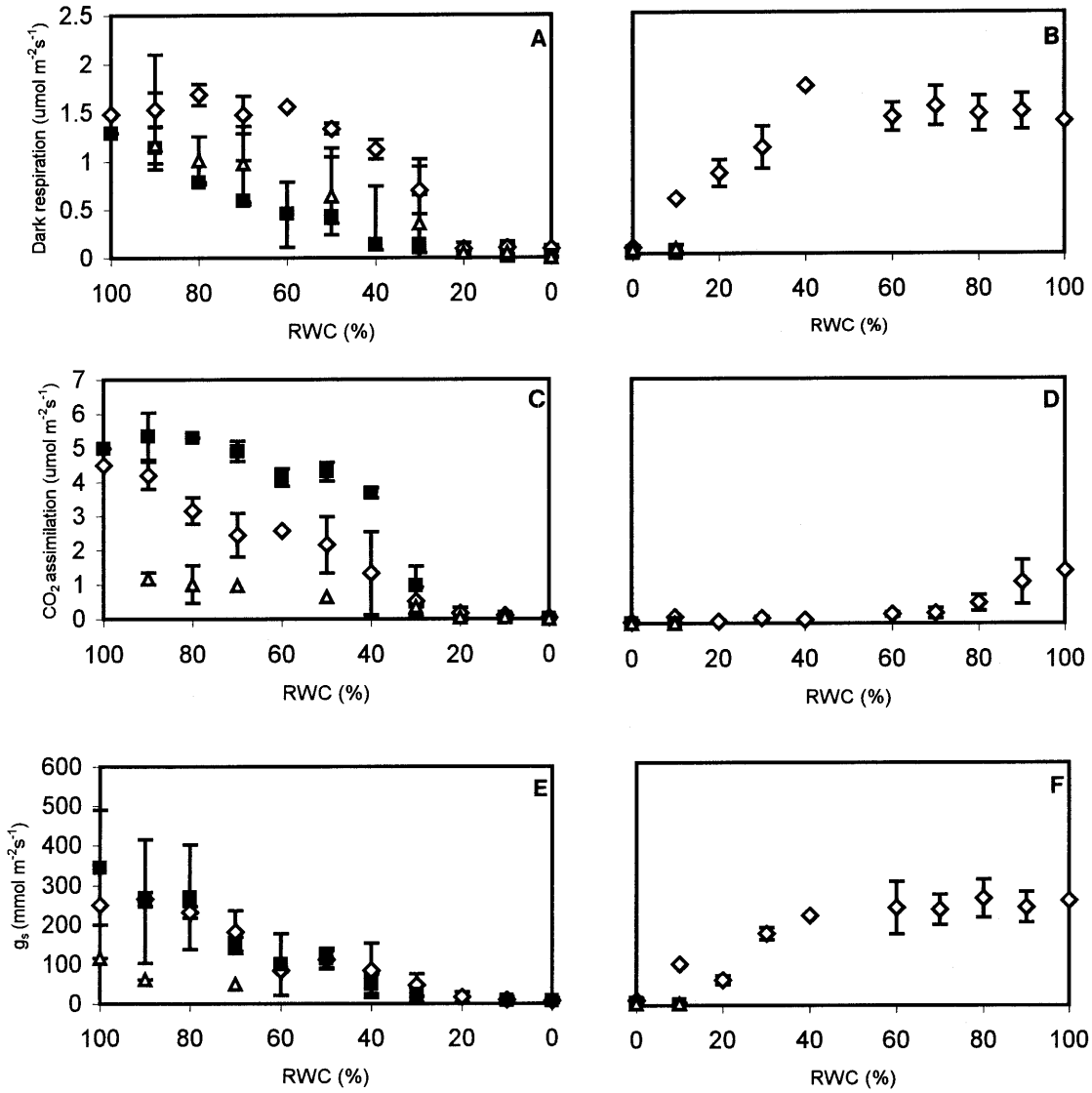


*Figure 4.* Gas exchange at a light intensity of 2 000 $\mu$mol $m^{-2}$ $s^{-1}$ of inner (◇) and outer (△) leaves of *E. nindensis* and inner leaves of *E. curvula* (■): stomatal conductance (a and b), respiration (c and d) and net photosynthesis (e and f) during dehydration and rehydration respectively. Error bars represent standard errors for each 10% RWC.

outer leaves of this species, or in *E. curvula* (Figure 4b, d, f).

## Discussion

Both *E. nindensis* and *E. curvula* have Kranz anatomy typical of all $C_4$ species (Gaff and Ellis 1974; Puliga et al. 1996). Ultrastructural observations suggest that *E. nindensis* belongs to the NAD-ME biochemical subtype (Prendergast and Hattersley 1987) unlike the tolerant *S. stapfianus,*which uses the PEP-CK pathway (Dalla Vecchia et al. 1998). The sensitive *E. curvula* has the same biochemical pathway as *E. nindendsis* (NAD-ME (Gutierrez et al. 1974)). Interestingly, of these two $C_4$subtypes found in Eragrostoideae, the NAD-ME pathway is more prevalent in low rainfall regions (Ellis 1980).

