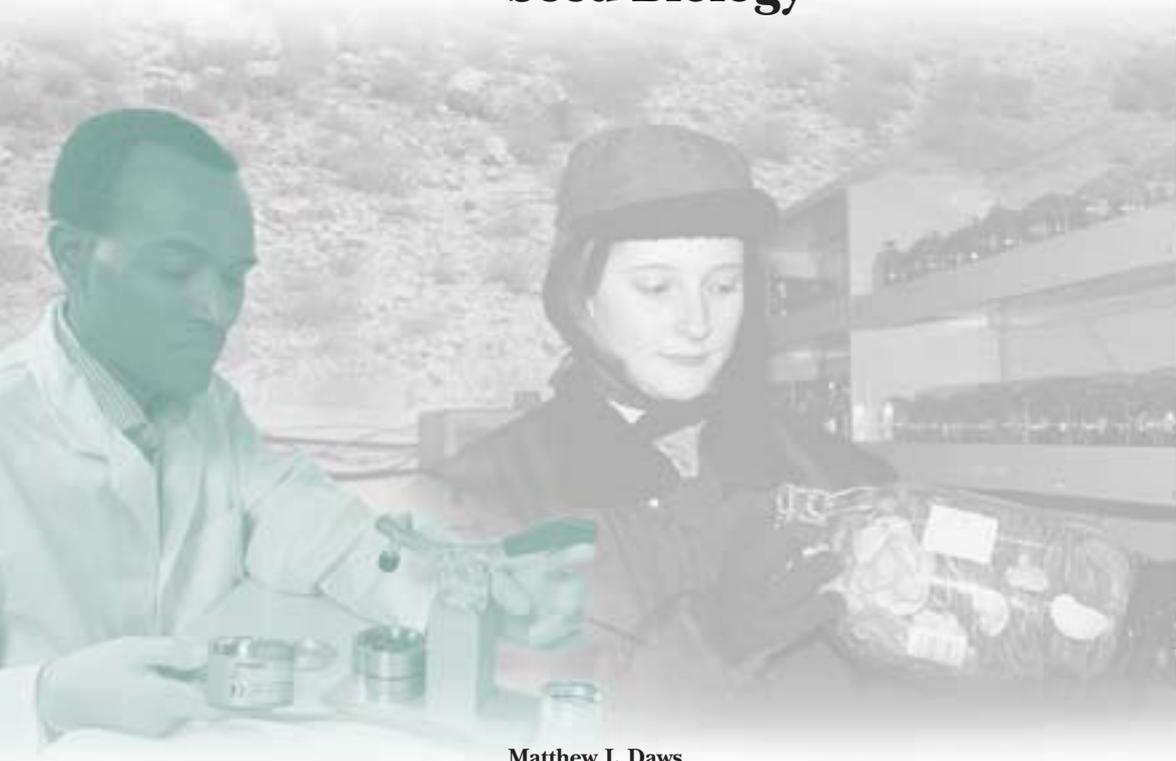


Some Ecological and Conservation Aspects of *Warburgia salutaris* Seed Biology



Matthew I. Daws

Seed Conservation Department, Royal Botanic Gardens, Kew, Wakehurst Place, Ardingly, Sussex RH17 6TN, UK

William Omondi

Kenya Forestry Research Institute, P.O. Box 20412, Nairobi, Kenya

Hugh W. Pritchard

Seed Conservation Department, Royal Botanic Gardens, Kew, Wakehurst Place, Ardingly, Sussex RH17 6TN, UK

Patricia Berjak

School of Life and Environmental Sciences, University of Natal, Durban 4041, South Africa

Summary

Warburgia salutaris is one of the most highly used medicinal plant species in Southern Africa and is threatened with extinction. Seed biology information on this species is scarce. This study investigated allometric relationships between fruit size and seed production as well as the effects of desiccation and -20°C treatments on seed viability. Results indicate that large fruits produce larger seeds than small fruits. As large seeds generally have a higher probability of establishing successful seedlings than small seeds, seeds from the largest fruits should be used for replanting schemes. Seeds tolerated desiccation to 7% moisture content (fresh weight basis), equivalent to about 14% moisture in the embryonic axis. Seeds at c. 17% moisture content tolerated storage at -20°C for 3 months but not for 12 months, probably as a result of the slow, cumulative effects of ice nucleation and crystal growth. Longer-term seed storage experiments in the dry state are needed.

