

Seed Quality Studies in the Kenyan Shrub *Millettia leucantha*



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Summary

Parameters of seed development were monitored in two populations of the Kenyan, evergreen, dryland shrub *Millettia leucantha* Vatke. Whilst precise changes in pod and seed characteristics were not reliable markers of seed maturity, the broad colour change of seeds from green to brown was a good indicator that seeds could be stored. Of the parameters of development recorded, a sharp downward inflection in average seed fresh weight correlated best with the timing of mass maturity (MM), which occurred around 150 days after flowering. Desiccation tolerance was acquired over a period of several weeks, reflecting wide seed-to-seed variation and generally slow seed development in this species. Measurement of the raffinose content of fresh seeds could be used as an indirect measure of seed quality. However, the presence or absence of raffinose in fresh or dried seeds was no indication of their ability to withstand desiccation. The effects of different drying methods on the quality of seeds harvested at four stages of maturity was also investigated. There were no consistent differences in seed quality attributed to the low-technology drying methods: sun drying; shade drying; shade for 10 d followed by sun drying. At each stage of maturity, seeds dried under gene bank dry room conditions (15% relative humidity and 20°C) were of lower quality compared with seeds dried by other methods. Evidence suggests that this was due to deterioration resulting from inadequate ventilation when seeds were dried inside a sisal bag.

Seed Development

1. Introduction

Millettia leucantha Vatke is an evergreen shrub endemic to the eastern drylands of Kenya. Despite being used by local people as dry season fodder, fuel wood and a source of mulch, *M. leucantha* has been designated vulnerable (Beentje, 1988; 1994) as plants are increasingly cleared for the cultivation of crops. Consequently, the Herbarium Department of the National Museums of Kenya (NMK) has identified *M. leucantha* as a priority for *ex-situ* conservation.

Although there have been a number of recent studies of the acquisition of desiccation tolerance and seed longevity, covering a range of species (for a recent review see Probert and Hay (2000) and Hay and Smith, 2003 – Chapter 6) there is no published information on the seed biology of *M. leucantha* to inform a seed conservation strategy. The sequential flowering pattern of *M. leucantha* and a total period from flowering to seed dispersal of approximately 200 d means that there is a high probability of seed collections intended for conservation containing a range of seed maturities.

The aim of this study was to understand the pattern of seed development in *M. leucantha* so that markers of seed quality could be identified to better inform conservationists on the optimum time for seed collecting.

Seed development was tracked in two populations over two years by recording and measuring, at regular intervals, a variety of parameters including:

- visual changes in seed and pod morphology;
- seed mass and moisture status;
- the germination of freshly harvested and dried seeds to identify the timing of the acquisition of desiccation tolerance; and
- levels of the oligosaccharide, raffinose, in freshly harvested and dried seeds.

2. Materials and Methods

2.1. Sampling

In 1997 and 1998 seed pods were sampled at regular intervals after flowering from two populations of *M. leucantha*. The populations, located at Mwingi, 0°56'S 38°03'E (altitude 997 m) and Makueni, 1°49'S 37°48'E (altitude 1199 m) were separated by at least 100 km. Two methods were used to ensure that each sample of pods represented a uniform stage of development. In 1997, inflorescences were tagged with tier labels at the time of flowering. In 1998, because of the difficulty in locating tagged pods and the need to maximise sample sizes, morphologically similar pods were collected at each harvest. As the season progressed, pods were selected to represent distinct and increasing stages of physiological maturity.

2.2. Morphology of pods/seeds

The colour of pods and seeds was recorded according to a British fungi colour chart (HMSO, 1969). Mean pod length and width was derived from random samples of 20 pods. Using seeds extracted from the same pods the mean length of random samples of twenty seeds was also measured using a ruler (mm).

