



Seed mass variation potentially masks a single critical water content in recalcitrant seeds

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Abstract

For recalcitrant seeds, mortality curves of germination versus water content typically imply a wide range of desiccation sensitivities within a seed population. However, seed to seed differences in water content, during desiccation, may confound our interpretation of these mortality plots. Here, we illustrate this problem for two batches of *Vitellaria paradoxa* (*Sapotaceae*) seeds collected in 1996 and 2002. Whole seeds were desiccated to various target water contents (TWCs) using silica gel. During desiccation, smaller seeds in the population dried most rapidly. Consequently, there was a significant linear relationship between whole-seed water content and seed mass during the drying process. In addition, following desiccation to low TWCs, only the largest seeds in the population retained viability. Taken together, this suggests that the larger seeds survived, not as a consequence of great relative desiccation tolerance, but as a result of taking longer to desiccate. Subsequently, the critical water content (CWC) for viability loss was calculated, based on the assumptions that in the seed population whole-seed water content during desiccation was normally distributed and the smallest, and hence driest, seeds were killed first. Using this approach, the driest seeds in the population that were killed, at each TWC, were always below a single CWC (c. 20% and 26% in 1996 and 2002, respectively). In subsequent experiments the effect of seed size variation on the response to desiccation was confirmed by conducting desiccation screens on seeds sorted into

two discrete size classes, i.e. the seed-lot heterogeneity in mass was reduced. Using this approach, the mortality curves had a steeper slope. Furthermore, data for 24 tropical tree species from the Database of Tropical Tree Seed Research (DABATTS) revealed that seed lots with less variability in mass had steeper mortality curves. Thus, taken together, the data suggest that, at least for whole seeds, the wide range of desiccation sensitivities typically inferred is an artefact of seed to seed variation in mass, and hence water contents, during drying.

Keywords: critical water content, desiccation, seed mass, *Vitellaria paradoxa*

Introduction

Desiccation of seeds, to a range of water contents, followed by germination tests is the usual method for assessing whether or not a species has desiccation-sensitive seeds (Hong and Ellis, 1996; Pritchard, 2004; Pritchard *et al.*, 2004). For recalcitrant-seeded species, this approach typically results in sigmoidally shaped mortality curves. The sigmoid shape of these curves implies that, within a seed population, there is seed-to-seed variation in sensitivity to desiccation that follows a normal distribution. This pattern can be explained by several mechanisms.

First, during seed development, the desiccation tolerance of recalcitrant seeds increases (Hong and Ellis, 1990; Pammenter *et al.*, 1991; Berjak *et al.*, 1993; Tompsett and Pritchard, 1993; Farrant and Walters, 1998), albeit not to the same extent as in orthodox seeds. Consequently, a distribution of individual seed desiccation tolerances could imply that a seed lot was heterogeneous with respect to seed maturity.

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Secondly, it has been proposed, for a number of recalcitrant species, that the rate at which seeds/excised embryonic axes are dried can affect the relative level of desiccation tolerance observed: axes that are dried rapidly spend less time at intermediate water contents where deleterious aqueous-based processes can occur, and hence survive to lower water contents than more slowly dried axes (Farrant *et al.*, 1985; Pammenter *et al.*, 1991, 1998; Pritchard, 1991; Berjak *et al.*, 1992, 1993; Pritchard and Manger, 1998). Individual seed drying rates may be influenced by, among other factors, seed size and seed coat thickness. Consequently, apparent differences between individual seeds in desiccation tolerance may reflect a range of drying rates for seeds within the population: small, rapidly drying seeds might be expected to survive to lower water contents than larger, more slowly drying seeds. Therefore, small seeds may represent the tail (i.e. most desiccation-tolerant proportion of the population) on mortality curves. However, this provides a conundrum, since the smaller seeds in the population might be assumed to be the least developed, and hence the most desiccation sensitive.

Thirdly, it has been suggested that the apparent high level of desiccation tolerance observed in some seeds within a population may reflect these seeds either starting at high relative water contents before desiccation or drying more slowly, perhaps due to greater size. In this scenario, the apparent survival of some individual seeds to comparatively low water contents is explained by seeds having a range of water contents at any given point during drying, and, consequently, some individual seeds have a water content significantly higher than expected (and therefore surviving to low 'apparent' water contents). This phenomenon has been suggested for seeds of both *Zizania palustris* (Probert and Longley, 1989) and *Acer pseudoplatanus* (Dickie *et al.*, 1991). For example, Probert and Longley (1989) found that some seeds of *Z. palustris* appeared to tolerate embryo water contents as low as 10%, whereas other seeds were killed by water contents as high as 30–40%. They attributed this wide range of apparent responses to desiccation to a large coefficient of variation in individual seed water contents during desiccation. Dickie *et al.* (1991) also found a large degree of variation in response to desiccation for *A. pseudoplatanus*, and attributed this variation to differences in water content between individual seeds at any given mean water content. However, neither study tested these propositions directly. For *Quercus robur*, during desiccation, Finch-Savage (1992) found there was a wide range of individual seed water contents at any given mean water content, and suggested that this may mask the existence of a single critical water content (CWC). This hypothesis was reinforced by germination experiments on part seeds:

one part was used for water content determination, the other for germination. Matching individual seed water contents to germination revealed that desiccation damage and death occurred over a reduced range of water contents; viability was reduced from 95 to 5% over a water content range of 9%, when using individual seed water contents (assuming an initial water content of 43%), compared to 25% when using mean water contents during desiccation (Finch-Savage, 1992). However, apart from studies by Finch-Savage (1992), the cause of the sigmoid shape of mortality curves has not been examined in detail (Pritchard, 2004).