Introduction

Warburgia salutaris (Bertol. f.) Chiov. has a scattered distribution in southern Africa (Palgrave, 1983). It is one of the most highly utilised medicinal plants in tropical and sub-tropical Africa with uses ranging from treating toothache to malaria. However, it is almost extinct in parts of its range, e.g., in KwaZulu Natal, mainly as a consequence of over-exploitation for its bark (Oldfield *et al.*, 1998).

Conservation of this species is hampered by a lack of basic knowledge about seed biology. For example, it is not known if the seeds can be conserved *ex situ* under typical gene-bank conditions (about -20°C after seed drying to c. 15% RH). Previous studies on seed storage responses in *Warburgia salutaris* have yielded contradictory results. Albrecht (1993) noted that the seeds are sensitive to desiccation and recommended that they be stored in moist sawdust in open containers in a cold room at 3°C . Kamondo *et al.* (1999) also stated that the seeds are desiccation sensitive, being killed by drying to low (c. 7–10%) moisture contents. However, other reports have suggested tolerance of this level of dehydration (Pritchard and Daws, 1997; Kioko *et al.*, 1998). If seeds are desiccation sensitive they can only be stored at near to full hydration (Pritchard *et al.*, 1995; Tompsett and Pritchard, 1998; Pammenter and Berjak, 1998). Consequently, low (sub-zero) temperature storage would result in the formation of damaging ice crystals, and warm temperatures would allow the seeds to progress towards germination (Tompsett and Pritchard, 1998; Pammenter and Berjak, 1999). On the other hand, tolerance of at least partial dehydration could allow transfer of the seeds to ultra-low temperatures with reduced risk of ice formation. Such a conservation technology has been investigated by Kioko *et al.*, (1998), who reported that 30% of seeds rapidly

dried to an axis moisture content of c. 7% (fresh mass basis) could withstand cryopreservation in liquid nitrogen for 1 h.

In addition, there is a lack of basic knowledge of seed ecology for this species in relation to seed production. The fruit is a spherical to egg-shaped berry, up to about 4 to 5 cm in diameter, with a leathery skin, turning from green to purplish-black when mature (Oct–Jan) (Palgrave, 1983; Albrecht, 1993). Each fruit contains numerous flatish, yellow-brown seeds in a pulpy medium (Albrecht, 1993). Life-history theory predicts that plants should produce seeds of a single optimum size (Smith and Fretwell, 1974; Lloyd, 1987; McGinley *et al.* 1987; Haig and Westoby, 1988). This premise is based on the assumption that offspring smaller than the optimum will be non-viable or have low fitness, whereas those greater than the optimum usurp resources that could have been used to provision more offspring. Accordingly, it is expected that plants should alter offspring number rather than size to ensure that all seeds are equally provisioned (Vaughton and Ramsey, 1997). However, this premise has rarely been tested and furthermore an understanding of resource allocation may be of use for determining collection strategies for material to be used in reintroduction programmes.

In this paper, we explore the relationships between fruit mass and seed production for *Warburgia*. In particular, we test whether there are differences in resource allocation to seeds in large versus small fruits. In addition, we report the results of a desiccation screen of seeds of *Warburgia* conducted in three different laboratories, but with seeds from the same source and the result of storage of seeds at -20°C. Finally, we suggest future avenues for seed conservation research on this species.

Materials and Methods

1. Seed Lot Details

Green fruits of *Warburgia salutaris* were collected from Muguga near Nairobi, Kenya in February 1997 and shipped to the Royal Botanic Gardens, Kew (United Kingdom). A further seed lot collected from the same source in 1998 was dispatched to the University of Natal (Republic of South Africa, RSA). Upon receipt at Kew, fruits were divided into mature and immature. Immature fruits were hard to the touch and were discarded, mature fruits were soft to the touch. Seeds were extracted from the fleshy fruits by splitting the fruits open with a scalpel and scooping out the seeds. Seeds were then gently rubbed on tissue paper to remove any remaining fruit tissue. During the cleaning process the fresh weight of each individual fruit was determined along with the number of seeds per fruit and the total mass of seeds per fruit.