2.3. Moisture content

Seed fresh weight, dry weight, moisture content and weight of water were determined at each harvest using a minimum of five replicates of 10 seeds. Moisture content (MC) expressed on a fresh weight basis was determined gravimetrically according to the International Seed Testing Association procedure for oily seeds (ISTA, 1985).

2.4. Germination

Four replicate samples of 25 seeds each were sown on plain water agar (10 g l⁻¹) held in 90 mm Sterilin Petri dishes. Germination tests were incubated in cooled incubators (24–26°C) with illumination for 12 h d⁻¹ provided by four warm white fluorescent tubes. To avoid imbibition damage, seeds were conditioned above distilled water at room temperature for 20–24 h prior to germination. Germination was recorded at regular intervals when radicles were at least 2 mm in length. Scoring was continued until no further germination occurred.

2.5. Desiccation tolerance

In 1997, pods were dried above re-activated silica gel (1:1; pods : silica gel) enclosed in a polycarbonate box (55 cm l × 38 cm w × 18 cm h), with a clear perspex lid. Seeds dehiscid from pods were further dried to <10% mc using the same ratio of silica gel. In 1998, freshly harvested pods were dried in the Kenya Gene Bank dry room (15% RH and 20°C).

2.6. Raffinose extraction

Raffinose was analysed using the ultra violet (UV) method (Boehringer, 1998). The protocol is based on the principle that NADH has a linear relationship to the amount of raffinose:

- 1) Raffinose + H₂O α-galactosidase → D-galactose + sucrose
- 2) D-galactose + NAD⁺ β-galactose dehydrogenase → D-galactonic acid + NADH + H⁺.

3. Results and Discussion

3.1. Pod and seed characteristics

As the observations for pod and seed morphology were broadly similar for both populations for convenience only data for the Makueni population are reported in detail.

In 1997, the total period from flowering to seed dispersal was approximately 200 d (Table 7.1). As expected, significant changes in the colour and dimensions of pods and seeds were recorded. Seed mass data indicated that seeds had attained mass maturity (MM) 153 d after flowering (DAF) in 1997 (Figure 7.1a) and by the fourth harvest on 1 September in 1998. Comparing the observations of pod and seed colour for both years at these times shows that whilst colour changes occurred around the time of MM, there was not a precise, and therefore reliable, change. However, the broad colour change of seeds from green to brown is a strong indication that seeds have passed MM and are therefore likely to be desiccation tolerant and capable of being stored. Pod and seed dimensions on the other hand are not good indicators of seed quality.

3.2. Seed development and the acquisition of desiccation tolerance

The pattern of changes in seed development parameters recorded in 1997 was similar to that reported for several taxonomically related crop species from the *Fabaceae* (Ellis *et al.*, 1987). Of the three parameters, fresh weight, dry weight and weight of water, whose values peaked at 153 d indicating the time of MM, the sharp downward inflection in mean fresh weight was most marked (Figure 7.1a). This trend was repeated in 1998 (Figure 7.2a) suggesting that for this species at least fresh weight change is the most sensitive indicator of the timing of MM. This fact has important practical implications because fresh weight is the easiest parameter to monitor, requiring only a reliable balance. Because moisture content falls continuously during the maturation phase, concomitant with the

Table 7.1 Morphological changes in pods and seeds in *Milletia leucantha* Vatke (Makueni population). \pm sd was calculated from the mean of 20 pods and seeds. Shaded rows indicate the timing of mass maturity determined from seed mass measurements (Figure 7.1)

