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THE
REGENERATION OF
Eucalyptus pauciflora Sieb. ex Spreng.
FROM SEED

by

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CHAPTER 3

INNATE DORMANCY

CHAPTER 3

The fate of a seed reaching the ground will be determined both by the physical and physiological characteristics of the seed and by the environment that it encounters. This chapter follows two lines of investigation; the first examines the differences in the physical and the physiological states of the seed from a range of altitudes, and the second looks at the potential of environmental factors to modify seed dormancy during dispersal.

Eucalyptus pauciflora has a variable proportion of dormant seed (Pryor 1954; Boland et al. 1980) which tends to increase with altitude (Grose 1957). Dormancy has been associated with properties of the seed coat (Boden 1957, Bachelard 1967b) and may be broken by moist treatment at low temperatures (Pryor 1954). The precise mechanism which produces the dormant state is unknown and is peripheral to the scope of this thesis which attempts to describe the response of the seed of E. pauciflora to its environment. Possible mechanisms causing seed dormancy in E. pauciflora are discussed in Section 8.3.

Seed size has been associated with seed dormancy in several Eucalyptus species. The size of a seed provides an indication of the reserves that it carries and has been shown to be an important factor influencing the outcome of competition between individuals of the same species (Black, 1956). Grose (1963) investigated the influence of seed size on dormancy in E. delegatensis and found that there was no difference in the dormancy of five seed populations, selected for the differences in their seed size distribution.

There were considerable differences in the dormancy of seed collected from individual trees in a particular locality and there was an increase in dormancy associated with decreasing seed size within seed collected from individual trees (Grose 1963). The variation in the dormancy of the seed of individual trees was not investigated in the studies described here but care was taken to take seed from at least twenty trees over an area of around 1 hectare at a seed collection site in order to sample the variation in the population.

Grose (1957) identified two patterns of germination with time in E. pauciflora seed associated with seed from low and high altitudes. The germination curve of the 'dormant', higher altitude type typically shows a rapid attainment (within 10 to 20 days at 15°C) of final germination capacity with a proportion of the seed remaining in a dormant state. In contrast, Grose reported that E. pauciflora seed from low altitudes (30 m, 365 m, 457 m) exhibits 'partial dormancy', which he described as "the attainment of full germination (germination capacity close to 100 %) at very slow rates (more than 35 days)". Seed samples exhibiting both the 'dormant' and the 'partially dormant' seed responded to stratification with decreases in the times-to-germination.

Grose (1960) found that maternal site factors such as altitude, aspect and dominance had no apparent effect on the dormancy of the seed of E. delegatensis in Victoria. Grose attributed this phenomenon to the absence of any selective advantage associated with an increase in dormancy in the range of environments considered; the major cause of deaths in autumn was found to be frost damage and all of the environments experienced severe radiation frosting during autumn.

The dormancy of seed may also be influenced by the conditions under which seed is shed from the capsule. The dormancy which may be induced in seed by environmental conditions prior to, and during, dispersal may not be strictly classified as innate; however, it is convenient to include it in this chapter. Grose (1960) noted that the dormancy of E. delegatensis seed shed from capsules, which were dried at high temperatures on the hearth by a fire, was greater than those which were permitted to dry in the air under ambient conditions. Boland et al.(1980) also mention that high temperatures may result in increased dormancy of the seed shed from Eucalyptus species. These observations confirm that environmental factors have the capacity to influence the dormancy of Eucalyptus seed during the process of seed shedding. The potential for changes in the dormancy of dry E. pauciflora seed directly after shedding is shown by the increases in dormancy of E. pauciflora seed held under laboratory conditions for 12 weeks following extraction (Boden 1957, quoting unpublished work by Pryor).

In summary, it seems that E. pauciflora seed is likely to show changes in dormancy with increasing altitude of seed source. The state of dormancy could be expected to be influenced by the temperature of extraction of the seed and by the size of the seed within a seed lot. The experiments reported in this chapter seek to define the range of innate dormancy which occurs in E. pauciflora and the role of the environment in modifying dormancy during seed shedding through investigations of the changes in the innate dormancy of the species in relation to altitude of seed source, seed size, and the conditions during extraction of the seed from the capsule.

3.1 Changes in innate dormancy with altitude

Methods:

Capsules were collected at five sites (see Figure 1.1) near Waste Point (960 m), K13 (1310 m), K15 (1510 m), Smiggins Holes (1730 m) and Mt. Perisher (2000 m) in December 1982. Replicates of the cleaned seed (Waste Point 0.3 g, 49 viable seeds; K13 0.3 g, 65 viable seeds; K15 0.2 g, 48 viable seeds; Smiggins Holes 0.2 g, 61 viable seeds; Mt. Perisher 0.15 g, 36 viable seeds) were set to germinate at 15°C.

Results and Discussion:

The germination capacities of seed from different altitudes were significantly different, but there were no consistent trends with altitude (Table 3.1, Figure 3.1).

TABLE 3.1

Summary of analysis of deviance for the germination capacity of seed collected at four altitudes and germinated at 15°C.

