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Reliability of germination testing procedures and germination performance of stored *Eucalyptus camaldulensis* seed of different ages

E.R. WILLIAMS[1], B.V.W. GUNN[2], D.J. SOLOMON[3] AND J.C. DORAN[2]

[1] Statistical Consulting Unit, The Australian National University, Canberra ACT 0200
(E-mail: Emlyn.Williams@anu.edu.au)
[2] Ensis, PO Box E4008, Kingston, ACT 2604, Australia
[3] CSIRO Plant Industry, GPO Box 1600, ACT 2601, Australia

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Summary

Eucalyptus camaldulensis Dehnh. is an important species for environmental and commercial forestry and there is a strong demand for seed. Stored seed must be tested in order to monitor losses in germination rate and vigour over time, which might adversely affect nursery recovery rates. Typically, seed storage facilities undertake germination testing in growth cabinets in which temperature and light are controlled. It is important to ensure that testing procedures are reliable; for example, the use of different germination cabinets should not affect the results. Here, we have assessed the germination performance of *E. camaldulensis* seedlots that have been stored for periods of 1, 6, 12 and 17 years, employing two germination cabinets routinely used for testing seed. A detailed experimental design was developed to determine the consistency of germination within and between germination cabinets. The normal practice of undertaking laboratory germination studies of limited size in a single germination cabinet does not appear to compromise the results. It is, however, important if multiple shelves are to be used within a cabinet, that an experimental design is employed so that adjustments can be made in across-shelf comparisons of germination capacity. Seed collected in 1985 and stored for 17 years had a significantly lower germination rate than younger seedlots.

Introduction

Seed of *Eucalyptus camaldulensis* Dehnh. is in high demand for environmental and commercial tree plantings in the wet/dry tropical to warm temperate parts of the world (Eldridge *et al.*, 1993). The Australian Tree Seed Centre (ATSC) is a major supplier of authentic, source-identified *E. camaldulensis* seed suitable for provenance/progeny trials and tree improvement work (ATSC 2004). The ATSC collection of *E. camaldulensis* seedlots spans a wide range of age classes, as the systematic sampling of provenances of this wide-ranging species has taken many years to accumulate.

Eucalyptus camaldulensis has orthodox seed (Ellis and Roberts 1980), which previous studies have shown to give good medium-term storage characteristics with a reduction in germination rate of only 13% after 10 years of storage at room temperature (Doran *et al.*, 1987; Gunn 2001). We need to ensure, however, that stored seed is regularly tested to assess any loss in germination rate. We also need to periodically evaluate our procedures for determining seed viability and in particular, our use of single germination cabinets

for germination tests. There are very few studies in the literature of the effect of using different cabinets on germination test results. Davidson (1991) found that cabinet effect was not significant in a study of germination of *Abies amabilis* [Dougl.] Forbes. Ashton *et al.* (2002) reported a shelf and dish placement effect within cabinets (2) in a study of *E. regnans* F. Muell.

Here we present results of the germination performance of *E. camaldulensis* seedlots that have been stored for periods of 1, 6, 12 and 17 years. The germination trial was designed to assess the consistency within and between germination cabinets when applying ATSC's routine seed viability testing procedures, as well as investigating the effects of storage time on germination performance.

Materials and methods

Thirty seedlots representing single seed trees and six bulk seedlots of *E. camaldulensis* from nine different provenances across four collection dates (1985, 1990, 1996, 2001) were germinated. Summary details of each seedlot are given in table 1. The bulk CSIRO seedlot 20618, was used as a control and was replicated five times more frequently as the other seedlots making a total of 40 trial seedlots; seedlot numbers 42-46 refer to the control. Note that the seedlots were numbered 7 to 46 to maintain consistency with the glasshouse and field components of this project.

Table 1. *E. camaldulensis* seedlots by provenance used in the germination experiment, including locations and collection dates.