1997 (days after flowering DAF)	Pod colour	Mean pod length (mm)	Mean pod width (mm)	Seed colour	Mean seed length (mm)
62	Leaf green	82 \pm 2.0	15 \pm 0.5	Leaf green	7 \pm 0.7
91	Leaf green	93 \pm 2.7	28 \pm 0.6	Leaf green	14 \pm 0.4
120	Sulphur yellow	100 \pm 3.1	28 \pm 0.4	Leaf green	18 \pm 0.4
153	Sulphur yellow	91 \pm 2.4	27 \pm 0.4	Herbage green	19 \pm 0.2
171	Brown/grey	91 \pm 2.7	27 \pm 0.8	Milk coffee	17 \pm 0.6
194	Brown/grey	-	-	Milk coffee	-
1998 (harvest no¹, date)					
1. (3 Jun)	Leaf green	94 \pm 2.2	29 \pm 0.6	Leaf green	17 \pm 0.4
3. (4 Aug)	Yellow green	95 \pm 2.5	28 \pm 0.7	Leaf green/yellow	18 \pm 0.4
4. (1 Sep)	Brown/grey	101 \pm 2.6	26 \pm 0.4	Milk coffee	19 \pm 0.4
5. (27 Sep)	Brown/grey	96 \pm 2.8	26 \pm 0.54	Milk coffee/grey	19 \pm 0.37

¹ Morphological data were not collected for harvest 2 (29 June) (see Figure 7.2).

increase in dry weight as food reserves accumulate, this parameter is the least reliable indicator of MM. This is accentuated in *M. leucantha* because the seeds are held inside fleshy pods which slow down the rate of moisture loss as seeds enter the post abscission phase. Ellis *et. al.*, (1987) also showed that change in seed moisture content was not a good indicator of the timing of MM in six grain crops from the same family as *M. leucantha*. A similar finding was also reported for the unrelated species *Digitalis purpurea* L. (Hay and Probert, 1995).

In 1997, a small proportion of fresh seeds germinated at 120 DAF, some 33 d prior to MM (Figure 7.1b). For the Mwingi population (data not presented), the germination of fresh seeds began even earlier, some 60 d prior to MM. However, in both cases at MM, higher levels of germination were recorded in dried seeds compared to fresh seeds. This unexpected result which was not repeated in the Makueni population in 1998 (Figure 7.2b) might be explained by the relatively slow drying of seeds inside pods which could have enabled a continuation of seed ripening. In 1997, seeds were dried in closed containers above silica gel whereas in 1998, pods were dried more rapidly under open conditions in a gene bank dry room.