2. Seed Desiccation

Seeds were desiccated at 26°C, using silica gel at a gel to seed ratio of c. 1:1. For the experiment in the UK, seeds were dried to the following moisture contents: 55, 42, 30, 15, 11, 10 and 7%. Similarly, for the desiccation trials in Kenya and South Africa seeds were dried to six or seven moisture contents. Following desiccation, seeds (4 replicates of 20 seeds per moisture content) were sown on the surface of 1% agar in water in sandwich boxes and placed in an incubator at a constant 26°C (12 h light/12 h dark). In addition, the whole seed moisture content was determined for 25 individual seeds. Seeds were scored for germination when the radicle had protruded from the seed by at least 2 mm. Subsequent to the desiccation trial, 25 individual seeds were desiccated to various moisture contents between 55 and 4% using identical conditions to the desiccation trial. The moisture content of the component parts of the seed (endosperm and seed coat and embryonic axis) was then determined to enable the embryonic axis moisture content of seeds in the desiccation trial to be estimated.

3. De-sorption Isotherm

A de-sorption isotherm for whole seeds and embryonic axes of *Warburgia* was constructed by equilibrating 5 whole seeds and 5 embryonic axes at 6 values of relative humidity above saturated salt solutions (15, 30, 45, 60 and 80%) or silica gel (c. 5%), at a constant 21°C for a period of seven weeks.

4. Storage at -20°C

In Kenya, seeds (n = 25) were dried to two moisture contents (16.5 and 17.8%), sealed in aluminium foil bags and then frozen at -20°C for 3 and 12 months. Upon thawing seeds were germinated at approximately 28°C.

5. Statistical Analysis

For the allometry data, the slope of fitted regression lines was compared with the slope of null lines using the following *t*-test (Underwood, 1997):

$$t = \frac{b - H}{SE_b} \quad \text{with } (n - 2) \text{ degrees of freedom.}$$

Where *n* is the number of samples, *t* is the test statistic, *b* is the slope of the fitted line, *SE_b* is the standard error of *b* and *H* is the slope of the null line.

Results

1. Allometry

Fruit fresh mass, the number of seeds per fruit and the total mass of seeds per fruit were determined for 138 individual fruits. Figure 23.1 indicates that there was a linear relationship between the logarithms of fruit fresh mass and total mass of seeds per fruit ($R^2 = 0.527$, $P < 0.001$). The slope of this line was not significantly different from a null line of slope 1 ($P > 0.05$). In comparison, while there was a significant linear relationship between $\log(\text{number of seeds per fruit})$ and $\log(\text{fruit mass})$ (Figure 23.2; $R^2 = 0.1784$, $P < 0.001$) the slope of the fitted line was significantly less than a null line of slope 1 ($P < 0.001$). There was also a linear relationship between $\log(\text{individual seed mass})$ and $\log(\text{fruit mass})$ (Figure 23.3; $R^2 = 0.3129$, $P < 0.001$). The slope of this line was significantly different from null lines of slope 0 or 1 ($P < 0.001$).