It has been suggested recently that there are discrete levels of critical water potential for the onset of seed viability loss: -1.8 , -5 , -12 , -50 and -180 MPa (Walters, 1998) and -4 , -8 , -12 , -23 and -73 MPa (Sun and Liang, 2001). The existence of discrete levels of critical water potential suggests that cells experience specific stresses at each water potential range during desiccation, and desiccation tolerance depends on the ability to tolerate the particular stresses that operate around each water potential level. While data for these critical water potentials are generally based on work with isolated axes, the fact that desiccation-induced mortality may occur over a narrow range of water potentials suggests that a narrow range of CWCs, as opposed to the wide range inferred from mortality curves, should also be observed. None the less, such data are rarely observed.

Exceptions appear to be three *Coffea* species (Dussert *et al.*, 1999) and one study on *Quercus robur* (Finch-Savage, 1992). However, the existence of single CWCs has not been clearly established, and our understanding of CWCs has been clouded by the use of water contents corresponding to the onset of viability loss (e.g. Pritchard *et al.*, 1995a; Tompsett and Kemp, 1996; Walters, 1998; Sun and Liang, 2001), 50% viability loss (e.g. Dussert *et al.*, 1999) and complete viability loss (e.g. Bonner, 1996) to describe seed lot responses to desiccation.

The aim of this study was to investigate whether variation in seed mass, and hence drying rates, influence our assessment of the CWC for viability loss during desiccation of whole seeds of *Vitellaria paradoxa* (Sapotaceae). This species is suitable for this kind of study since its seed size extends over a wide range (approximately 3–17 g fresh weight), and has previously been shown to exhibit recalcitrant seed storage behaviour (Pritchard *et al.*, 1997; Danthu *et al.*, 2000). In addition, data for 24 recalcitrant tree species (from Tompsett and Kemp, 1996) for which seed mass, initial water content and the slope of the relationship between germination and water content during desiccation were available, were further used to examine the role of variation in seed mass and initial water content in our assessment of desiccation tolerance.

Materials and methods

Seed lot details

Seeds of *Vitellaria paradoxa* were collected from Poa, Burkina Faso (grid reference: 12°15'N 2°07'W) in June 1996 (batch 1) and sent immediately to Wakehurst Place, UK. In addition, a further seed lot was collected in July 2002 (batch 2) from Saponé, Burkina Faso (grid reference: 12°03'N 1°43'W) and also sent immediately to Wakehurst Place. On both occasions, seeds were collected from a minimum of 25 trees. Upon receipt, seeds were placed in plastic bags and stored fully hydrated, in the dark, at $15 \pm 1^\circ\text{C}$. An initial water content was determined on 25 (batch 1) or 50 (batch 2) seeds for the seed coat and embryos separately. The dry mass was determined by drying seed components in an oven at 103°C for 17 h (ISTA, 1999).

Desiccation and germination of *V. paradoxa* seeds

For dehydration, six or seven equal aliquots of seeds were placed in polythene bags with an equal weight of freshly regenerated silica gel desiccant. Each aliquot contained enough seeds (at least 125) to permit water content and germination assessment. The bags were held in an incubator at 26°C and periodic re-weighing of the seeds, separated from the silica gel, allowed target weights and hence water contents to be achieved.

For batch 1, seeds were not sorted into size classes prior to desiccation. However, for batch 2, desiccation experiments were performed on a non-sorted subset of the seed lot as well as two sorted subsets. For the sorted subsets, seeds were split into classes based on seed fresh mass (4–6 g and 8–10 g) and then subjected to desiccation. During the desiccation experiments, 25 (batch 1), 40 (sorted, batch 2) or 50 (unsorted, batch 2) seeds were used for water content determinations. Concurrent with the desiccation experiments, hydrated seeds were held as a control, at 26°C , in polythene bags containing an equal weight of expanded mica. These seeds were also used for water content determination and germination, the latter being sown at the same time intervals as desiccated seeds. Seeds (five replicates of 20) were sown for germination on the surface of 1% agar in water in sandwich boxes ($6 \times 11 \times 17$ cm) at 26°C (12-hour photoperiod) and scored for germination every 2–3 d until no further seeds germinated for two consecutive time intervals. Germination was defined as radicle emergence by at least 2 mm. In addition, once desiccated seeds from batch 1 had fully re-hydrated and as germination commenced, the fresh mass of 320 seeds was determined following removal of surface water by blotting on filter paper. This enabled a comparison to be made of the fresh mass of the

whole-seed population with the mass of those seeds that germinated after desiccation.

For batch 2, 20 seeds, selected to represent the entire seed mass range (fresh mass 2.8–16.6 g), were desiccated under conditions identical to the main desiccation experiments. These seeds were weighed daily to enable the rate of water loss to be tracked. After 20 d of desiccation, seed dry mass was determined.

Desiccation and mass determination for species from DABATTS

Values in the Database of Tropical Tree Seed Research (DABATTS; Tompsett and Kemp, 1996) for the slope of the fitted line between probit germination and water content were obtained for 24 species from seven genera within the *Dipterocarpaceae* and *Meliaceae*. In these studies seeds were dried as a mono-layer at 15°C and *c.* 15% relative humidity. Seeds were removed at intervals for water content determination (3–6 individual seeds) and germination tests. For germination tests, two replicates of more than 10 seeds were sown as above. For dry mass determination, 10–20 individual seeds were used.