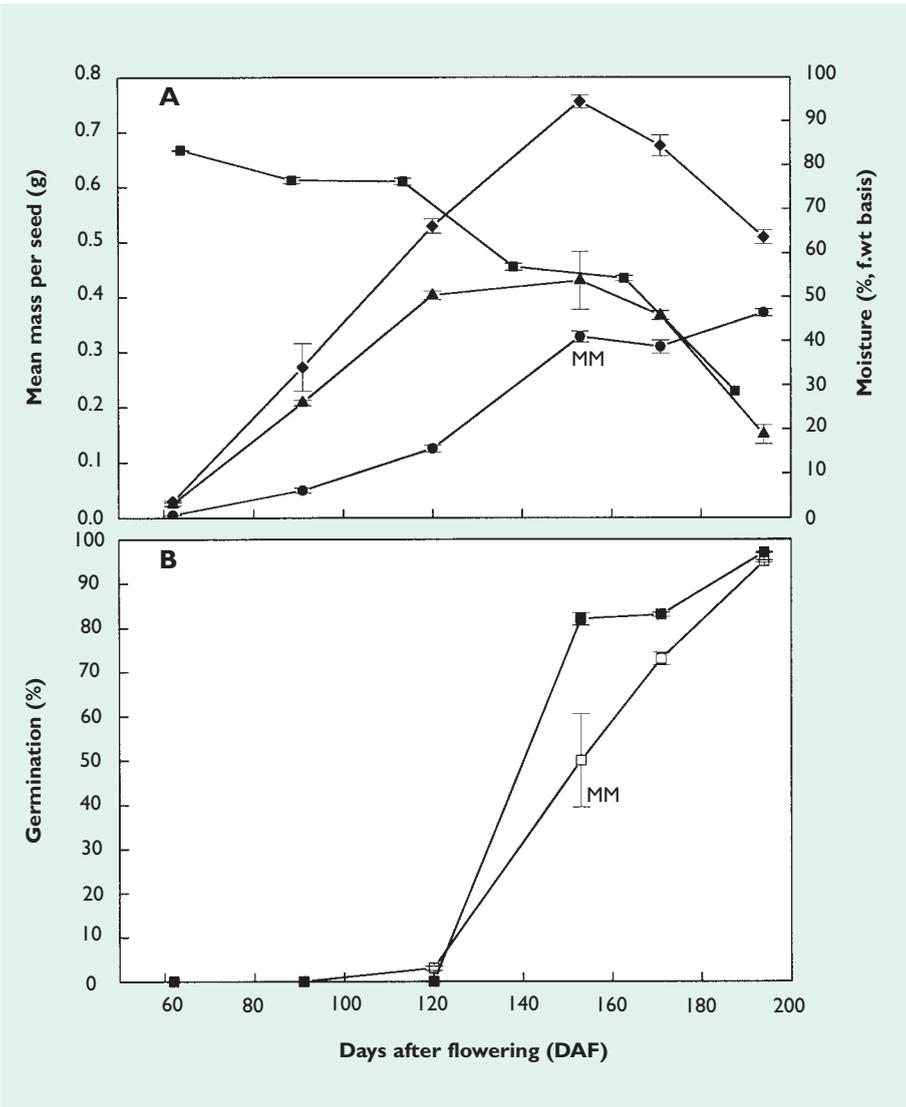
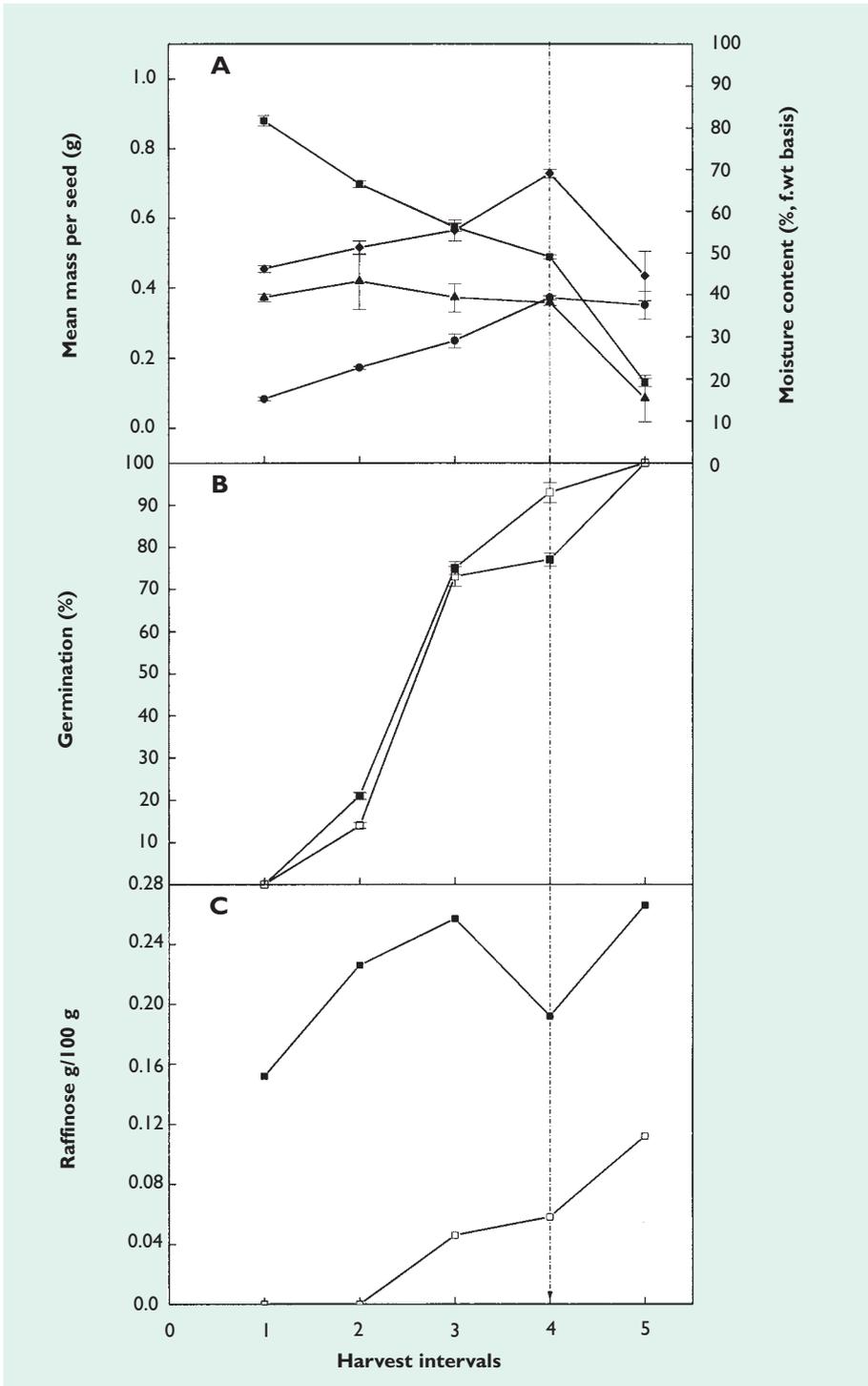