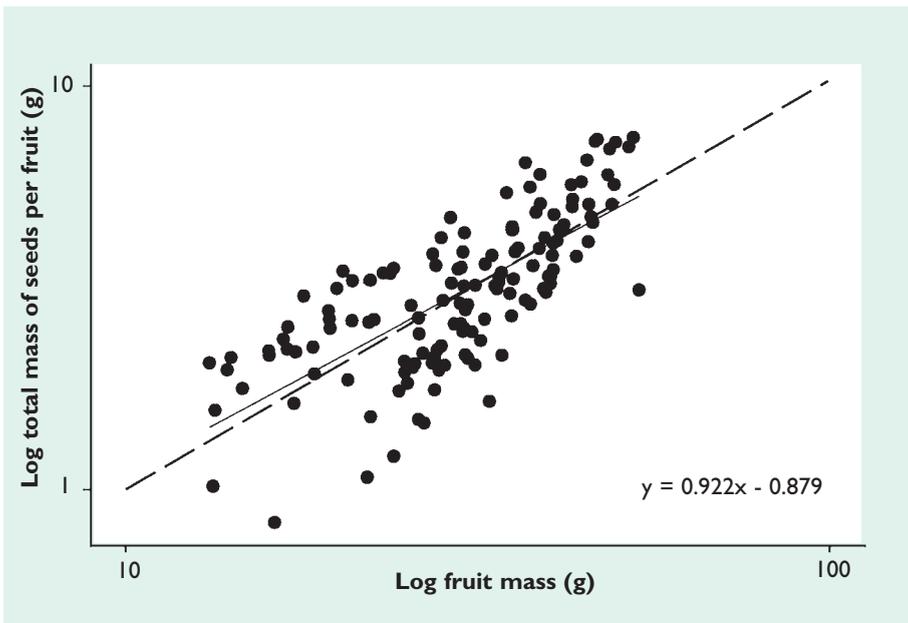


Figure 23.1 The relationship between fruit mass and the total fresh mass of seeds per fruit. The dashed line represents a null line of slope 1.

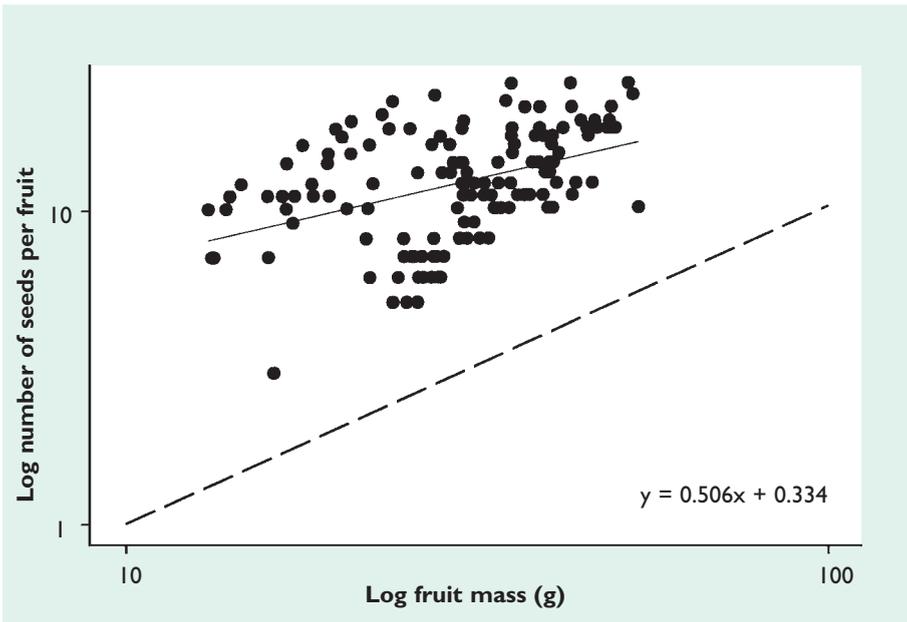


Figure 23.2 The relationship between fruit mass and the number of seeds per fruit. The dashed line represents a null line of slope 1.