Trial Seedlot Number (seedlot)	CSIRO Seedlot Number (provenance)	Latitude	Longitude	Altitude (m)	Location	Collection Date
7	15024	360800	1415800	75	Lake Hindmarsh-SE	1985
8-17	15025	355700	1415200	75	Lake Hindmarsh-NW	1985
18	15031	352600	1415700	45	Lake Agnes	1985
19	18114	354500	1422300	60	Lake Coorong	1990
20-29	19708	354500	1425800	150	Lake Albacutya	1996
30-39	20561	354500	1415800	80	Lake Albacutya	2001
40	20562	355730	1415130	73	Lake Hindmarsh-NW	2001
41	15022	365500	1424000	170	Wimmera Glenorchy	1985
42-46	20618	380200	1433800	100	Cressy	2001

Harvested fruit of *E. camaldulensis* were air dried prior to extraction of the 'seed' consisting of small viable seeds and a much larger fraction of unfertilized ovules referred to as chaff (Boland *et al.*, 1980). Following cleaning and fumigation, seedlots were packaged and stored at temperatures between 23°C – 27°C in metal containers with air tight lids.

Storage occurred within one to two months after field collection. One exception to this was seedlot 19 (bulk CSIRO seedlot 18114), which was placed into storage at room

temperature 18 months after collection, with no prior knowledge of its handling. While moisture content of seedlots was not determined at the time of storage or subsequently, seed moisture content for this species following field extraction is typically in the range of 8 to 11%. Seeds were sprinkled on moist filter paper over 30ml of A-grade vermiculite in a 90mm glass Petri dish to which 28ml of gel-filtrated water was added. Each of the four replicate dishes received 0.1g ± 0.01g of seed and chaff, consistent with normal laboratory procedures for determining germination capacity with this species (ISTA 1996, Gunn 2001). The dishes were placed at 25°C ± 2°C in two Linder and May© germination cabinets which were set to alternate between 12 hours of light and 12 hours of darkness. The cabinets differed in age and light direction. Cabinet 1 was a 1990 model with the two fluorescent white lights mounted vertically at the rear of the cabinet. Light intensity ranged from 100 LUX in the front of the cabinet to 570 LUX at the rear of the cabinet. Cabinet 2 was a 2002 model with the two fluorescent white lights mounted vertically in the front door providing a range of light intensity from 150 LUX at the rear of the cabinet to 750 LUX nearest the light. There were no marked differences in light intensity between the shelves in both cabinets, as determined when no germination dishes were present.

The germination cabinets accommodated 20 dishes on a shelf (four rows and five columns) and had eight shelves. Hence a three-dimensional experimental design was required in order to adjust for possible variation between rows and columns within each cabinet and between shelves. For each germination cabinet, a four-replicate latinized row-column design (see Williams *et al.*, 2002, Chapter 7) was generated using the software package CycDesigN (Whitaker *et al.*, 2002). This design was then adapted to give a three-dimensional construction. Features of this design are that each pair of shelves constitutes a complete replicate of the seedlots. The five dishes in row 1 of each of the eight shelves also make up a replicate of the seedlots, as do the dishes in rows 2, 3 and 4. There are also design properties for rows and columns within each shelf and so the layout allows us to detect and adjust for a variety of possible sources of systematic variation within the germination cabinets.

Germination counts in any one dish commenced when the first cotyledons emerged, usually from day three. Healthy germinated seedlings were recorded daily (from day three onwards) with the exception of days 15 and 16, which coincided with a weekend. Germination counts were completed at day 21; this provided ample time for germination to be completed. The 17 daily count variates were labelled $n_1 ... n_{17}$. At the completion of the test, germinated seed with seed coats still attached were included in the calculation of germination count, but abnormals were excluded.

Germination measurements were converted into the following variates in order to analyse germination performance:

1. *Total* – the total of the 17 counts $n_1 ... n_{17}$
2. *Rate* – the germination rate $\Sigma n_i t_i / \Sigma n_i$ where t_i is the number of days to the *ith* count starting at 11/09/2002 – a high value indicates a slow germination rate
3. *Peak* – $\max((\dot{\Sigma}_j n_j) / t_i)$
4. *Speed* – $\Sigma n_i / t_i$

The variates *Total*, *Rate* and *Peak* follow Fox and Davies (1983) and *Speed* from de Matos Malavasi *et al.* (1985).