Figure 7.1 Seed development in *M. leucantha* (Makueni population) in 1997. A: Changes in moisture content (fr. wt. basis) (■); fresh weight (◆); dry weight (●) and weight of water (▲). B: Germination of fresh (□) and dried (■) seeds. Points represent the mean of five replicates of 10 seeds each (A) or four replicates of 25 seeds (\pm s.e.) (B). MM denotes the timing of mass maturity.



In both populations and in both years, despite the fact that tolerance to desiccation was acquired in some individuals prior to MM, the time when all seeds were capable of germination after drying occurred after MM. A similar pattern has been reported for a range of diverse species including grain legumes (Ellis *et al.*, 1987), *Acer platanoides* (Hong and Ellis 1990; Dickie *et al.*, 1991); pepper (Demir and Ellis, 1992) and *Digitalis purpurea* (Hay and Probert, 1995). In all cases, the time lapse between the first individuals in the population being able to withstand drying and all individuals having acquired desiccation tolerance appears to indicate variation between individuals in the physiological age when this trait is acquired. However, it seems more likely that much of this apparent variation is due to developmental variation between individuals at each sample point. Despite, efforts to reduce this variation by tagging flowers or selecting morphologically similar fruits, as in this study, it is inevitable that samples will contain individuals at different stages of maturity. Such variation will appear to be less in species with short developmental periods and exaggerated in species like *M. leucantha* where seeds develop slowly over a period of several months.

3.3. The accumulation of raffinose in fresh and dried seeds

From the second to the fifth and final harvest, there was a steady increase in the level of raffinose in freshly harvested seeds which paralleled the increase in the proportion of seeds capable of germinating with or without drying (Figures 7.2b and c). Although there was an overall increase in raffinose levels in dried seeds the correlation with germinability was less strong.

Although a proportion of seeds were capable of withstanding desiccation at the time of the second harvest, there was no detectable raffinose in fresh seeds at this time. By contrast, comparatively high levels of raffinose were detected in dried seeds even in the first harvest when no seeds were capable of germination. This confirms a previous report that there is no simple link between the presence of raffinose and the ability of seeds to withstand desiccation. Black *et al.* (1999) showed that desiccation tolerance could be induced in immature wheat embryos by slight desiccation at a time when the raffinose signal in fresh seeds was not detectable.

