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Desiccation tolerance is switched on and off in the resurrection fern, *Morhia caffrorum*

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however, when non-dormant *S. alterniflora* seeds were dried, there was a burst in FOX-positive products between 80 and 60% WC (DWB). The FOX burst disappeared before the seeds reached their critical water content (40% WC), and does not appear to be related to recalcitrance. Organic and inorganic leachates were measured to estimate overall membrane damage. When whole *S. alterniflora* seeds were dried, neither organic nor inorganic leachates increased, suggesting that membranes are intact throughout the dry down. Protein carbonyl amounts were measured as an indicator of protein oxidation, and when *S. alterniflora* seeds were dried, carbonyl amounts increased significantly from 2.0 nmol mg⁻¹ protein to over 5.0 nmol mg⁻¹ protein. Protein carbonyl amounts did not increase significantly when *S. pectinata* seeds were dried; however, the baseline carbonyl amounts in *S. pectinata* were higher than the baseline in *S. alterniflora* seeds. The total antioxidant capacity also changed significantly when *S. alterniflora* seeds were dried, decreasing from 35% in fully hydrated seeds, to c. 12% in seeds with a water content <15%. The antioxidant capacity of *S. pectinata* did not change significantly during drying, yet the baseline antioxidant capacity was lower than that of *S. alterniflora* seeds. These results suggest that lipid peroxidation and membrane damage do not play a role in death due to drying, and without proper techniques, lipid peroxidation values may be falsely inflated. Protein oxidation and loss of antioxidant capacity may contribute to loss of viability as *S. alterniflora* seeds are dried.

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***In vitro* regeneration of recalcitrant embryonic axes: Effects on the biomass characteristics of resulting plants**

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In vitro regeneration of the embryonic axes of recalcitrant seeds is a necessary step in practices such as cryopreservation of zygotic germplasm, micropropagation, and the production of disease-free plants. As recalcitrant seeds usually harbour surface and tissue-borne microflora, axes used for *in vitro* culture are invariably subjected to decontamination treatments such as surface-sterilisation with antiseptic substances, treatment with surface- and systemic fungicides and antibiotics, and culturing on variously-modified media formulations. The effects of these treatments are usually evaluated only on the basis of the level of decontamination achieved, with little information available on how these decontamination treatments affect the establishment and/or biomass characteristics of the plants subsequently produced. This study investigated the effects of different *in vitro* culture media, surface-sterilants, phenolic-controlling compounds, systemic fungicides and antibiotics on biomass accumulation and partitioning of seedlings obtained from embryonic axes of *Syzygium cordatum*, a recalcitrant-seeded multipurpose tree species indigenous to eastern and southern

Africa. Embryonic axes were found to be infected with tissue-borne fungi at harvest, and any development after *in vitro* culture depended on decontamination. The decontamination treatments tested were most effective when axes were cultured on a medium containing MS nutrients. Even though the systemic fungicides tested (propamocarb-HCl, triazole and benzimidazole) and the antibiotic, kanamycin, were effective in curtailing the incidence of microbial infection, the axes treated with these compounds developed into stunted plantlets. The decontamination treatments, and the nutrient media on which the axes were originally cultured, also significantly affected the specific leaf area, leaf area ratio, leaf mass ratio, and unit leaf rate of seedlings assessed three months after *in vitro* culture. These results indicate that *in vitro* procedures applied to excised embryonic axes affect growth strategies and competitiveness of the plantlets produced. Future studies will seek to ascertain the basis of (particularly) the adverse effects of the fungicides used, and test alternative products having different active ingredients. (In this context, see current Abstract and Poster of Reddy et al.).

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Desiccation sensitivity in the Arecaceae: Correlates and frequency

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Many palm species are under threat of extinction. However, palms are under-represented in *ex situ* seed banks because of a lack of knowledge about their seed biology. To assess the conservation potential of palm seeds the *Semina Palmarum* project has been established to examine the desiccation tolerance of 200 species. A screening method using 100 seeds has been developed which quantifies seed water content, relative humidity, morphometry, initial germination percentage and rate, responses to short-term storage, desiccation tolerance and seedling morphology. The project has currently generated data for >150 species from c. 70 genera, many of which are new to seed conservation biology. Approximately a third of the species studied had desiccation tolerant seeds, which if extrapolated suggests routine *ex situ* conservation may be possible for c. 1000 species from this family. To enable predictions for seed responses to desiccation for previously unstudied species, here we explore, in a phylogenetic context, correlates of seed desiccation tolerance including germination morphology, seed mass and plant habitat.