Model terms	d.f.	Residual deviance	RMD	Change d.f.	Change deviance	Significance Level
Total	24	40.38	1.68			
Source	20	21.04	1.05	4	19.34	p<0.005

Source = Seed source

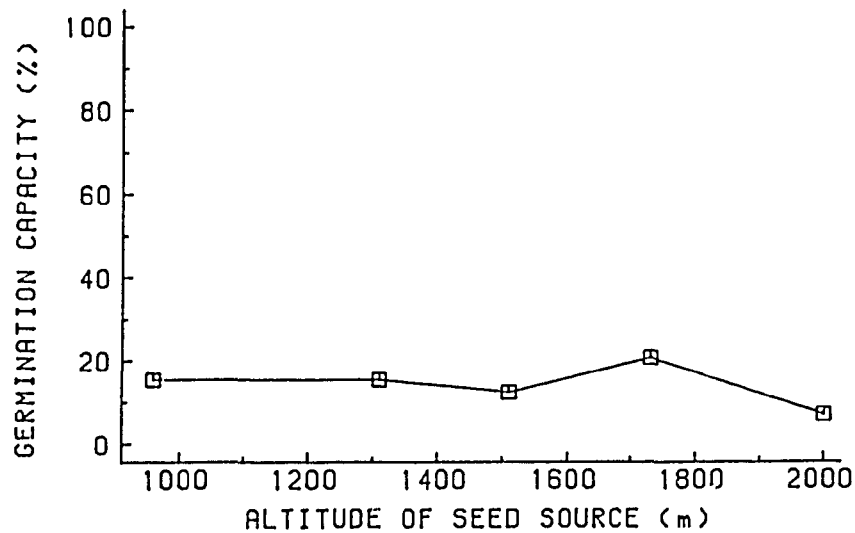


Figure 3.1 The germination capacity of *E. pauciflora* seed from 5 altitudes set to germinate at 15°C.

The germination capacities of seed from the four lower altitudes, Waste Point, K13, K15 and Smiggins Holes were not significantly different; however, the seed from Mt. Perisher (2000 m) had a significantly lower germination capacity (7.2%). Low germination capacities have also been observed for seed collected at treeline at Thredbo (1900 m) (Section 3.3.1). There may well be an increase in the degree of innate dormancy in seed collected near treeline but full elucidation of the trend with altitude would have required more extensive sampling of treeline populations of *E. pauciflora* than was permitted by the resources available.

3.2 Changes in innate dormancy with seed size

A general reduction in seed size with increasing altitude of seed source has been found by many workers (Tranquillini 1979, Wardle 1981). However, changes in the physical characteristics of Eucalyptus pauciflora seed with altitude have never been reported in the literature.

The term 'seed', in the experiments of this section, will refer to all the material that was extracted from the capsules, and includes both viable seed and 'chaff' (aborted ovules).

Methods:

Four replicates (10 g) of seed from each of the sources used in Section 3.1.1 were separated into nine contiguous size classes using a series of sieves (2000, 1680, 1410, 1190, 1000, 841, 707, 595, 0 μm mesh size) placed on a mechanical shaker.

The seed remaining on the top of each sieve was weighed and classified according to seed source and sieve size; the data were then subjected to an analysis of variance. As there was only enough seed from the highest seed source (2000 m) for three replicates of the experiment, the balance of the design was maintained for the analysis by estimating the weights of the missing replicate in the analysis.

The dormancy of seed from the various size classes was assessed by analysis of germination capacity following either 0 or 20 days stratification. The method used for seed preparation varied from that already described (Chapter 2) in that the cleaning step was omitted and

the seed from the various size classes was divided into units depending on the amount of seed available (0.2 to 0.5 g).

The number of viable seeds in each size class was obtained by multiplying the number of viable seeds per gram of seed for each size class by the weight of seed in that class. The weight of seed in each class was obtained from the table of means associated with the analysis of variance.

Results and Discussion:

(i) Seed size and altitude.

The size distribution of the components of the seed sample decreased with increasing altitude of seed source (Table 3.2, Figure 3.2). Analysis of the residuals following analysis of variance of the raw data showed that the residuals increased as the fitted values increased indicating heterogenous variance; this was subsequently corrected by a square root transformation.

There were equal amounts of seed from each source used in the experiment and the analysis should, therefore, have removed the main effect of seed source. However, the weighing of the seed left on the sieves had greater accuracy (± 0.00005 g) than the original weighing of the replicates (± 0.05 g) and this greater accuracy resulted in differences in the mean weight of seed from the different sources.

TABLE 3.2

Summary of analysis of variance for the mass of seed of various size classes in samples of seed from four altitudes.