Figure 7.2

Seed development and the accumulation of raffinose in *M. leucantha* (Makueni population) in 1998. A: Changes moisture content (fr. wt. basis) (■); fresh weight (◆); dry weight (●) and weight of water (▲). B: the germination of fresh (□) and dried (■) seeds. C: The raffinose content of fresh (□) and dried (■) seeds. Points represent the mean of five replicates of 10 seeds each (A) or four replicates of 25 seeds (± s.e.) (B). Raffinose values represent the mean of two assays on 0.1 g each of dried seeds and 0.5 g each of fresh seeds. These ground tissue samples were from approximately 10 seeds. The broken line denotes the timing of mass maturity.

4. Conclusions

- Whilst the description of pod and seed colour can be somewhat subjective, the striking change from green to brown, particularly in seeds of *M. leucantha*, is a good indication that most seeds are likely to be desiccation tolerant and therefore capable of being stored.
- In this study, a sharp downward inflection in seed fresh weight at MM was found. Conservationists wishing to monitor the development of seed quality in *M. leucantha* could therefore track this parameter using, for example, a simple laboratory balance.
- We suggest that the most reliable field indicator of high seed quality are signs that pods have changed colour and are beginning to split.
- Measurement of the raffinose content of fresh seeds could be used as an indirect measure of seed quality. However, the presence or absence of raffinose in fresh or dried seeds is no indication of their ability to withstand desiccation.

Drying Method and Potential Longevity

1. Introduction

When seeds are harvested prior to the attainment of maximum ripeness, improvements in final seed quality can be achieved by delayed or slow initial seed drying (Hopkinson *et al.*, 1988; Hay and Probert, 1995; Hay *et al.*, 1997; Probert and Hay, 2000; Hay and Smith, 2003 – Chapter 6). Ripening events are thought to continue during such treatments. Like other wild plants that have been studied in detail, maximum seed quality in *M. leucantha* is attained close to natural seed dispersal.

This study examined the effects on seed quality of a variety of different post-harvest handling techniques when seeds of *M. leucantha* were harvested at different time intervals during the post-abscission phase.

2. Materials and Methods

2.1. Seed collection and drying

Bulk collections of pods were made on 4 August, 12 August, 1 September and 27 September 1998 from a population of *M. leucantha* located in the Makueni district, Kenya (1° 49'S, 37° 48'E). After each collection, pods were selected in the laboratory according to prevalent morphological features so that the pods

represented a distinct physiological stage with minimum developmental variation between pods. For each harvest, pods were then divided into four equal portions and allocated to the following drying methods:

Sun drying to equilibrium

Pods were held on a wire grid supported about 12 cm above the floor of a well-ventilated concrete drying frame. The dryer with its removable fibreglass cover was inclined away from the sun. Diffused sunlight was able to penetrate into the dryer resulting in solar gain and thus an acceleration of seed drying rate. Each day at about 8.00 a.m., pods were mixed. After about 30 d, dehisced seeds were transferred to cotton bags (30 cm $l \times$ 15 cm w) and allowed to equilibrate to local ambient conditions for about 10 d prior to transfer to a dry room (15% RH and 20°C).

Shade drying to equilibrium

Pods were placed on a 1 m \times 1 m wire grid on a wooden support in an open potting house at the National Museums of Kenya (NMK). Each day, pods were mixed as described above for sun drying. Dehisced seeds were handled as described above.

Shade drying for 10 d followed by sun drying

Pods were initially dried in the shade for 10 d as described above. Pods were then transferred to sisal bags (100 cm $l \times$ 70 cm l) which were placed in the sun drying frame for 30 d. Seeds were then transferred to a dry room as described above.