Initially, analyses were carried out separately for each cabinet using a mixed-effects model (see Williams *et al.*, 2002, Chapter 8) with seedlots, shelves, cabinet rows and columns as fixed effects and rows and columns within shelves as random effects. The statistical package GenStat was used for all analyses. Estimated seedlot means were then compared for consistency across the two cabinets using analyses of variance as outlined by Williams *et al.* (2002, Chapter 5) to investigate the size of the cabinet by seedlot interaction. At the same time the between seedlot variation was partitioned into components within and between provenances.

A complicating factor in relating storage time and viability of the different seedlots is that they can vary in the amount of seed and chaff in each seedlot sample. Hence separate 0.3g samples were retrospectively taken from just 12 of the seedlots; a number of the seedlots were no longer available and so seedlots were chosen on seed availability and to span the range of collection dates. The number and weight of seeds were recorded in order to provide information on relationships between seed size, chaff content and storage time.

Results

The mixed-model analyses of both germination cabinets allowed an assessment of the level and nature of within-cabinet variation. There was considerable variation within cabinets as exhibited by differences between cabinet shelves, rows and columns for the four variates but there was no consistent pattern. The main variation occurred between shelves of the cabinets, particularly for cabinet 1 where differences were highly significant ($P<0.001$) for all variates. For cabinet 2 there were highly significant differences for *Total* and *Rate* but less variation for *Speed* ($P<0.05$) and *Peak* (not significant). The estimated shelf means are plotted in figure 1, with a 5% least significant difference (LSD) pooled over both cabinets. For *Rate* there is evidence that germination has been slower on the top shelves (i.e. a higher value for germination rate). Also in cabinet 1 the middle shelves have demonstrated good germination performance for *Total*, *Peak* and *Speed*.

In cabinet 2 there were significant row differences for *Rate* ($P<0.001$), *Peak* and *Speed* ($P<0.01$). For these variates the trend was for better germination performance near the front of the cabinet (near the light source). Cabinet 1 also showed evidence of better germination nearer the light source (this time at the back of the cabinet) but the differences were not statistically significant for any of the variates. There were some significant column differences, the strongest being in cabinet 1 for *Peak* ($P<0.001$), *Speed* ($P<0.01$), *Total* and *Rate* ($P<0.05$). The major cause for the differences was due to column 1 (at the side where the door opens) which had poorer germination performance than the other columns. As a measure of the overall variation accounted for by fitting shelves, rows and columns in the analyses, it is useful to compare the residual mean squares for a randomized complete block analysis of each variate with the corresponding mixed-model analysis. This is done in table 2 and shows a substantial reduction in residual mean squares, hence leading to a more precise comparison of estimated seedlot means.