Statistical analysis

Mortality curves on the co-plots of germination versus water content were analysed by binary logistic regression, using a probit link function implemented in Minitab 13 (Minitab Inc., Pennsylvania, USA). This is a quantal approach and assumes a normal distribution of desiccation tolerances within the population, and that each individual seed in the population is a statistically independent unit (since each individual seed can either live or die following desiccation). Quantal models have been applied previously to desiccation curves (e.g. Dussert *et al.*, 1999). The goodness of fit of these models was assessed using Wald tests (Tabachnick and Fidell, 2001). In addition, the slope of the fitted curves, at the point at which 50% of viability was lost, was used as a measure of the variability in the population response to desiccation.

The water content distribution of individual seeds in the desiccation experiments was tested for normality using the Kolmogorov–Smirnov test (Sokal and Rohlf, 1995). At each water content during desiccation, the following assumptions were made: (1) that the seeds in the germination test had water contents following a normal distribution, with mean and standard deviation the same as the seeds used for water content determination; and (2) that the seeds killed by desiccation at each water content were those with the lowest water content. Subsequently, using the mean and standard deviation of the whole-seed

water content at each target water content (TWC; from Table 1), the NORMINV function in Microsoft Excel 97 (Microsoft Corporation, Redmond, Washington, USA) was used to calculate the water content below which the dead subset of the population (smallest and hence driest seeds) fell.

For the species in DABATTS, the slope of the regression line between probit germination and water content was determined using probit regression in the GLIM statistical package (Crawley, 1993) and was used as a measure of the variability of the seed population response to desiccation.

Results

Effect of desiccation on germination

Desiccation of both batches of *V. paradoxa* resulted in a steady decline in germination, which dropped from *c.* 100% to 0% as the whole-seed water content decreased from *c.* 35% to 15% (Fig. 1). Quantal models with a probit link function were fitted to the mortality curves. For all four mortality curves the model gave a highly significant fit (Wald test, $P < 0.001$), implying a normal distribution of responses to desiccation within the population. Between the two study years, the median water content for 50% of viability loss differed by about 6% (20% and 26%, batch 1 and 2, respectively; Fig. 1). The unsorted seeds (both batches) had mortality curves with a similar slope (3.81 and 3.90% mc^{-1} , batch 1 and 2, respectively). However, for the size-sorted seeds (i.e. less variation in seed mass) the slopes of the mortality curves were steeper (6.40 and 4.85% mc^{-1} for 4–6 and 8–10 g, respectively). The control seeds held moist at 26°C maintained high (>90%) viability throughout the 14-day course of the desiccation experiments (data not shown).

Effect of seed size on water content during desiccation

For non-desiccated seeds there was no relationship between whole seed water content and seed dry mass (Figs 2A and 3A). However, during desiccation, significant linear relationships between whole-seed water content and dry mass developed (Figs 2B–G and 3C–E, Table 1), although by the lowest TWCs this relationship disappeared (Figs 2H and 3F and G). These significant relationships imply that, during desiccation, the smallest seeds in the population dried most rapidly. However, when similar graphs were plotted for the size-sorted seeds, only two significant relationships were found (TWC = 26% for both size classes; $P < 0.05$, Table 1), presumably as a result of the very limited range of seed masses on the *x*-axis.

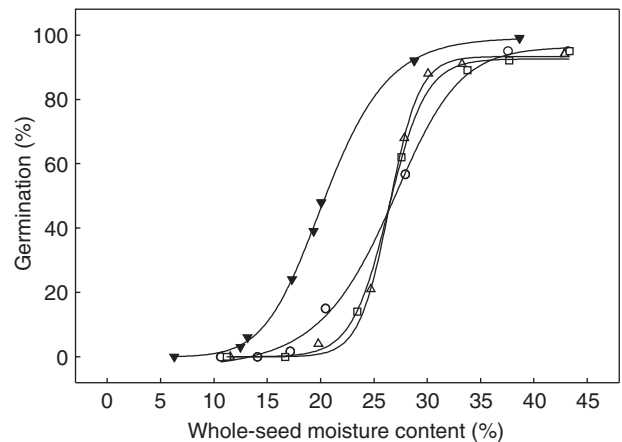


Figure 1. The effect of drying whole seeds of *Vitellaria paradoxa* on seed viability for seeds of batch 1 (▼) and batch 2 (open symbols). Seeds of batch 2 were either desiccated as a bulk non-sorted collection (○) or divided into two mass classes 4–6 g (□) or 8–10 g (△).

For 20 individual seeds, the masses of which were monitored during desiccation, this pattern of the smallest seeds in the population drying most rapidly was also observed. Thus, sample drying curves demonstrate that the smaller seeds in the population dried most rapidly (Fig. 4)

Determination of critical water contents

Using a Kolmogorov–Smirnov test, the distribution of individual seed water contents at each TWC during desiccation was tested for normality. For all samples [except the last but one TWC for batch 2, 8–10 g (i.e. 12%)], there was a normal distribution of water contents (Table 1, $P > 0.05$).

During desiccation there was an increase in the mean fresh mass of those seeds that retained viability at each subsequent TWC (Fig. 5). Furthermore, for batch 2 only the larger seeds in the population survived desiccation to low mean TWCs (M. I. Daws, personal observation). The CWCs calculated using the NORMINV function of Microsoft Excel 97 fell within a narrow range of values, although there were differences in the CWC between the two years (18.2–20.8% versus 25.2–27.37% for batch 1 and 2, respectively) (Table 1).