Source of Variation	d.f	Sum of Squares	Mean Square	Variance Ratio	Significance Level
Size	8	71.675	8.959	8367.9	p<0.001
Source	4	0.075	0.018	17.6	p<0.001
Size . Source	32	13.469	0.421	393.1	p<0.001
Residual	126	0.135	0.001		
Total	170	85.355			

Size =Sieve opening (2000,1680,1410,1190,1000,841,707,595,0 μm)
 Source =Seed source (980 m, 1340 m, 1510 m, 1730 m, 2000 m)

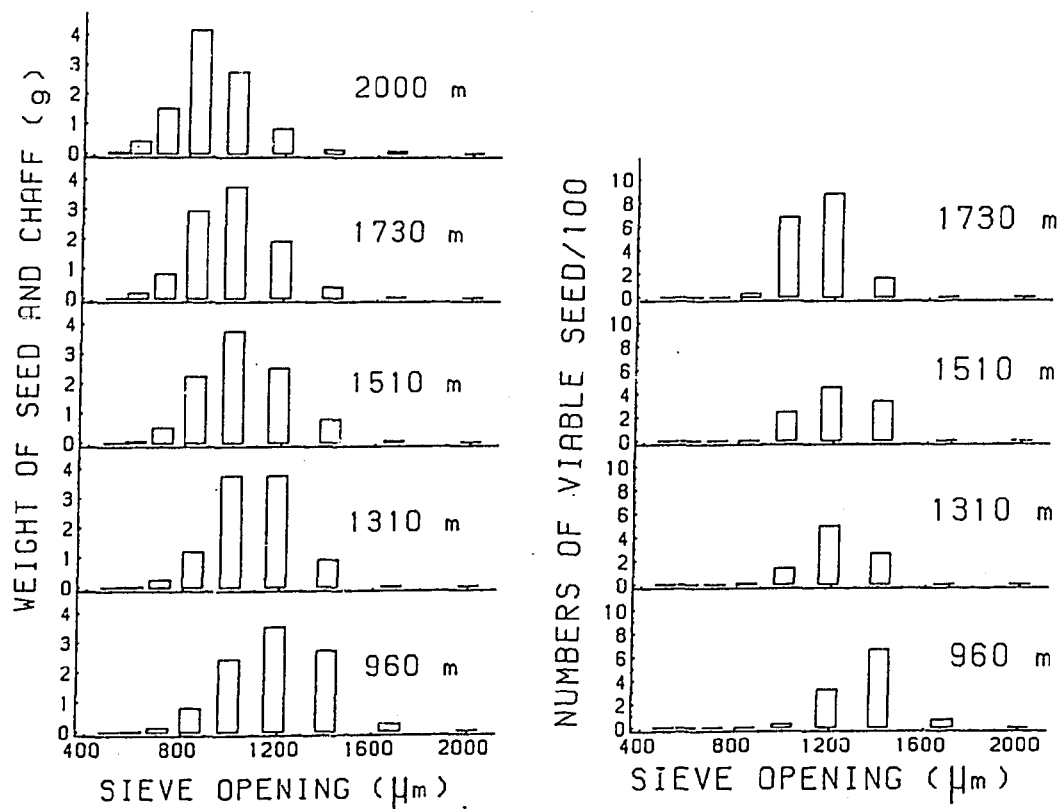


Figure 3.2 The size profiles for (a) weight of seed and (b) viable seed collected from trees at four altitudes.

The increase in the weight of the smaller components of the seed sample with increasing altitude was significant (Table 3.2, [Size.Source]) and was associated with a reduction in the size of the viable seed (Figure 3.2b).

The distribution of viable seed was largely restricted to four size classes (representing the seed remaining on top of the 1000, 1190, 1410, 1680 μm sieves) and also showed a trend of decreasing seed size with increasing altitude. Virtually none of the seed which passed through the 1000 μm sieve was viable (Figure 3.2b); however the proportion of the capsule contents in these smaller size classes increased markedly with altitude (Figure 3.2a).

The reduction in the size of viable seed with increasing altitude could have been genetically determined, or it could have resulted from a shorter growing season with a reduced period of seed-filling at higher altitudes. The increase in the proportion of the smaller, non-viable elements of the seed sample could either be a consequence of reduced seed-filling or could be due to a failure in the pollination and fertilization stages of seed production at higher altitudes. Regardless of the cause, the outcome is that, at higher altitudes, the viable seed reaching the ground is likely to be smaller and therefore have less reserves to draw upon during the seedling establishment phase.

(ii) Seed size and germination capacity

Comparisons of the relationship between seed size and germination capacity were restricted to seed from the four lower altitudes (960, 1310, 1510, 1730 m) because of a shortage of seed from the highest altitude. The experiment was further restricted because the number of viable seeds in the smaller (841, 707, 595, 0 μm) and larger (2000, 1680 μm) size classes was not sufficient for reliable estimates of germination capacity to be made.

The analysis of germination capacity is discussed in detail for this experiment in order to further clarify the method outlined in Section 2.4.1. The objective of the analysis was to quantify the effects of the experimental treatments on the germination capacity of the seed by selecting an appropriate statistical model, and to examine the parameters of the model in order to determine the effects of the treatments. The presentation of the results of the analysis is also discussed. A detailed discussion of the statistical analysis follows; the results of this analysis are summarized on page 57.