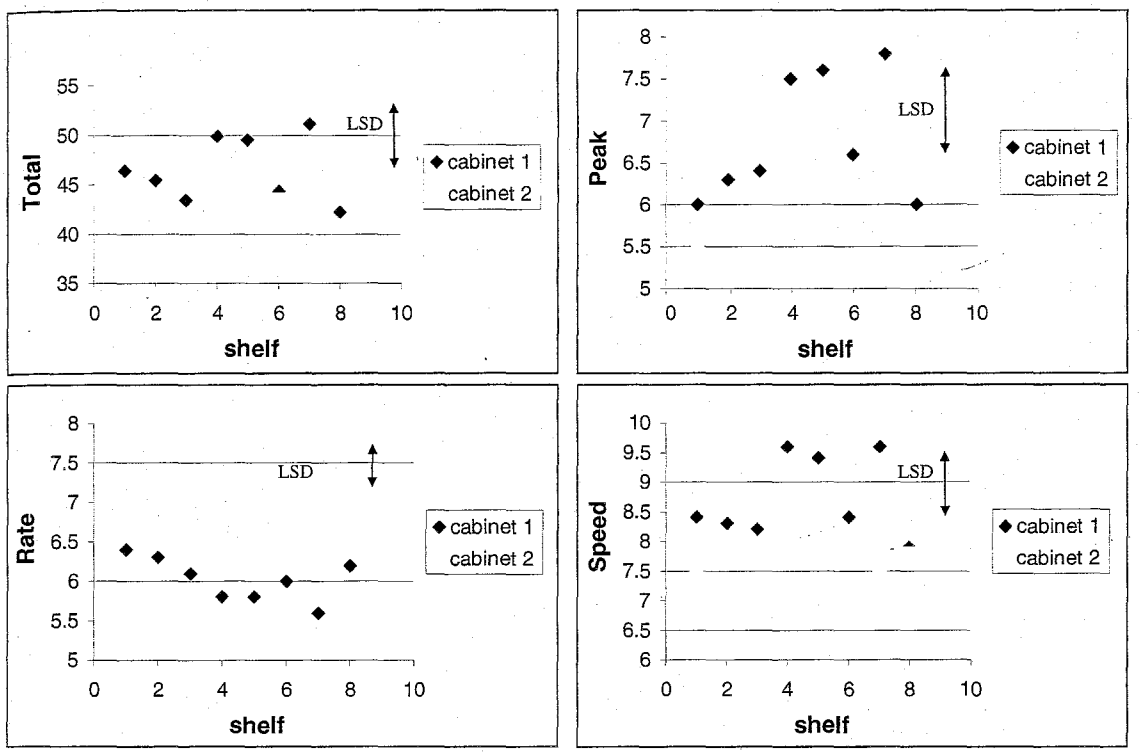
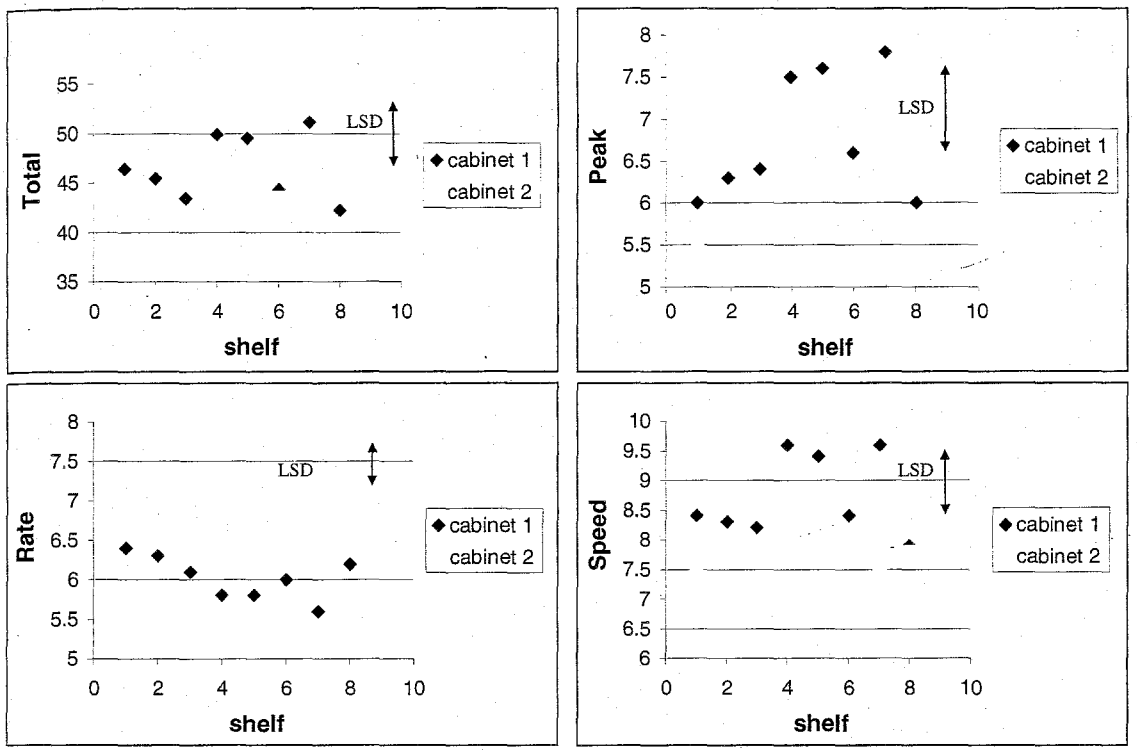


Figure 1. Plots of estimated shelf means by germination variate for *E. camaldulensis* seedlots.