Gene bank drying

Pods were enclosed in sisal bags to prevent the loss of seeds as a result of explosive dehiscence (100 cm $l \times$ 70 cm w) and sent immediately to a dry room (15% RH and 20°C). During drying, efforts were made to spread the pods in a thin layer within the sisal bag. Following pod dehiscence, seeds were sorted manually then transferred to cotton bags (30 cm $l \times$ 15 cm w) and allowed to equilibrate for 20 d in the dry room. When all seed lots had attained equilibrium they were air-freighted to Wakehurst Place (WP). On arrival, seeds were re-assessed for moisture status and either dried further or used immediately in controlled ageing experiments.

2.2. Controlled ageing experiments

To ensure that seeds were at the same level of water activity, seeds were rehydrated in a sealed polycarbonate box (Electrospeed) over a saturated salt solution of magnesium nitrate, $[\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}]$ at 6 °C. Seeds were supported above the salt solution on a wire grid held on plastic containers. Differences in moisture content between individual seeds, across different processing methods, was therefore minimised prior to transfer to the ageing environment. For ageing, seeds were held in 520 ml open aluminium cans in a humidity controlled oven (LEEC, Model SFC 2) at 60% RH and 50°C. This open system was adopted to eliminate the risk of variation in moisture status between samples.

3. Analysis of Results

The viability equation (Ellis and Roberts, 1980):

$$v = K_i - p/\sigma$$

where v = probit percentage germination, K_i is the seed lot constant (y intercept of survival curve), p = days in storage and σ = standard deviation of seed deaths in time, was fitted to the seed survival data using probit analysis using the Glim 4 Computer Package (Francis *et al.*, 1993).

4. Results and Discussion

As expected, seed quality increased between the first and last harvests (Figure 7.3); for example, mean P_{50} across the four drying treatments increased from 4.1 d to 12.9 d (Table 7.2). Differences in seed quality between the three drying methods: shade drying, shade followed by sun drying, and sun drying were comparatively small and not consistent. This is emphasised by pods harvested on 1 September, when no differences between these methods were evident and regressions could be constrained to a single line without a significant increase in residual deviance ($P > 0.05$, Figure 7.3c). Unexpectedly, the quality of seeds dried in the dry room, was consistently lower than for the other processing methods at every harvest. There are two possible explanations:

(a) K_i failed to increase during dry room drying

This could be supported if seeds dried too rapidly in the dry room thus curtailing maturation events which continued in the other (slower) drying methods. Although direct measurements of the drying rate of seeds within pods were not made, this explanation seems unlikely since seeds were dried inside pods held within sisal bags. Observations showed that it took several days before any pods began to dehisce when more rapid drying could begin.

Table 7.2 The time (d) for viability to fall by 50% (P_{50}) for seeds of *Milletia leucantha* harvested at four time intervals in 1998 and then dried within pods under four conditions

	Harvest 1 (4 August)	Harvest 2 (12 August)	Harvest 3 (1 September)	Harvest 4 (27 September)
Dry room	3	5.1	3	10.1
Shade	3.7	7.8	7.5	12.9
Shade/sun	5.1	8.7	8	13.5
Sun	4.7	10.3	8.5	15