The relationship between germination capacity (calculated as the ratio of the number of seeds which germinated to the number of viable seeds in a treatment) and seed size, seed source and stratification treatment is shown in Figure 3.3. The responses to both seed size and seed source were most marked and consistent in seed which had been stratified for 20 days. The seed from high altitudes (1510 m, 1730 m) which had been stratified for 20 days had a lower germination capacity than the seed from the two lower altitudes (960 m, 1310 m) although there is little evidence of such differences in unstratified seed. The seed stratified for 20 days also shows a general trend for increases in

germination capacity with increasing seed size, a trend which is only apparent in the unstratified seed from low altitudes.

The statistical model (Section 2.4.1) relates the logarithm of the odds ratio ($G / 1-G$), where G is the probability of germination, to a linear combination of covariates, and is a particular case of a class of models known as Generalized Linear Models (Nelder & McCullagh 1983). The selection of the covariates to be included in the final model depends on the amount of variation which they explain.

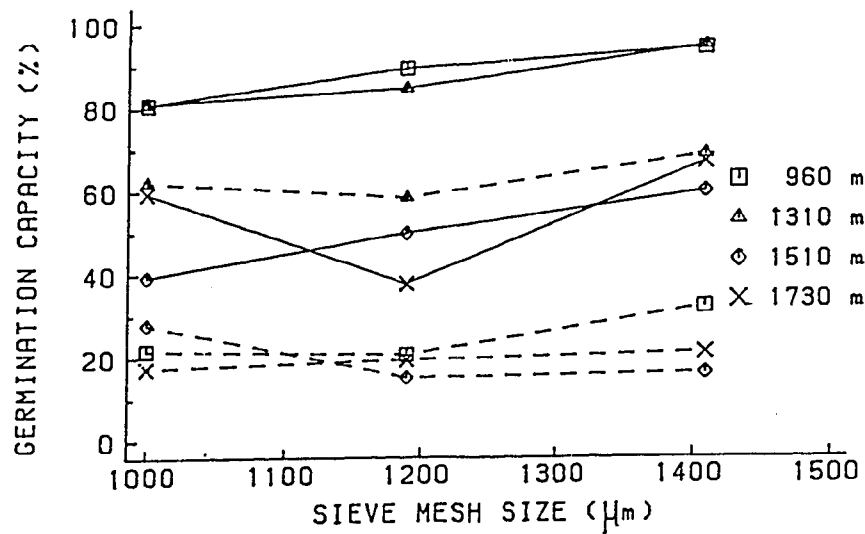


Figure 3.3 The germination capacity of three sizes of seed from four altitudes stratified for either 0 (---) or 20 (—) days.

The selection process is based on the systematic fitting of models with an increasing number of covariates. The number of covariates in the model may include the total number of treatment combinations but is more usually a smaller sub-set representing the treatments and a restricted set of the interactions between treatments.

The first step in developing an appropriate model was to determine the variation inherent in the data by fitting a model which includes the mean and none of the covariates.

$$\text{logit}(G) = \mu \quad \dots(1)$$

The covariates seed size, source and stratification treatment were all found to influence the germination capacity and the contribution of these covariates to reducing the deviance is determined by including them as factors in the model.

$$\text{logit}(G_{ijk}) = \mu + \text{SZ}_i + \text{SO}_j + \text{ST}_k \quad \dots(2)$$

In this model SZ is the seed size, SO the source and ST the duration of stratification. The subscripts refer to the levels within a covariate which are represented by dummy variables in the model; for example, in the case of seed size, three dummy variables, $i=1,2,3$ representing the sizes classes,

1000 μm < size₁ < 1190 μm < size₂ < 1410 μm < size₃ < 1680 μm respectively were used.

The analysis of deviance differs from an analysis of variance in that the comparisons are not orthogonal, and therefore the significance of a factor or interaction between factors cannot be assessed in isolation, but rather is assessed by the change in deviance when this element is removed from the model. In practice, this means that the model (2) must be refitted with each of SZ, SO, ST removed in order to determine the change in deviance due to each of these factors.

The observed changes in deviance are usually compared with the critical value of χ^2 associated with the change in the degrees of freedom; however, the analysis showed that this test is not appropriate because there is evidence that the data in this experiment is not Binomially distributed. When the model (4), which takes account of all the known sources of variation in the experiment is fitted to the data, the variation remaining cannot be wholly attributed to the Binomial distribution, since the residual mean deviance (RMD) is greater than 1 (RMD = $301.4/96 = 3.14$) (Table 3.3). In this case, a more conservative test of significance is employed which allows for the residual variation not accounted for by the Binomial distribution. The test is similar to the F-test of analysis of variance, the F-ratio being calculated as the ratio of the mean change in deviance (MCD) to the residual mean deviance (RMD) for the full model.

The summary of the analysis (Table 3.3) shows that each of the main effects accounts for a significant proportion of the deviance. There are likely to be significant interactions between the main effects, and the next step is to check these by adding the three two-factor interaction terms to the model (3).

$$\text{logit}(G_{ijk}) = \mu + \text{SZ}_i + \text{SO}_j + \text{ST}_k + (\text{SZ.SO})_{ij} + (\text{SZ.ST})_{ik} + (\text{SO.ST})_{jk} \dots (3)$$

Removal of each of the two factor interactions from the model shows that only [SZ.ST] ($p=0.004$) and [SO.ST] ($p<0.001$) are significant (Table 3.3). The response to stratification was thus different for seed of different sizes and seed from different sources, but the response of germination capacity to seed size was not significantly different for the four seed sources [SZ.SO] ($p=0.141$).