Table 2. Overall means and residual mean squares by germination variate for *E. camaldulensis* seedlots from Randomized Complete Block (RCB) and mixed-model analyses of data from each cabinet.

	Cabinet 1			Cabinet 2		
		Residual mean squares			Residual mean squares	
Variate	Mean	RCB	Mixed-model	Mean	RCB	Mixed-model
Total	46.5	109.8	92.3	44.7	116	89.8
Rate	6.01	0.32	0.22	6.65	0.69	0.48
Peak	6.77	2.58	1.81	5.71	2.75	2.21
Speed	8.71	3.92	2.98	7.5	3.97	2.97

In figure 2 the seedlot means from the two cabinets are plotted for the four variates demonstrating consistency across cabinets. This is quantified in the non-orthogonal analyses of variance (table 3) where the residual mean square for testing has been obtained by pooling the residuals from the individual cabinet mixed-model analyses. The results show that the interaction of provenances and cabinets is not significant for all variates except *Rate*. Further investigation of the cause of this interaction shows that it derives from the bulk seedlot 7 and to a lesser extent seedlot 41 (CSIRO seedlot numbers 15024 and 15022 respectively); both of these seedlots were stored from 1985 and had poor germination rates. The analyses also demonstrate that there is variation between individual seedlots within the *E. camaldulensis* provenances in all variates and that the interaction of seedlots



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within provenances and cabinets is not significant. Interestingly seedlot 19 (which was placed in storage 18 months after collection) had the worst germination performance for all variates except *Rate*.

For the 0.3g extra samples, there was considerable variation in seed numbers (table 4) and the correlation between seed number and weight was strong (r^2=0.76). The regression of seed number on age was, however, not significant. The variates *Total, Peak* and *Speed* are all influenced by different numbers of seed and hence cannot be used to assess germination performance over time; *Rate*, however, is normalized for seed number and can be used for this purpose. Incorporating collection dates into the table 3 analysis of *Rate* shows highly significant differences (P<0.001), due mainly to the slow germination rate of the 1985 seedlots. The estimated means for *Rate* are 9.22, 4.80, 4.07 and 5.49 for seed stored for 17, 12, 6 and 1 years respectively (LSD=0.91).