The effect of seed-lot heterogeneity on response to desiccation for 24 tree species

Plotting the slope of the mortality curves (probits mc^{-1}) for 24 species from DABATTS (Tompsett and Kemp, 1996) against the variability in seed dry mass for each seed lot generated a significant negative

Table 1. Parameters required to calculate the critical water content (CWC) for seeds of *Vitellaria paradoxa* [batch 1 unsorted, batch 2 unsorted and batch 2 sorted (4–6 and 8–10 g)]. The table includes target water contents (TWCs) during drying, the corresponding actual mean water content (WC) and its standard deviation (SD). To calculate the CWC, at each TWC, the actual water contents, their standard deviations and the proportion of seeds killed were entered into the NORMINV function of Microsoft Excel. The remaining columns provide support to the assumptions used to calculate the CWC. Thus, the equations of the fitted lines refer to a plot of whole-seed water content against whole-seed dry mass. The equations, r^2 and associated P -value indicate whether or not the smallest seeds in the population dried, and hence reached the CWC most rapidly (see Figs 2 and 3). The value for A^2 and associated P -value refer to the results of the Kolmogorov–Smirnov test for normality applied to the distribution of water contents at each TWC

TWC (%)	Actual WC (%)	SD	Germination (%)	Proportion killed	CWC	Equation of fitted line	r^2	A^2
Batch 1 unsorted (8.10 ± 2.10 g)								
Initial	38.66	4.32	99	0.000	N/A	$y = -0.17x + 39.47$	0.002 ^{ns}	0.251 ^{ns}
30	28.79	5.41	92	0.071	20.83	$y = 2.21x + 18.48$	0.228*	0.282 ^{ns}
20	20.06	4.75	48	0.515	20.24	$y = 3.24x + 5.60$	0.495**	0.322 ^{ns}
16	19.35	5.27	39	0.606	20.77	$y = 1.98x + 7.71$	0.317**	0.086 ^{ns}
13	17.33	4.38	24	0.758	20.44	$y = 2.43x + 4.89$	0.491**	0.198 ^{ns}
10	13.16	3.28	6	0.939	18.24	$y = 2.55x + 0.52$	0.433**	0.672 ^{ns}
8	12.49	4.19	3	0.970	20.35	$y = 2.17x + 1.51$	0.312**	0.070 ^{ns}
5	6.286	1.16	0	1.000	N/A	$y = 0.05x + 6.04$	0.002 ^{ns}	0.335 ^{ns}
					Mean	20.15 ± 0.96		
Batch 2 unsorted (10.18 ± 2.76 g)								
Initial	43.23	4.88	95.0	0.000	N/A	$y = -0.30x + 44.80$	0.090 ^{ns}	0.391 ^{ns}
40	37.52	5.83	95.0	0.000	N/A	$y = -0.74x + 40.67$	0.022 ^{ns}	0.480 ^{ns}
33	27.95	4.20	56.7	0.403	26.92	$y = 0.98x + 23.45$	0.079*	0.293 ^{ns}
26	20.46	4.73	21.7	0.772	25.20	$y = 3.20x + 7.01$	0.540**	0.247 ^{ns}
19	17.16	4.12	6.70	0.929	25.81	$y = 3.23x + 3.85$	0.639**	0.409 ^{ns}
12	14.09	3.03	0.00	1.000	N/A	$y = 0.67x + 11.24$	0.074 ^{ns}	0.430 ^{ns}
7	10.65	2.11	0.00	1.000	N/A	$y = 0.19x + 9.81$	0.010 ^{ns}	0.297 ^{ns}
					Mean	25.98 ± 0.87		
Batch 2 sorted 4–6 g (5.40 ± 1.02 g)								
Initial	42.89	4.16	94	0.000	N/A	$y = -1.61x + 47.45$	0.054 ^{ns}	0.296 ^{ns}
40	33.24	3.76	91	0.032	26.27	$y = 0.87x + 30.67$	0.015 ^{ns}	0.650 ^{ns}
33	30.10	2.81	88	0.064	25.82	$y = 0.54x + 28.46$	0.040 ^{ns}	0.355 ^{ns}
26	27.76	2.7	68	0.277	26.11	$y = 1.64x + 23.31$	0.139*	0.361 ^{ns}
19	24.72	3.007	21	0.777	27.01	$y = -0.49x + 28.09$	0.004 ^{ns}	0.720 ^{ns}
12	19.80	3.24	4	0.957	25.38	$y = -0.27x + 20.57$	0.002 ^{ns}	0.379 ^{ns}
7	11.54	2.50	0	1.000	N/A	$y = 0.50x + 10.07$	0.019 ^{ns}	0.295 ^{ns}
					Mean	26.12 ± 0.60		
Batch 2 sorted 8–10g (8.93 ± 1.41 g)								
Initial	42.82	5.53	95	0.000	N/A	$y = 1.47x + 36.09$	0.036 ^{ns}	0.314 ^{ns}
40	37.74	5.58	92	0.032	27.37	$y = -1.23x + 43.50$	0.031 ^{ns}	0.300 ^{ns}
33	33.95	4.78	89	0.063	26.64	$y = 1.78x + 26.99$	0.053 ^{ns}	0.210 ^{ns}
26	27.62	3.70	62	0.347	26.17	$y = 2.40x + 12.20$	0.334**	0.334 ^{ns}
19	23.22	2.54	14	0.853	25.88	$y = 0.69x + 20.31$	0.080 ^{ns}	0.531 ^{ns}
12	16.48	2.67	0	1.000	N/A	$y = -0.22x + 17.46$	0.031 ^{ns}	1.210**
7	10.92	2.397	0	1.000	N/A	$y = 0.06x + 10.67$	0.009 ^{ns}	0.722 ^{ns}
					Mean	26.52 ± 0.65		

N/A, not applicable; ns, not significant; * $P < 0.05$; ** $P < 0.01$.

linear relationship ($r^2 = 0.220$, degrees of freedom (df) = 22, $P < 0.05$; Fig. 6A). Thus, the slope of the mortality curve increased as the variation in seed mass within the seed lot decreased.