TABLE 3.3

Summary of the analysis of deviance for the germination capacity of three sizes of seed collected at four altitudes and stratified for 0 days or 20 days.

Model terms	d.f.	Residual deviance	RMD	Change d.f.	Change deviance	MCD	F Ratio	P
Total	119	3241.0	27.23					
Main Factors								
<u>+SZ +SO +ST</u>	113	568.3	5.03	6	2672.7	445.45	141.9	<0.001
-SZ	115	663.9	5.77	2	95.6	47.80	15.2	<0.001 ¹
-SO	116	1700.0	14.65	3	1131.7	377.23	120.1	<0.001 ¹
-ST	114	1984.0	17.40	1	1415.7	1415.7	450.9	<0.001 ¹
Main Factors +Interactions (2 factor)								
<u>+SZ.SO+SZ.ST+SO.ST</u>	102	344.8	3.38	11	223.5	20.31	6.5	<0.001
-SZ.SO	108	375.9	3.48	6	31.1	5.18	1.7	0.141 ²
-SZ.ST	104	381.0	3.66	2	36.2	18.10	5.8	0.004 ²
-SO.ST	105	467.8	4.46	3	123.0	41.00	13.1	<0.001 ²
Main Factors +Interactions (2 factor +3 factor)								
<u>+SZ .SO .ST</u>	96	301.4	3.14	6	43.4	7.23	2.3	0.040

SZ =Sieve opening (1000, 1190, 1410 μ m)

SO =Seed source (960 m, 1310 m, 1510 m, 1730 m)

ST =Duration of stratification (0, 20 days)

¹ compared with SZ+SO+ST

² compared with SZ+SO+ST+SZ.SO+SZ.ST+SO.ST

RMD= Residual mean deviance

MCD= Mean change in deviance

Finally, the three factor interaction is added to the model (4).

$$\text{logit}(G_{ijk}) = \mu + \text{SZ}_i + \text{SO}_j + \text{ST}_k + (\text{SZ.SO})_{ij} + (\text{SZ.ST})_{ik} + (\text{SO.ST})_{jk} + (\text{SO.SZ.ST})_{ijk} \dots (4)$$

The three factor interaction [SZ.SO.ST] was not significant (Table 3.3, p=0.04) and therefore the most appropriate model (5) for the data

is the one including the mean, main effects and the two factor interactions which are significant (Note: except where otherwise stated $p=0.01$ is used as the critical level for significance).

$$\text{logit}(G_{ijk}) = \mu + \text{SZ}_i + \text{SO}_j + \text{ST}_k + (\text{SZ.ST})_{ik} + (\text{SO.ST})_{jk} \quad \dots(5)$$

The next step in the analysis is the determination of the response of $\text{logit}(G)$ to the covariates in the selected model. The statistical model for the prediction of germination capacity is based on the coefficients given in Table 3.4. Conventionally, the coefficient of level one of a factor is set to zero and is not included in the table of coefficients.

TABLE 3.4

The coefficients of the model relating germination capacity to seed size, source and stratification treatment.

	ESTIMATE	s.e.
Constant	-0.832	0.087
SiZe ₂	-0.264	0.084
SiZe ₃	-0.106	0.121
SOUrce ₂	1.553	0.112
SOUrce ₃	-0.648	0.114
SOUrce ₄	-0.430	0.113
STrat ₂	3.745	0.174
SOUrce ₂ .STrat ₂	-1.810	0.218
SOUrce ₃ .STrat ₂	-1.706	0.193
SOUrce ₄ .STrat ₂	-1.944	0.193
SiZe ₂ .STrat ₂	-0.614	0.118
SiZe ₃ .STrat ₂	-0.317	0.163

The coefficients in Table 3.4 relate the covariates to the logit of germination capacity of the seed; for example, the germination capacity, G , of seed of SiZe₂ ($1190 \mu\text{m} < \text{size} < 1410 \mu\text{m}$), collected at 960 m (SOUrce₁) and stratified for 20 days (STrat₂) is given by

$$\begin{aligned}
 \text{logit}(G_{2,2,2}) &= \mu + SZ_2 + SO_1 + ST_2 + (SO.ST)_{1,2} + (SZ.ST)_{2,2} \\
 &= -0.832 - 0.264 + 0 + 3.745 + 0 - 0.614 \\
 &= 2.037
 \end{aligned}$$

In this equation G is the probability of germination and the terms to the right of the equality are termed the linear predictor of $\text{logit}(G_{2,2,2})$.

$$\text{Since,} \quad \text{logit}(G) = \ln \frac{G}{1-G}$$

$$\text{then,} \quad G_{2,2,2} = \frac{e^{2.0372}}{1 + e^{2.0372}}$$

$$G = 0.88$$

The presence of two factor interactions which are significant shows that the response of the germination capacity of the seed to stratification depends on both the source and the size of the seed. The method of examining the nature of the significant two factor interactions will now be discussed.