This is in keeping with the observation that *E. curvula* is fairly drought tolerant and can continue growing even at relatively low leaf water potentials (Puliga et al. 1996). The leaves of this grass restrict

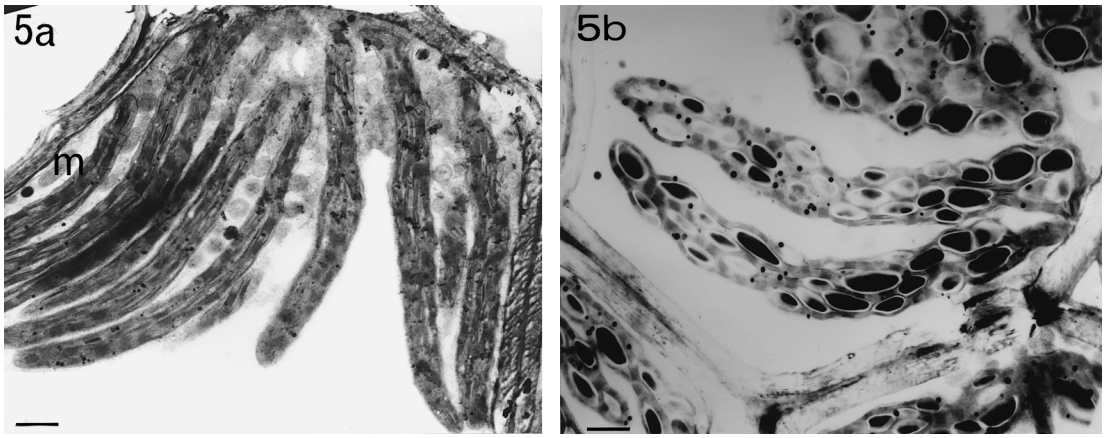


*Figure 5.* Bundle sheath cells of hydrated (a) *E. nindensis* (inner) and (b) *E. curvula*(inner) leaves. Note the (centrifugal) orientation of the chloroplasts at one side of the cell. Mitochondria (m) are interspersed among the chloroplasts in *E. nindensis* and numerous starch grains are evident in *E. curvula.*

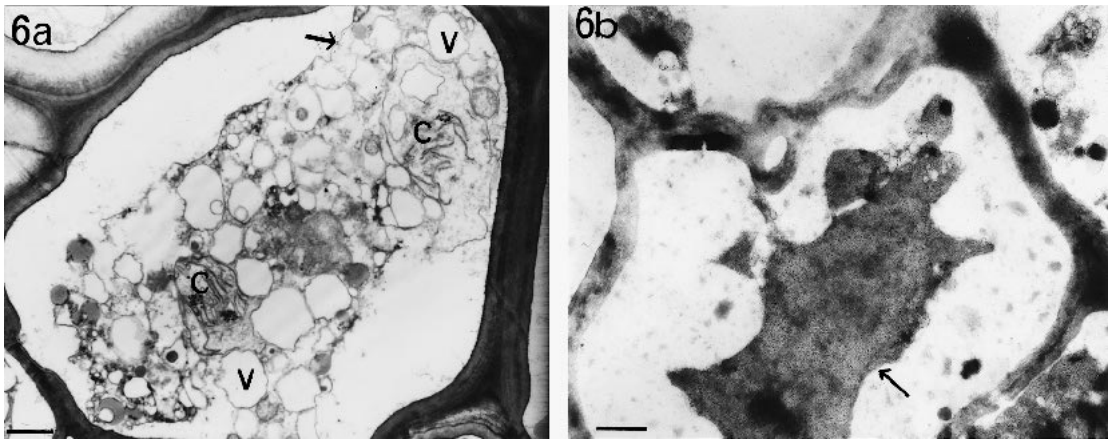


*Figure 6.* Bundle sheath cells of dehydrated (a) *E. nindensis* (inner) and (b) *E. curvula* (outer) leaves. In *E. nindensis* the plasma membrane (arrowed) has withdrawn from the cell wall, but appears intact. Chloroplasts (c) are dispersed in the cytoplasm which contains numerous small vacuoles (v). Thylakoid membranes have become unstacked and vesiculated. Considerable plasma membrane withdrawal is evident (arrowed) in *E. curvula* and the densely stained cytoplasm contains no distinguishable organelles.

water loss by transpiration as soon as RWC drops below about 90%, but they do not have the capacity to survive severe desiccation and remain metabolically active until irreparable damage is incurred at RWCs below about 40% (Figures 2, 3 and 4 and Figure 6b). It was noticed that a colouration suggestive of anthocyanins did accumulate around the base of tillers, but no new growth was observed from the meristem when plants were rewatered following severe water stresses.