An alternative explanation for the variability in the slopes of the mortality curves is variability in the

initial seed water contents prior to desiccation. This possibility was tested, although there was no significant relationship between the slope of the mortality curves and the standard deviation of the initial seed lot water content ($r^2 = 0.002$, df = 20, $P = 0.837$; Fig. 6B).

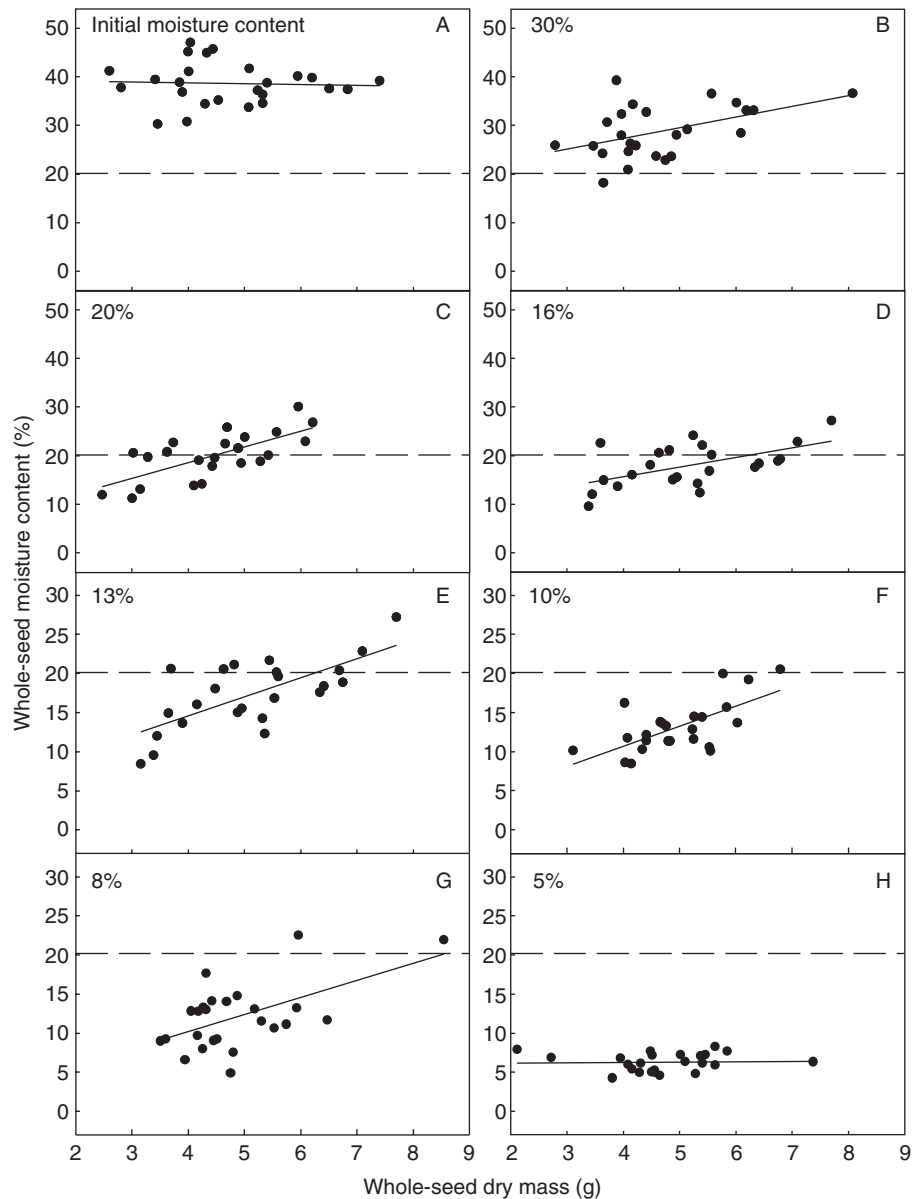


Figure 2. (A–H) The distribution of individual seed water contents in relation to seed dry mass, for batch 1, at each target water content during drying. Note that the target water content is given on each graph. The dashed horizontal lines correspond to the mean whole-seed critical water content, given in Table 1. The equations of the fitted lines and their statistical significance are given in Table 1.

Discussion

Desiccation sensitivity

These current results reinforce the hypothesis that seeds of *V. paradoxa* exhibit recalcitrant seed storage behaviour (cf. Pritchard *et al.*, 1997; Danthu *et al.*, 2000). Desiccation from a whole-seed water content of *c.* 30% resulted in a steady decline in viability, with the water content for 50% viability loss falling in the

region 20–26%. Similarly, for this species, Danthu *et al.* (2000) reported a water content of 22% for 50% viability loss. As viability in the wet-stored controls was maintained, viability loss was a consequence of desiccation *per se* rather than the storage duration, with the overall desiccation sensitivity comparable to that observed for a wide range of species in other studies (e.g. Dickie *et al.*, 1991; Finch-Savage, 1992; Tompsett and Pritchard, 1993; Tompsett and Kemp, 1996; Normah *et al.*, 1997).