The nature of the two factor interactions cannot be determined from the coefficients in Table 3.4 because the statistical model is not orthogonal and the interaction terms cannot be considered in isolation from the main effects. During the fitting of the model, the program GENSTAT can generate an array of the fitted values of 'r' for each 'r' in the data (the statistical model is being fitted to data of the form 'r' germinated seeds out of 'n' viable seeds where the ratio r/n is the probability of germination). These predicted values of 'r' can then be summed to give the predicted numbers of germinated seeds for each of the components of the two factor interactions to be considered, averaged over all of the levels of the third factor not included in the two factor interaction.

TABLE 3.5

The derivation of the nature of the seed Source by Stratification interaction from the predicted number of germinated seeds ('r') out of 'n' viable seeds.

STRATIFICATION	a. r.		b. n		c. r/n	
	0	20	0	20	0	20
SOURCE						
960 m	200	678	693	730	.29	.93
1310 m	533	787	817	868	.65	.91
1510 m	214	646	1261	1195	.17	.54
1730 m	307	811	1594	1684	.19	.48

For example, in the case of the interaction between seed source and stratification [SO.ST] the predicted number of germinated seeds is determined for each source X stratification class by summing the predicted values of 'r' for each of the four seed sizes in each source X stratification class (Table 3.5a). The values of 'n' from the original data are then summed over the same treatments (Table 3.5b) and the ratios of the corresponding cells in Table 3.5a and Table 3.5b are shown in Table 3.5c.

The germination capacities presented in Figure 3.4 show that the interaction between stratification and seed source results from differences in the strength of the dormancy of seed from the various seed sources. Experiments with cleaned, unsieved seed suggest that the degree of innate dormancy either does not change, or increases only slightly with altitude (Section 3.1), and that the strength of dormancy increases with altitude of seed source (Section 4.2.1).

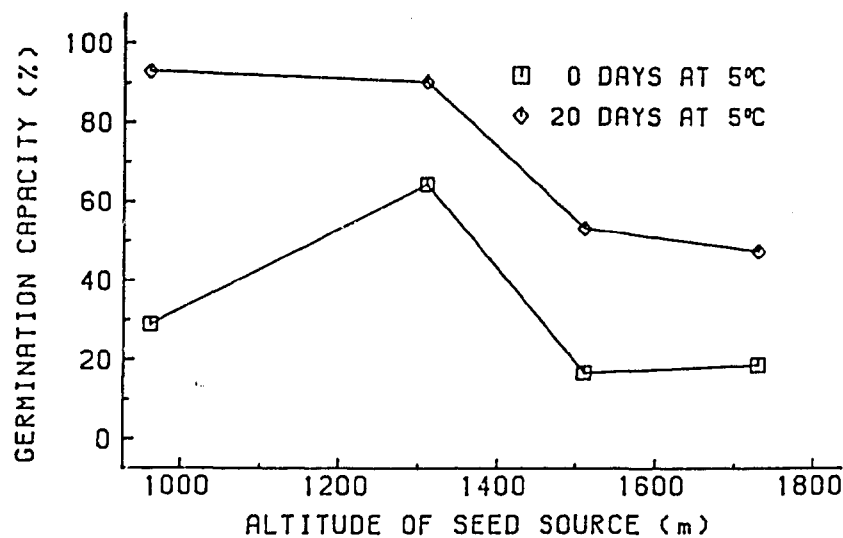


Figure 3.4 The predicted effect on germination capacity of seed source and duration of stratification, averaged over the seed sizes.

The germination capacity of seed which has not been stratified would be expected to show little or no variation with altitude and the germination capacity after 20 days should show a decline with altitude of seed source. The response in Figure 3.4 would, therefore, be expected to show two converging lines. This is the case except for the seed from 1310 m which has a much higher germination capacity than would be expected of seed with no stratification from that altitude.

The differences in the germination capacity of the seed in relation to seed source are broadly consistent with the other data for changes in dormancy with altitude. However, in this particular experiment care should be taken in the interpretation of the interaction between seed source and stratification because the proportion of the seed sizes in the raw seed has been changed by the sampling procedure (replicates of 0.4 g of seed regardless of seed size). The analysis of the source X stratification interaction considers the effect averaged over all the seed sizes.

Any differences in the dormancy of the seed sources (due, for example, to varying proportions of seed of different sizes, with different characteristics of dormancy) may be unreliably represented by this interaction. The high germination capacity of unstratified seed from 1310 m is unlikely to be due to changes in the representation of seed of different sizes because the germination capacity is uniformly high, regardless of seed size (Figure 3.3). It seems that the seed from this source had become less dormant in the year between the experiment reported in Section 3.1 and this experiment.

The other two factor interaction which was significant involves the effect of stratification on the germination capacity of seed of different sizes (Figure 3.5). Seed which was not stratified showed no trend in germination capacity with seed size.