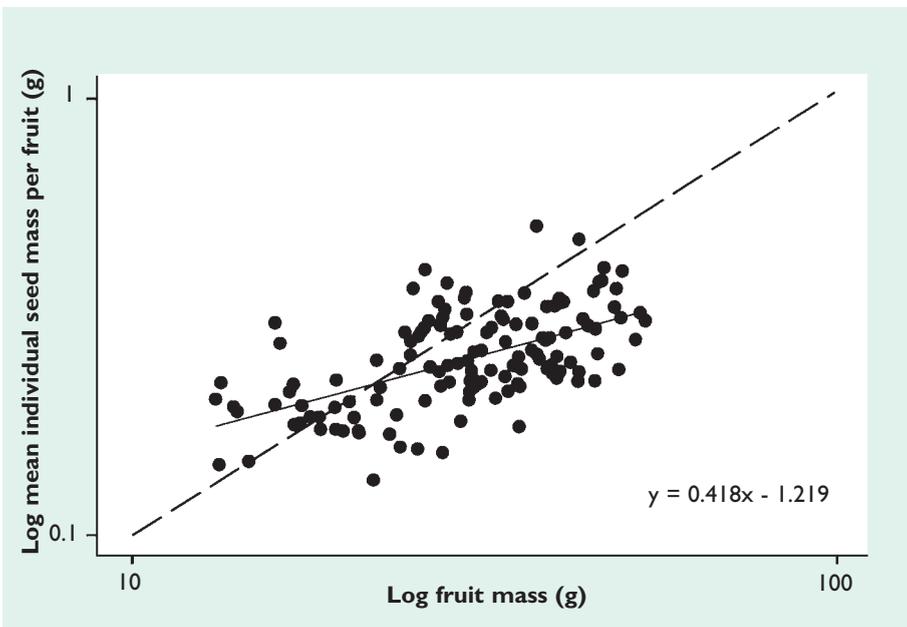


Figure 23.3 The relationship between fruit mass and mean fresh seed mass per fruit. The dashed line represents a null line of slope 1.