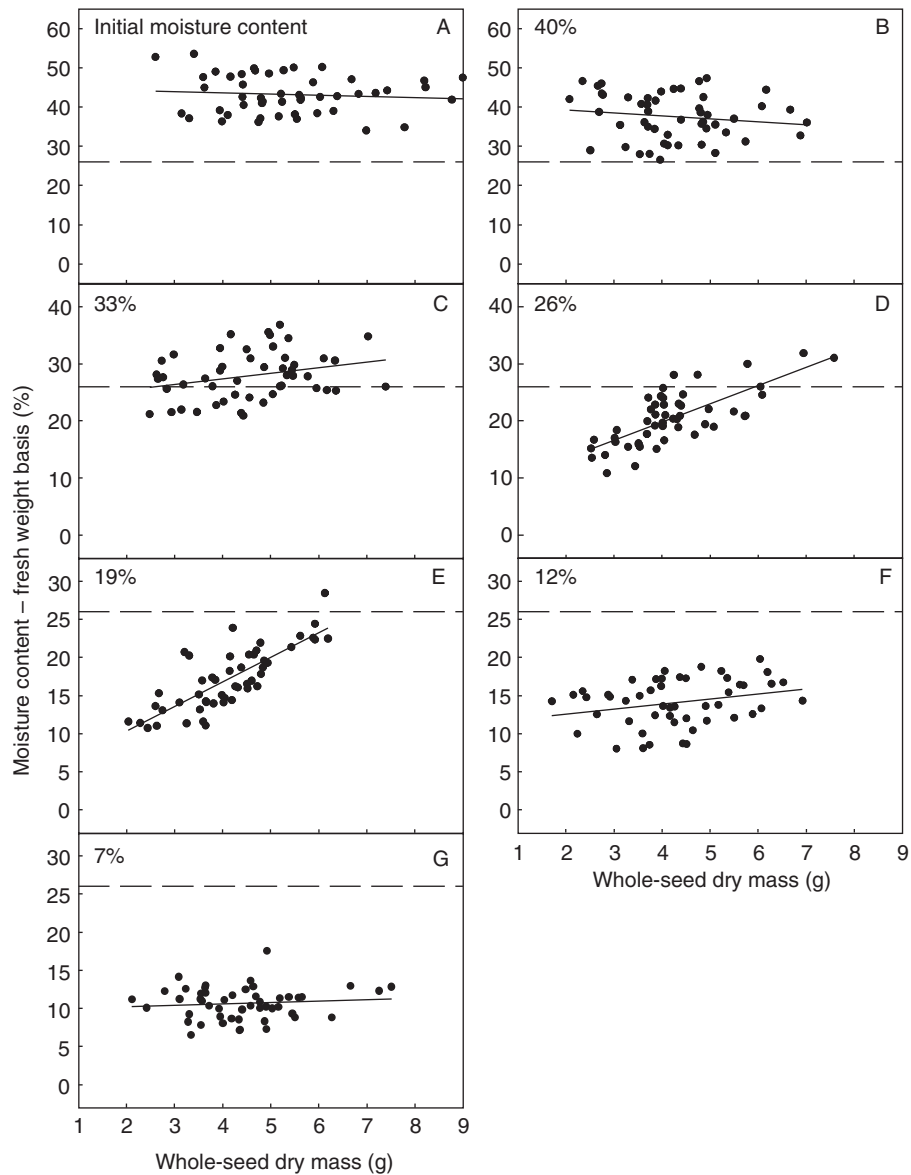


Figure 3. (A–G) The distribution of individual seed water contents in relation to seed dry mass, for the non-sorted batch 2 seeds, at each target water content during drying. Note that the target water content is given on each graph. The dashed horizontal lines correspond to the mean whole-seed critical water content, given in Table 1. The equations of the fitted lines and their statistical significance are given in Table 1.

The water content for 50% viability loss exhibited differences between the two study years (c. 20 and 26% for batch 1 and 2, respectively). One explanation for these differences is that the seed lots differed in developmental status, with batch 1 being more developed and, hence, more tolerant of desiccation. An effect of developmental status on seed desiccation tolerance has been observed for a range of recalcitrant species (Hong and Ellis, 1990; Pammenter *et al.*, 1991; Berjak *et al.*, 1993; Tompsett and Pritchard, 1993; Farrant and Walters, 1998). This concept is reinforced

by the lower initial water content of the 1996 batch (38.7 versus 43.2%), suggesting that development had progressed further (Kermode and Finch-Savage, 2002). The potential impact of local environmental conditions on phenotypic plasticity in recalcitrant seed development and responses has recently been shown for *Aesculus hippocastanum* seeds harvested across Europe (Daws *et al.*, 2004). Therefore it is possible that differences in desiccation tolerance in *V. paradoxa*, between years, is due to climate-dependent developmental changes.