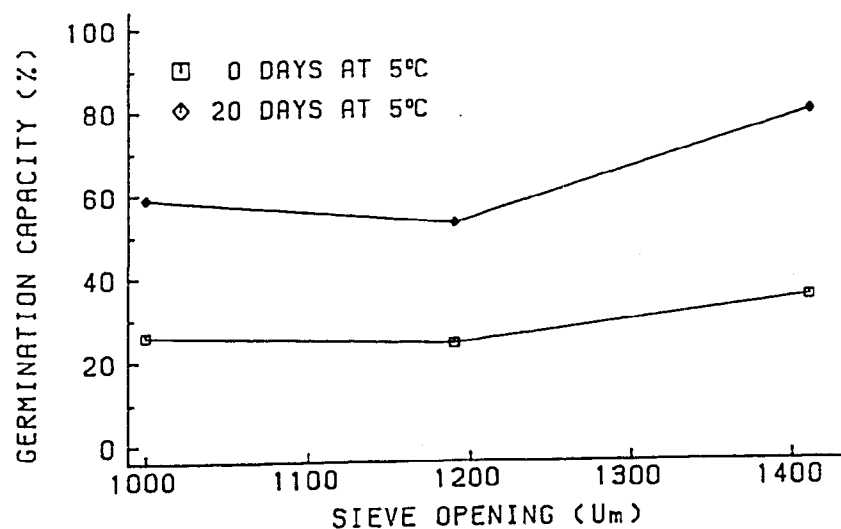


Figure 3.5

The predicted effect on germination capacity of seed size and duration of stratification [SZ.ST], averaged over the four altitudes of seed source.

However the largest seed stratified for 20 days showed a higher germination capacity than the smaller seed. The germination capacity of the medium sized seed ($1190 \mu\text{m} < \text{size} < 1410 \mu\text{m}$) does not conform to this trend since it had a lower germination capacity than either the smaller or the larger seed. The low germination capacity of middle-sized seed from the highest seed source (1730 m) is likely to be the cause of this inconsistency in the response of germination capacity to seed size since the seed from all other sources conformed to the trend (Figure 3.3).

In summary, the analyses above have shown that the germination capacity of the seed is affected by seed size, seed source and duration of stratification. The germination capacity of unstratified seed showed no trend with altitude, however, seed stratified for 20 days showed a decrease in germination capacity with increases in altitude of seed source. This increase in the strength of dormancy with altitude did not follow a smooth trend; seed from 960 m and 1310 m had similar, but weaker, dormancy than seed from 1510 m and 1730 m. Unstratified seeds of all sizes have a similar germination capacity but the strength of dormancy decreases with increasing seed size. The response of germination capacity to seed size was similar in all seed sources although the differences in dormancy between the seed sources could not be attributed to differences in their seed size distribution.

(iii) Seed size and times-to-germination

The Cox regression model was used to assess the effects of treatments on the times-to-germination and is presented and discussed in detail as an example of the method presented in Section 2.4.2. The analysis demonstrates that increases in both seed size and altitude of

seed source are associated with decreased times-to-germination.

The analysis follows the same steps as the analysis of germination capacity. The first step is the selection of an appropriate statistical model, and the second is the investigation of the coefficients of that model to elucidate treatment effects. The final step is concerned with the presentation of the results of the analysis in terms of germination curves (cumulative germination (%) plotted against time). The analysis is restricted to the same classes of seed as the analysis of the germination capacity.

The Cox model uses observations of the risk of germination (the number of seeds which germinated out of the number of viable seeds) at various times during the germination period and, for comparative purposes, requires that the times of observation are similar in all treatments. The germination periods of the experiments in this study varied between 20 days and 40 days and field-work commitments meant that occasionally the regular, daily monitoring of the germinated seeds was disrupted. The problem is compounded by the fact that the seed from different stratification treatments was set to germinate at different times, which means that the days of missed observation need not coincide for the treatments.

The lack of coincidence of observations, which may occur when the germination periods of the treatments in an experiment are not concurrent, can be overcome in two ways, depending on whether the reduction of the data set can be achieved without disrupting the analysis. Generally, the approach taken in this thesis is to fit the Cox model to a reduced data set formed by increasing the time interval between germination counts.

The risk of germination is then considered on the days of observation which are common to the stratification treatments being compared. However, in cases such as this where the set of days of observation which are common to all stratification treatments (1,2,3,4,5,6,7,8,9,11) was not sufficient to cover the full germination curve. The times-to-germination of each of the stratification treatments were considered in separate two-factor (Size and Source) Cox models rather than a single three factor (Size, Source, Stratification) model.

TABLE 3.6

Summary of analysis of deviance for the effect of seed size on the times-to-germination of seed from four seed sources, stratified for two durations.