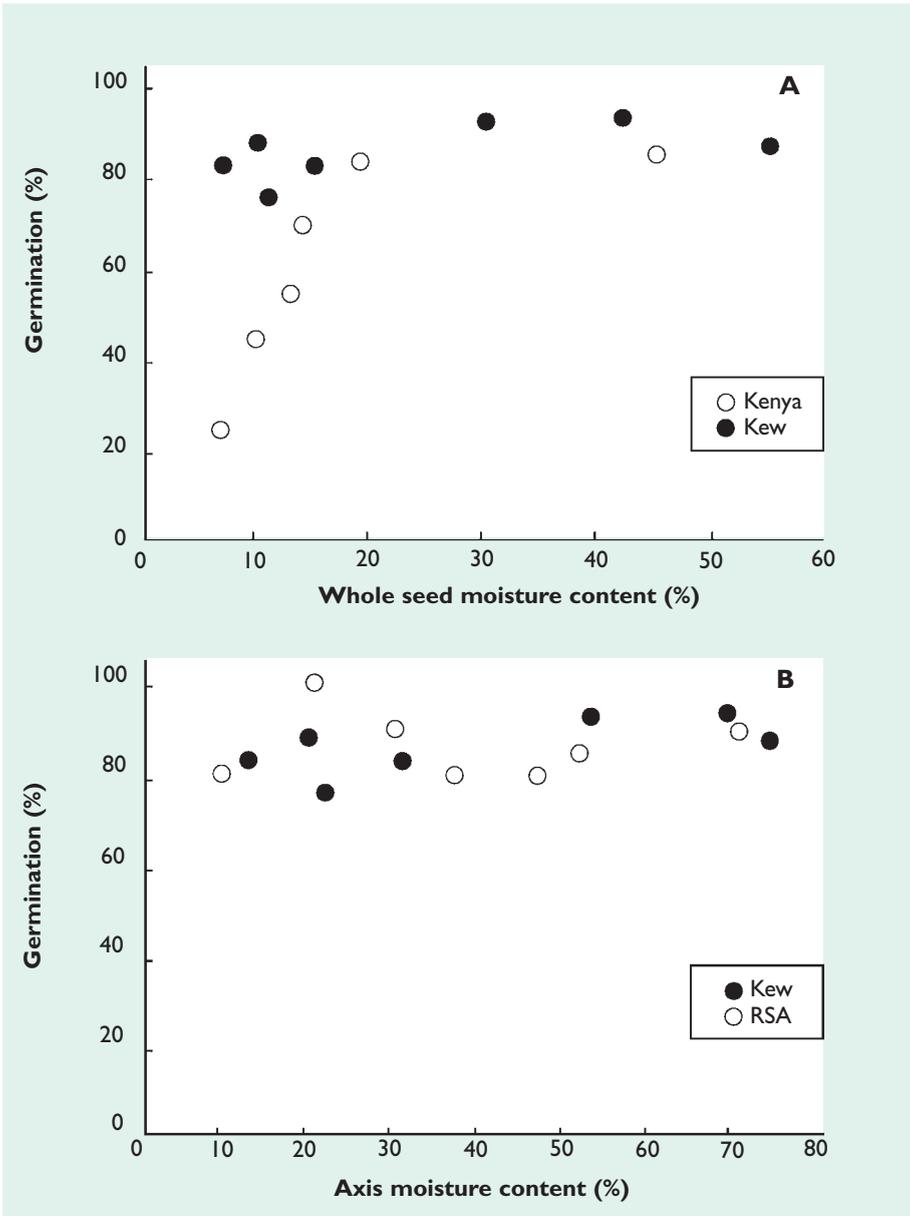


Figure 23.4 The effects of desiccation on seed germination of *Warburgia* seeds. Moisture contents are expressed on a fresh weight basis for (a) whole seeds and (b) the embryonic axis. Seeds used in RSA were from the 1998 collection; all others were from 1997.

2. Desiccation and Storage

Figure 23.4a indicates that seeds of *Warburgia* collected in 1997 from the same source and desiccated in Kew and Kenya exhibited different responses. Following desiccation at Kew, seeds exhibited no loss of viability following desiccation to a whole seed moisture content of 7% (Figure 23.4a). In contrast, seeds desiccated in Kenya exhibited a reduction in total germination from 86% to 25% following dehydration to 7% moisture content (whole seed basis) (Figure 23.4a).

Desiccation of seeds of the 1998 batch in South Africa revealed no loss in seed viability following desiccation to 10% moisture content (embryonic axis basis) (Figure 23.4b). Figure 23.5 is the relationship between embryonic axis moisture content and whole seed moisture content determined on 1997 seed at Kew. Using this relationship the results of the desiccation trial at Kew were plotted in terms of embryonic axis moisture content for comparison with the 1998 data (Figure 23.4 b). This revealed that both seed lots were similarly capable of tolerating desiccation to embryonic axis moisture contents of between 10 and 13%.

The de-sorption isotherm for *Warburgia* seeds and embryonic axes is presented in Figure 23.6. This revealed that seeds desiccated to a whole seed moisture content of 7% and embryonic axis moisture content of 13% were at c. 60% relative humidity.

When seeds from 1997 that were partially dried to c. 17% moisture content and stored at -20°C, few lost viability by 3 months (Table 23.1). However, no seeds survived 12 months storage (Table 23.1).

Discussion

1. Allometric Relationships

In this species, fruit size has no effect on the amount of resources that are allocated to seed production. Thus, while large fruits produce a greater mass of seeds than small fruits, the approximate slope of 1 of the regression line in Figure 23.1 indicates that proportionately, small fruits invest the same resources to seed production as large fruits. Consequently, irrespective of fruit size the same resources are expended per unit mass of seeds. The corollary of this is that the amount of fruit tissue per unit mass of seed is similar across the range of fruit sizes with approximately 90% of the fruit being devoted to flesh and hence attracting dispersers. Wheelwright *et al.* (1984) determined the proportion of fruit that was allocated to seeds for 115 Costa Rican tree and shrub species with fleshy fruits. Interestingly, if $\log(\text{total mass of seeds per}$