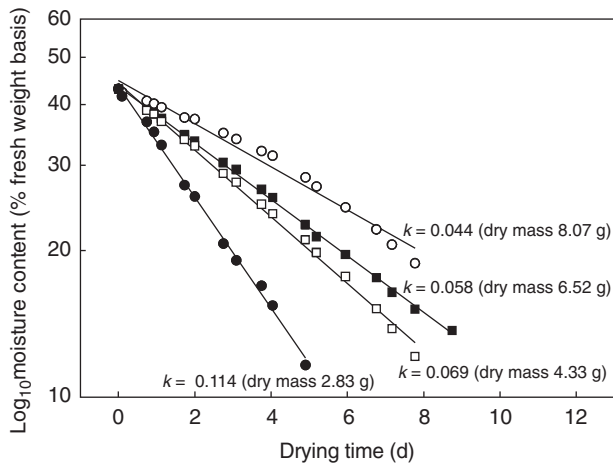


Figure 4. Representative drying curves for seeds of *Vitellaria paradoxa* (batch 2) covering the entire seed mass range. The slopes of the fitted lines (k) are given on the figure.

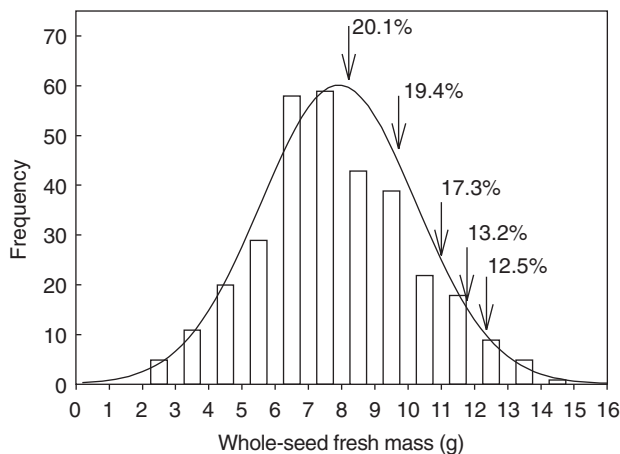


Figure 5. The distribution of 320 individual seed fresh weights (batch 1) for fully re-imbibed seeds of *Vitellaria paradoxa* after desiccation to a range of target water contents. Superimposed on the figure is a theoretical normal distribution with the same mean and standard deviation as the sampled seed population. The arrows on the figure correspond to the mean fresh weight of those seeds that germinated at the indicated mean water contents during desiccation.

The mortality curves for the non-size-sorted seeds imply a wide range of desiccation sensitivities within the two seed lots. Thus, for batch 1, some seeds appear to tolerate desiccation to water contents as low as 12%, while others are killed at water contents as high as 30% (Fig. 1). However, there are several lines of evidence that suggest that there is, in fact, a narrow range of tolerances within the population, with the apparent variability resulting from seed-lot hetero-

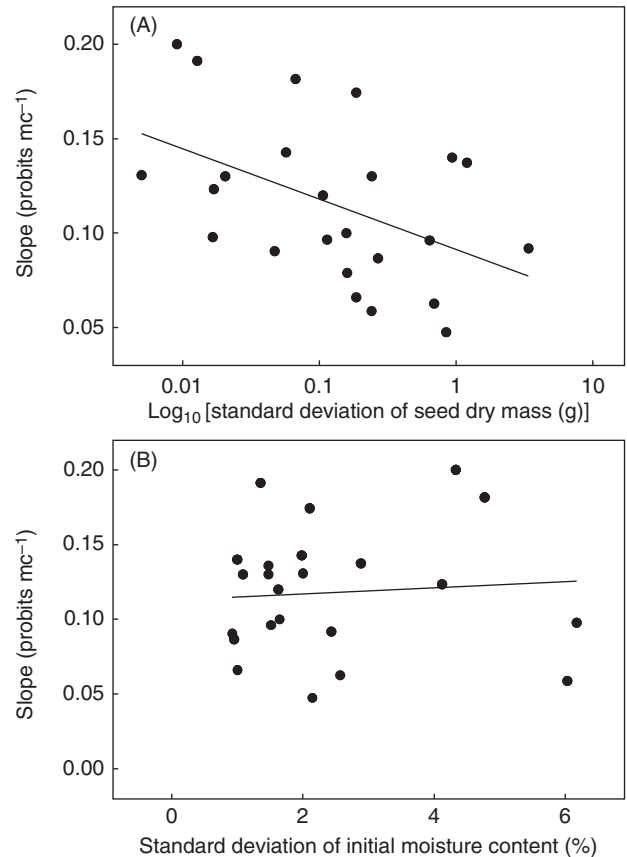


Figure 6. The effect of initial variability in (A) seed dry mass and (B) seed water contents on the slope of the relationship between probit (germination) and water content for 22 and 24 recalcitrant seeded tree species, respectively. Species included in the analysis were: *Dipterocarpus costatus*, *D. obtusifolius*, *D. turbinatus*, *D. zeylanicus*, *Dryobalanops keithii*, *D. lanceolata*, *Hopea mengarwan*, *Parashorea malaanonan*, *P. smythiesii*, *P. tomentalla*, *Shorea affinis*, *S. amplexicaulis*, *S. congestiflora*, *S. ferruginea*, *S. macrophylla*, *S. macroptera*, *S. obtusa*, *S. ovalis*, *S. robusta*, *S. roxburgii*, *Trichilia monadelpha*, *T. tessmannii*, *Vatica mangachapo* and *V. odorata* ssp. *odorata*. Data for variability in the initial water content were unavailable for *D. costatus* and *P. malaanonan*.

geneity with respect to size (mass). First, reducing the seed-lot heterogeneity in mass of batch 2, by sorting seeds into fresh mass classes (4–6 and 8–10 g), resulted in steeper mortality curves, albeit with the same water content for 50% viability loss (Fig. 1). This narrowing of the water content range, over which viability loss occurred, presumably resulted from seeds drying at a more uniform rate, and hence, all were at a similar water content at each TWC during desiccation.