*E. nindensis* is reported to be poikilochlorophyllous (Gaff and Ellis 1974) and we have shown that chlorophyll is lost and thylakoids de-differentiate during drying (Figure 3a, Figure 6a). The ability to reversibly switch off metabolism has been suggested to be part of the mechanism of desiccation tolerance (Farrant 2000; Farrant et al. 1997; Pammenter and Berjak 1999; Sherwin and Farrant 1998). Thus the dismantling of the photosynthetic apparatus, associated with the cessation of photosynthesis, constitutes a part of a programmed suspension of physiological activity. In *E. curvula* $CO_2$ assimilation remains at relatively high values until a RWC of 40%, then further drying causes damage to the photosynthetic apparatus and assimilation ceases. The damage is evidenced by the inability of this process to recover. Poikilochlorophylly is an effective mechanism to prevent the consequences of photo-oxidation at intermediate and low water contents (Sherwin and Farrant 1998; Tuba et al. 1998). However, because of the ne-

cessity to resynthesise and reassemble the photosynthetic apparatus, it results in a lag before photosynthesis can resume (Figure 3b, Figure 4f). Most other resurrection grasses are homiochlorophyllous (Gaff and Ellis 1974) or intermediate (*S. stapfianus* (Quartacci et al. 1997)) and hence resume full metabolic activity sooner.

*E. nindensis* (inner leaves) is thus truly desiccation tolerant, whereas *E. curvula* is not. In addition to the light protection mechanisms, the former has many features in common with other resurrection plants that account for its ability to survive drying. These include the maintenance of membrane integrity including tonoplasts (Figure 2a, Figure 6a, Dace et al. (1998); Sherwin and Farrant (1996)); the formation of numerous smaller vacuoles (Figure 6a, Farrant (2000); Farrant et al. (1997); Farrant and Sherwin (1998); Pammenter and Berjak (1999)) and the ability to switch off metabolism (Figure 4a, c, e) respiration and photosynthesis) and recover it on rehydration (Figure 4b, d, f, Farrant et al. (1997); Farrant and Sherwin (1998); Pammenter and Berjak (1999); Vertucci and Farrant (1995)).

It has been suggested that because of the small proportion of desiccation-tolerant species in the *Eragrostis* genus, tolerance is a relatively recent evolutionary adaption (Gaff 1989). Furthermore, species in which desiccation-tolerance is restricted to the basal meristematic or immature tissue (for example, *E. hispida*) are said to be less advanced than those in which mature tissues can rehydrate (Gaff and Ellis 1974). It is thus interesting that although the seed and mature plants of *E. nindensis* are desiccation tolerant, the seedlings are not (personal observation). Although leaf senescence is common to all plants, like some other resurrection plants (Gaff 1989), dehydration also precipitates death in the oldest outer leaves of *E. nindensis*.

The older desiccation-sensitive leaves of *E. nindensis* are usually less photosynthetically active than the younger leaves (Figure 4f). During dehydration chlorophyll degradation and gas exchange patterns in the outer leaves follow the controlled shutdown observed in younger leaves (Figure 3a, Figure 4a, c, e). Although this seems to suggest that the ability to suspend metabolic activity observed in younger leaves has not been entirely lost, anthocyanin is not synthesised (Figure 3e) and membrane damage is incurred (Figure 2a) in the outer leaves during dehydration. Furthermore, like the desiccation-sensitive species, *E. curvula*, the outer leaves curl irregularly. It is possibly the ability of the inner leaves of *E. nindensis* to form a tight spiral, with anthocyanin abundant in the exposed adaxial tissue, that protects them from irreparable light damage during dehydration. The leaves of the resurrection grass *S. stapfianus* do not form tight spirals in the dry state (Dalla Vecchia et al. 1998). Since *E. nindensis* is poikilochlorophyllous and *S. stapfianus* is intermediate (Quartacci et al. 1997), it is surprising that, if leaf curling is an important light protective mechanism, it is not present in *S. stapfianus.* The uncontrolled leaf curling in the older leaves of *E. nindensis* may be a consequence rather than cause of irreparable membrane damage on drying.

The desiccation-sensitivity of rapidly-dried or detached leaves of some resurrection plants is a phenomenon that is of use in studying the mechanisms which confer tolerance in the natural situation (Farrant et al. 1999; Kuang et al. 1995; Quartacci et al. 1997). The comparison of desiccation-tolerant (inner) and desiccation-sensitive (outer) leaves on the same plant (*E. nindensis*) may constitute an even better system for investigating the mechanisms underlying this unusual phenomenon.

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