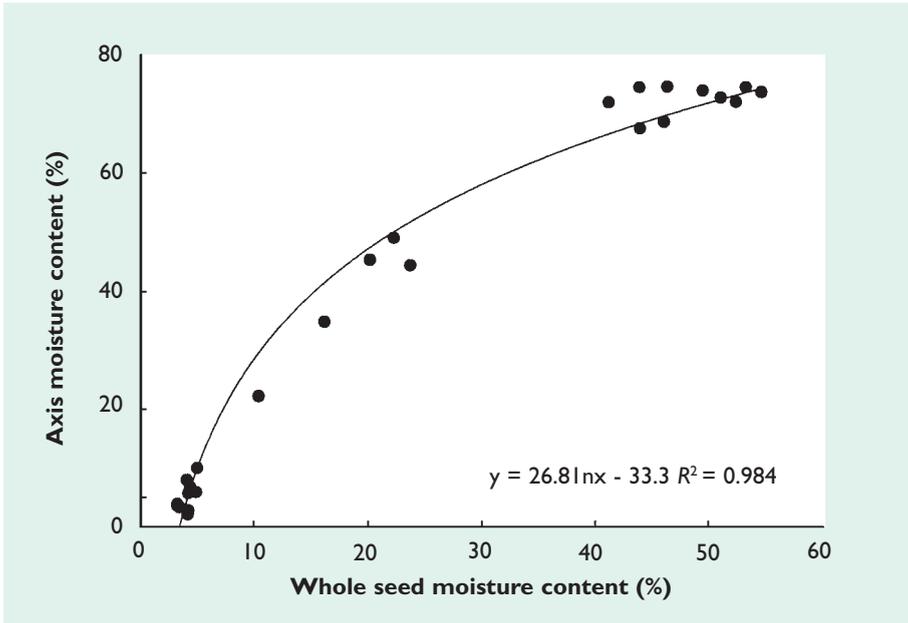


Figure 23.5 The relationship between whole seed and embryonic axis moisture content for *Warburgia* seeds.

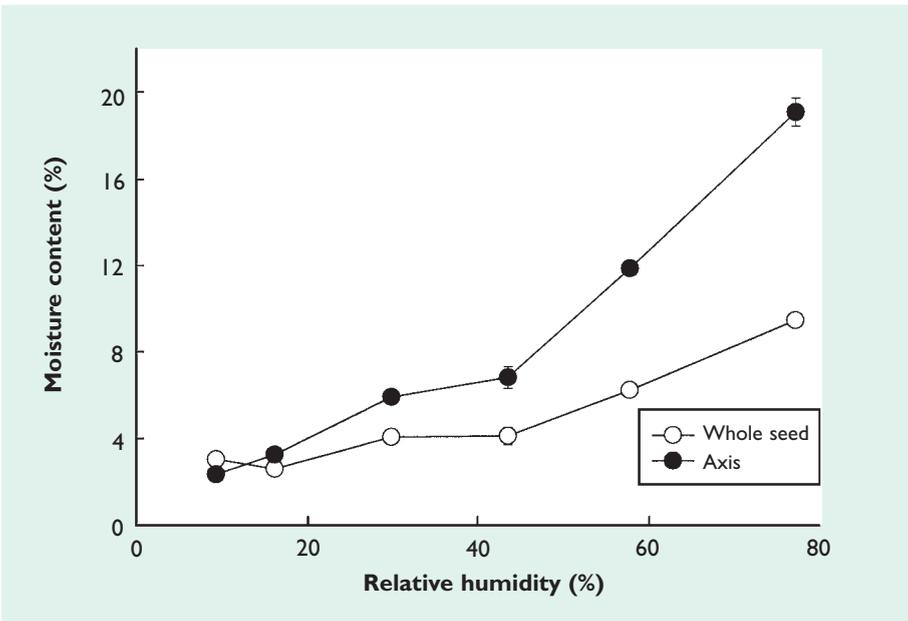


Figure 23.6 De-sorption isotherm at 21°C for *Warburgia* seeds.

Table 23.1 Survival of seeds (n = 25) after 3 and 12 month storage at -20°C

Storage duration (months)	Moisture content before storage (%)	Moisture content after storage (%)	Germination (%) after storage
3	16.5	15.5	57
3	17.8	15.5	85
12	16.5	17	0
12	17.8	18.7	0

fruit) is plotted against $\log(\text{fruit mass})$ using their tabulated data, a significant positive relationship with a slope of approximately one is produced. This fitted line is displaced on the y-axis, in the same way as the fitted line in Figure 23.1 with, on average for their species, 90% of fruit mass allocated to flesh and 10% to seed. Thus, the constant proportional allocation of resources to seeds and fruit indicates that with these species, there was no trade-off between fruit size and allocation of resources to seeds versus dispersal as has been suggested by Grubb (1998). Taken together, the study of Wheelwright *et al.* (1984) and this present study, suggest that across a wide range of species, the selection pressures operating on the balance between attracting seed dispersers and producing sufficient quantities of seeds are constant, irrespective of fruit size.