Secondly, it is possible to take this argument further, to the point where accounting for differences in seed mass, and hence in water content among

individual seeds during drying, allows the calculation of a single CWC. Based on assumptions outlined in the Materials and methods, a CWC was calculated for each TWC during desiccation. These CWC values fell within a narrow range of values (18.2–20.8%, 1996; 25.2–27.4%, 2002, sorted and non-sorted).

Thirdly, for an unrelated data set (from Tompsett and Kemp, 1996), there was a negative relationship, for 24 species, between the uniformity of the population response to desiccation (as assessed by the slope of the mortality curve) and the seed-lot heterogeneity with respect to mass. This implies that seed lots with little variability in mass, which presumably dry at more uniform rates, will have a steep mortality curve, i.e. mortality will occur over a narrow range of water contents. Furthermore, the lack of a relationship between the slope of the mortality curve and seed-lot heterogeneity in initial water content suggests that heterogeneity in mass is a more important determinant, for these species, of the slope of the mortality curve than heterogeneity in initial water content (cf. Probert and Longley, 1989). Thus, the heterogeneity in water contents during drying, observed in the study by Probert and Longley (1989), is more likely to have been a consequence of seed to seed differences in drying rates, resulting from differences in seed mass, or perhaps coat thickness, than variability in water contents at the outset of drying.

A narrow range of CWCs has been observed in comparatively few studies. However, Dussert *et al.* (1999), investigating nine *Coffea* species, observed a narrow range of water contents over which viability was lost in three of the species, although the variability in seed masses was not given. Finch-Savage (1992), when matching individual seed water content values and germination, found a reduced range of water contents over which viability loss occurred. We found that accounting for variability in mass reduced the range of water contents over which viability was lost. None the less, there was some variability in the CWC, which is perhaps not surprising, since some heterogeneity within a seed lot (e.g. in seed developmental status) is probably unavoidable.

Limitations to the current approach

A large number of studies have investigated the effect of drying rates on the level of tolerance to desiccation of seeds or embryonic axes. Typically, more rapid drying rates enabled tolerance of desiccation to lower water contents, presumably because of a reduction in deleterious aqueous-based reactions (Pammenter *et al.*, 1991; Pammenter and Berjak, 1999). More recently, from work on excised axes, an optimum drying rate has been suggested (Liang and Sun, 2000, 2002), with

extremely rapid and very slow drying rates having no beneficial effect on the CWC, compared to intermediate drying rates. However, the range of drying rates in the present study all fall within the range within which no beneficial effect on the CWC was evident in these two previous studies (Liang and Sun, 2000, 2002). In this current study we found no evidence that the smaller seeds in the population, which dried up to five times faster than the largest seeds, survived to lower water contents. Instead, the data are consistent with the hypothesis that mortality occurred once seeds had reached the CWC; the only effect of drying rate was that the small seeds reached the CWC faster than larger seeds.

Much of the research that has reported an effect of drying rates on the level of desiccation tolerance has been conducted on isolated axes, rather than whole seeds. With isolated axes, rapid drying rates can be achieved comparatively easily. However, the majority of the published literature on seed desiccation deals with the desiccation of whole seeds, as opposed to isolated axes, and, in support of the approach taken in this paper, generally fail to report a substantial effect of drying rate on the level of desiccation tolerance (e.g. Tompsett, 1982, 1984, 1987; Pritchard, 1991; Finch-Savage, 1992; Pritchard *et al.*, 1995b), presumably as a function of the limited range of drying rates that can be easily achieved with whole seeds. Consequently, our observed results are unlikely to be a consequence of shifts in the CWC resulting from seed-mass-driven differences in drying rate, and instead, reflect mortality over a narrow range of water contents. In addition, it should be remembered that the reported CWCs for *V. paradoxa* only correspond to desiccation at 26°C within a limited range of drying rates and, therefore, may not represent the maximum possible expression of desiccation tolerance for this species (Pammenter and Berjak, 1999). Consequently, the CWC may change under a different set of drying conditions.

This study presents a potential mechanism to explain the wide range of CWCs typically observed when drying whole seeds: variability in seed mass will result in a wide range of individual seed water contents during drying. The calculated CWC values all fall within a narrow range, coincident with the water content corresponding to 50% viability loss. Thus, it would appear that, at least for whole seeds, this water content is a better reflection of the seed lot response to desiccation than either the water content corresponding to the onset or end-point of seed viability loss. The water content at which the onset of viability loss occurs has been described as the lowest safe water content (LSWC; Tompsett, 1984; Tompsett and Kemp, 1996). However, our results suggest that the LSWC has no physiological basis, since, at least for *V. paradoxa*, all the seeds in the population

appeared to be able to survive desiccation to water contents below the LSWC. Instead, the LSWC is an artefact of the distribution of water contents during desiccation; those seeds apparently killed following desiccation to just below the LSWC are likely to be the smallest seeds in the population, and hence at a water content significantly lower than expected. In conclusion, this study found that seed-lot heterogeneity in mass, and hence drying rates, appears to mask the existence of a narrow range of CWCs for viability loss in seeds of *V. paradoxa*. Clearly, it would be useful to investigate the extent to which seed mass variability contributes to masking CWCs for other species.

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