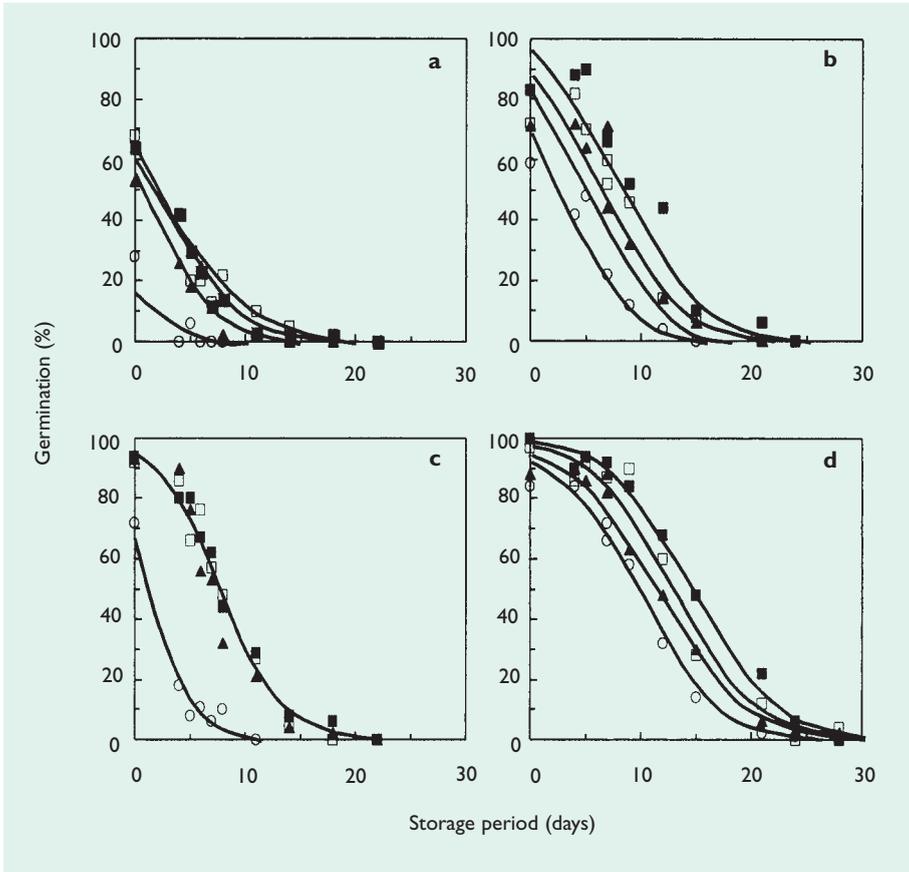


Figure 7.3 Survival curves fitted by probit analysis (best fit regressions) for seeds of *Millettia leucantha* Vatke harvested in 1998 at four time intervals during the post-abscission phase of development. a) Harvest 1 (4 August); b) harvest 2 (12 August); c) harvest 3 (1 September) and d) harvest 4 (27 September). Pods were dried in a dry room at 15% RH and 20°C (○), shade (▲), shade for 10 d followed by sun drying (□) and sun only (■). Seeds were aged 60% RH and 50°C. All points represent the mean of either 100 (4 × 25 replicates) or 50 seeds (2 × 25 replicates).

(b) K_i dropped during dry room drying

This would occur if seeds dried very slowly under dry room conditions, resulting in ageing. This explanation appears to be supported by the seed development data shown above. For seeds harvested at around the time of mass maturity (on 1 September 1998), percentage germination for fresh seeds prior to drying was 93%. By contrast, the K_i (initial viability) of dry room dried seeds, derived by probit analysis of seed ageing data, was 65.9%. This compared with 94.8%, 95.9% and 96.3% for sun, shade and shade/sun drying respectively. Evidently, K_i dropped while seeds dried in the dry room. As mentioned above, pods were enclosed in sisal bags to avoid seed loss arising from explosive dehiscence. The quantities of pods involved were large and in order for seeds to dry effectively, regular mixing and re-spreading was essential particularly for the first three harvests when pods were very moist. That K_i dropped during the gene bank drying treatment is further supported by the relatively small differences in quality between drying methods for the final harvest (Figure 7.3d), when pods were close to natural dehiscence and therefore much drier.

5. Conclusions

- For seeds of *M. leucantha*, harvested at four stages of maturity, no consistent differences in seed quality could be attributed to the different drying methods: sun, shade or shade followed by sun.
- All three 'low technology' methods resulted in higher final seed quality compared with seeds dried in a seed bank dry room at 15% RH and 20°C.
- Evidence suggests that the poor quality of dry room dried seeds resulted from deterioration probably caused by slow drying of pods enclosed in sisal bags.
- These results demonstrate that great care must be taken to ensure adequate ventilation during the drying of pods of *M. leucantha* that are not fully mature.

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