However, while the number of seeds per fruit increased with fruit mass it was not directly proportional to fruit mass, as large fruits produced less seeds than would be expected if $\log(\text{number of seeds per fruit})$ was directly proportional to $\log(\text{fruit mass})$. The corollary of large fruits having less seeds than expected is that seeds from large fruits are larger than those from small fruits (Figure 23.3). This suggests that there is a trade-off operating between seed size and number within fruits of different sizes. It is clearly advantageous to produce large seeds as seedlings from large seeds have a higher probability of successful establishment (Wulff, 1986). However, there is also an advantage to producing small seeds as these are more likely to be dispersed (Leishman, 2001). Thus, the production of seeds of a range of sizes may be advantageous by diversifying seed dispersal and seed germination in a temporally and spatially variable environment. Interestingly, these results are in contrast to those of Vaughton and Ramsey (1998) who found that in *Banksia marginata*, there was a negative relationship between the mass of individual seeds and the number of seeds per inflorescence. While some of the variation in seed size in *Warburgia* may have been a consequence of differences in developmental stages of fruits, this is unlikely to have been a major influence, as all fruits used in this study were soft to the touch, and hence ripe, on receipt at Kew. This suggests that further studies of patterns of resource allocation in *Warburgia* may be useful in terms of understanding the constraints that operate during seed development. In addition, since larger seeds are likely to produce seedlings with a higher probability of establishment, seeds should therefore be collected from large fruits for propagation purposes.

2. Desiccation Tolerance and Storage

Studies in two laboratories in consecutive years revealed that seeds of *Warburgia* are able to tolerate desiccation to low (c. 10%) axis moisture contents (Figure 23.4b) that, based on the de-sorption isotherm (Figure 23.6), are equivalent to an equilibrium RH of c. 60%. On this basis, seeds of *Warburgia* are not recalcitrant (Black and Pritchard, 2002). However, experiments in Kenya reported that seeds were desiccation sensitive below about 20% moisture content (Figure 23.4a). Such contradictory data for the seeds of this species has been reported previously (Kioko *et al.*, 1998; Kamondo *et al.*, 1999) and emphasises the importance of conducting duplicate experiments on seeds of species for which the seed storage performance is uncertain, assuming the availability of adequate seed numbers. As to the causes of such variability in response observed within seeds of the same batch, the relative level of desiccation tolerance in seeds is known to be a feature of post-harvest treatment (Tompsett and Pritchard, 1998; Pammenter and Berjak, 1999). Thus, subtle variations in the processing/cleaning and pre-storage conditions applied might explain the observed differences in seed quality. Clearly, more detailed studies should be pursued on the impact of processing, etc. on both initial and longer-term quality of *Warburgia* seeds.

Partially drying seeds to c. 17% moisture content, which resulted in the retention of viability in the majority of seed, followed by storage at -20°C was successful, but only for 3 months (Table 23.1). The moisture content of storage was equivalent to > 80% RH (Figure 23.6), which is in the region where freezeable water is observed in seeds (Vertucci, 1990). Thus, death of the seeds by 12 months storage could have resulted from the progressive formation of ice crystals. In contrast, Kioko *et al.* (1998) found that storage in liquid nitrogen for just 1 h resulted in a complete loss of seed viability at all axis moisture contents except 10%, and even at this moisture content viability was reduced from 80 to 30% following cooling and rewarming. Seed to seed variation in moisture content after dehydration, and at the time of cooling, may have ensured that some seeds froze. However, it is also possible that short-term chilling damage – a type of ‘cold shock’ (Morris *et al.*, 1983) – contributed to a reduction in viability. Evidently, further work is required with this species to determine optimum conditions for long-term seed storage.

In conclusion, seed size in the critically-endangered species *Warburgia salutaris* is positively related to fruit size, and given large seeds generally have a higher probability of establishing successful seedlings than small seeds, seeds from the largest fruits should be used for *in situ* and *ex situ* replanting schemes. The seeds can be dried to low (c. 7%) moisture contents with no appreciable loss of viability. However, optimum temperatures for cold storage *ex situ* remain to be identified.

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