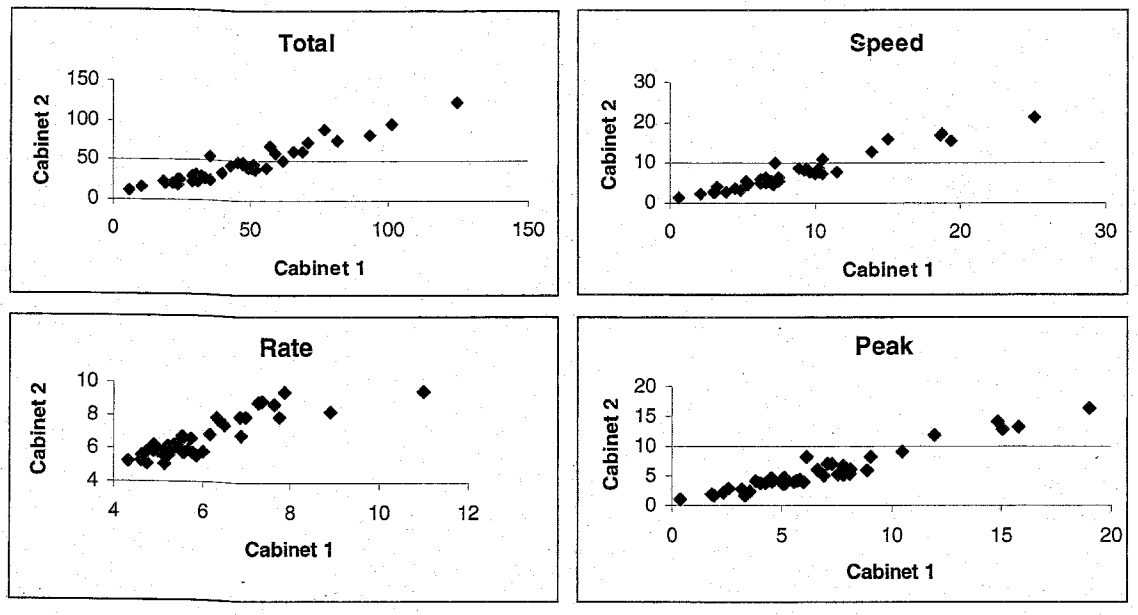

Figure 2. Comparison of estimated *E. camaldulensis* seedlot means from both cabinets.

Table 3. Across cabinet analyses of variance of germination results for *E. camaldulensis* seedlots.

		Mean Squares			
Source	d.f.	Total	Rate	Peak	Speed
Cabinet	1	64.0 ns	8.2***	22.2 ***	29.3 ***
Provenance	8	2675.1 ***	13.9***	77.5 ***	114.1 ***
Cabinet.Provenance	8	24.4 ns	0.6***	0.7 ns	1.2 ns
Provenance.Seedlot	31	849.7 ***	0.4***	15.9 ***	27.6 ***
Cabinet.Provenance.Seedlot	31	22.5 ns	0.1 ns	0.6 ns	0.9 ns
Residual	114	22.8	0.1	0.5	0.7

*** P<0.001; ns not significant

Table 4. Seed counts and weights from separate 0.3g samples of selected seedlots.

Trial Seedlot Number (seedlot)	CSIRO Seedlot Number (provenance)	Collection Date	Seed Count	Seed Weight (g)
8	15025	1985	562	0.067
10	15025	1985	312	0.046
11	15025	1985	455	0.058
13	15025	1985	236	0.042
17	15025	1985	165	0.034
20	19708	1996	439	0.073
26	19708	1996	438	0.075
27	19708	1996	270	0.074
33	20561	2001	145	0.038
38	20561	2001	518	0.118
41	15022	1985	202	0.035
42-46	20618	2001	217	0.044

Discussion

An understanding of the nature and level of any variation both between and within germination cabinets is very important in order to provide accurate seed germination information to clients. This trial showed very good consistency of germination results between the two cabinets used in the study. This confirms that seed testing procedures routinely conducted by ATSC, which typically rely on the use of a single germination cabinet per test, can provide reliable results. The detailed experimental design used within each cabinet illustrated, however, that consideration needs to be given to the position of germination dishes, with both cabinets showing substantial but different patterns of variation. The predominant source of variation was from shelf-to shelf with cabinet 2 also exhibiting a reduction in germination performance moving away from the light source. Hence, as much as possible, comparative studies of germination should be carried out on the same shelf, or experiments should be designed to allow for variation between shelves and for distance from the light source.

It is evident from these results that the germination rate for *E. camaldulensis* seedlots, stored routinely at room temperature in air-tight tins, rapidly lose vigour and germination capacity some time after about 12 years of storage to the extent that they may perform poorly in the nursery. This has implications for scheduling *E. camaldulensis* seed collecting activities aimed at replacing aging seed stocks. Clearly, the accumulating biochemical and cytological deterioration within the stored *E. camaldulensis* seeds has reached a stage by about the 12-year mark to have an effect on the ability of seeds to germinate normally. Temperature and moisture content are the two major factors determining the rate of ageing (Schmidt 2000). Where ex-situ conservation is a priority in storing rare or threatened provenances of *E. camaldulensis* (Eldridge *et al.*, 1993), it would be prudent, therefore, to store the seed in a domestic freezer (-18°C) in order to prolong storage life (Gunn 2001).

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