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Desiccation tolerance is switched on and off in the resurrection fern, *Morhia caffrorum*

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Resurrection plants are unique in that the vegetative tissues have the ability to dry to 5% relative water content (RWC) and recover full metabolism in existing tissues on rehydration. The mechanisms whereby this is achieved varies among the orders. In bryophytes, where drying and rehydration is rapid, tissues suffer damage during drying but is repaired upon rehydration. In the angiosperms, extensive protection is laid down during protracted drying and little by way of repair is required. The pteridophytes include several resurrection species, but it is not known whether survival is by protection, repair or a combination of both. The present study was undertaken to determine the mechanism of tolerance in the resurrection fern *Morhia caffrorum*. Plants were collected from Table Mountain in summer (the dry season) and maintained in a glass house before and during experimentation. Morphological (frond folding), anatomical and ultrastructural (SEM and TEM), physiological (relative water content [RWC] and electrolyte leakage) and biochemical (quantification of sugars, LEAs and antioxidants) were assessed during drying and rehydration of plants. Plants dried in summer were desiccation tolerant. Fronds dried to 5% RWC had minimal electrolyte leakage and recovered full turgor on rehydration. They curled inwards and chlorophyll shading occurred facilitated by a dense layer of adaxial scales. Sucrose levels increased and a number of heat stable LEA-like proteins were produced *de novo* during drying. These declined during rehydration to levels present in pre-dried fronds. The enzymic antioxidants, ascorbate peroxidase, catalase, glutathione reductase and superoxide dismutase, remained active during the desiccation and rehydration. Subcellular organisation was retained without evidence of damage. During the winter months, when rain is prevalent (albeit that they did not experience the rain), the plants lost the ability to recover from desiccation stress. Fronds did not curl during drying and full rehydration did not occur. Sucrose levels did not increase and no new heat stable proteins appeared upon dehydration. Antioxidant enzymes became denatured and lost activity upon dehydration. During winter, the plants produces spores which became desiccation tolerant and have all the same characteristics of desiccation tolerant "summer" fronds. Upon germination of these spores, an event which occurs in spring and early summer, the new ferns were once again desiccation tolerant. To our knowledge, this is the first report of a species in which desiccation tolerance is seasonal.

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Associations between the biophysics of water and desiccation stress in *Vitellaria paradoxa* seeds

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We previously showed for two batches (1996, 2002) of *Vitellaria paradoxa* (Sapotaceae) seeds that smaller seeds in the population dried most rapidly and that there was a significant linear relationship between whole-seed water content and seed mass during the drying process. Moreover, we showed that only the largest seeds in the population retained viability after drying to whole seed mean target moisture contents, i.e. larger seeds were not more desiccation tolerant but survived as a result of taking longer to desiccate. We then calculated the critical water content (CWC) for viability loss, assuming that the smallest seeds were killed first. Using this approach, we showed that surviving seeds were always above a single CWC, which was seed-lot-specific (c. 20% and 26% FWB in 1996 and 2002, respectively). Analysis of data for this species and 24 other tropical species revealed that a less steep slope for the desiccation mortality curve (% germination against % water content) was associated with increased seed-lot heterogeneity in mass. We concluded that the wide range of desiccation sensitivities typically inferred from such curves is an artefact of seed-to-seed variation in mass, and hence water contents, during drying. In this study we explore, in the same two seed lots of *Vitellaria paradoxa* previously investigated, whether critical water contents are related to: (1) specific biophysical events, such as turgor loss (based on isotherm analysis); (2) the status of water in the seed tissues (using differential scanning calorimetry); and (3) temporal and spatial variation of water across the seed during the drying process (using magnetic resonance imaging).

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Structural and metabolic changes associated with desiccation and rehydration of the roots of a dicot horticultural plant, *Ranunculus asiaticus*

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Ranunculus asiaticus is a horticultural dicot, the tuberous roots of which are able to tolerate desiccation (to 8–10% water content) during its annual life cycle. Root development prior to desiccation is accompanied by changes in the cell walls, with considerable thickening taking place. An increase in starch grains also occurs, and as the root undergoes desiccation there is also a substantial increase in protein. Upon rehydration of the roots, secondary walls expand rapidly upon water influx, presumably due to the extensive presence of pectin therein. As the aerial parts of the plant are formed from latent buds, and photosynthesis becomes established, there is depletion of the cell wall and cell contents of the roots, presumably to provide an early source of carbon and nitrogen. Recent studies have concentrated on the nature of the proteins that are present in the root during and following desiccation. There is a large increase in a particular low mol. mass