Model terms	Residual d.f.	Residual deviance	RMD	Change d.f.	Change deviance	Significance Level
0 days Stratification						
Total	738	4192.0	5.68			
Time	722	884.5	1.23	16	3307.5	p<0.001
+Size +Source	717	735.2	1.02	5	149.3	p<0.001
+Size .Source	711	705.6	0.99	6	29.6	p<0.001
20 days Stratification						
Total	701	5943.0	8.48			
Time	689	1186.0	1.72	12	4757.0	p<0.001
+Size +Source	684	1015.0	1.48	5	171.0	p<0.001
+Size .Source	678	981.2	1.45	6	33.8	p<0.001

The Cox model uses a complementary log-log link function to relate the probability of a seed, in factor combination ij , germinating on day m , $[F_{ij}(t_m)]$ to a linear combination of covariates (Section 2.4.1). The selection of the model follows the procedure discussed in Section 3.1.2 (iii) in which the importance of the terms added to the model is

judged from the reduction in the residual deviance. The procedure used to fit these models is similar to that used in the analysis of germination capacity and, again, the order of fitting the covariates is important because the treatment effects are not orthogonal. Time is always fitted first and the treatments are then fitted with their interactions (Table 3.6).

The summary table of the analysis of deviance shows that the changes in the times-to-germination in response to seed size were different in the four seed sources ([Size .Source], $p < 0.001$, Table 3.6). The most appropriate statistical model therefore includes the two factor interaction [Size.Source] (6).

$$\ln\{-\ln[1-F_{ij}(t_m)]\} = \mu + \text{TIME}_m + \text{SZ}_i + \text{SO}_j + (\text{SZ.SO})_{ij} \quad \dots(6)$$

The effects of the individual treatment combinations can now be elucidated by consideration of the treatment constants. The treatment constants, β_{ij} , are related to the risk of germination on day m , $F_{ij}(t_m)$ as follows.

$$F_{ij}(t_m) = 1 - \exp\{-\exp(\beta_{ij})[\exp(\gamma_1) + \exp(\gamma_2) + \dots + \exp(\gamma_m)]\} \quad \dots(7)$$

Consequently, for each time, m , as the value of β_{ij} increases so the value of $F_{ij}(t_m)$ increases. When all times are considered, an increase in β_{ij} signals an increase in the rate of germination, which is synonymous with a decrease in the times-to-germination. The treatment constants are derived from the coefficients of the Cox model (Table 3.7) in a similar way to the derivation of the linear predictor for $\text{logit}(G)$ which was described in Section 3.1.2 (iii).

TABLE 3.7

The coefficients of the Cox model fitted to the times-to-germination of seed which germinated after 20 days stratification.

TERM	ESTIMATE	s.e.
SiZe ₂	-0.523	0.089
SiZe ₃	-0.839	0.229
SOurce ₂	0.267	0.067
SOurce ₃	-0.263	0.072
SOurce ₄	0.158	0.089
SiZe ₂ .SOurce ₂	0.208	0.120
SiZe ₃ .SOurce ₂	-0.196	0.264
SiZe ₂ .SOurce ₃	0.409	0.123
SiZe ₃ .SOurce ₃	0.556	0.284
SiZe ₂ .SOurce ₄	0.044	0.127
SiZe ₃ .SOurce ₄	0.331	0.249

The treatment constants are derived by summing the relevant coefficients of the model (Table 3.7). For example, the treatment constant, $\beta_{2,1}$, for the seed of SiZe₂, 1190 μm < size < 1410 μm , from SOurce₁, Waste point (960 m), is given by

$$\begin{aligned}
 \beta_{2,1} &= \text{SiZe}_2 + \text{SOurce}_1 + \text{SiZe}_2 .\text{SOurce}_1 \\
 &= -0.5225 + 0 + 0 \\
 &= -0.5225
 \end{aligned}$$

The standard errors of the treatment constants are more difficult to determine, and the procedure depends on whether the full model is appropriate. If the full interaction term is significant, and therefore the full model is appropriate, then the treatment constants and the standard errors are derived by simply fitting all the treatment combinations as separate treatments in the model. The coefficients of the model and their standard errors are then equivalent to the treatment constants and their standard errors. In this example, the full model is appropriate and the three sizes and four sources can be

fitted as twelve treatments. If some subset of the full model was more appropriate then the standard errors would have been calculated from the variance/covariance matrix resulting from the fit of the model. The treatment constants and their standard errors (as error bars) are presented for both 0 days and 20 days stratification treatments in Figure 3.6.

The rate of germination of unstratified seed tends to increase with altitude of source regardless of seed size (Figure 3.6a). The lower germination capacities of the unstratified seed (Figure 3.3) resulted in the treatment constants in Figure 3.6a being based on fewer germinated seeds than those in Figure 3.6b; this may account for the larger error bars in Figure 3.6a and the absence of a consistent response to seed size. This hypothesis is supported by the behaviour of the unstratified seed from 1310 m, which had a large germination capacity and a response of times-to-germination to seed size which was similar to seed stratified for a longer time period.

The times-to-germination of seed stratified for 20 days have a very different response to seed size and altitude of seed source compared to unstratified seed. Generally, the larger seed exhibits more rapid germination (Figure 3.6b). There does not appear to be any trend in the times-to-germination of stratified seed with altitude, however, the response of times-to-germination to increases in seed size is different in the four seed sources. The difference between the times-to-germination of the largest and the smallest seed has a tendency to become smaller with increases in altitude. The reduction in the difference is brought about by a reduction in the rate of germination of the largest seed and an increase in the rate of germination of the smallest seed.

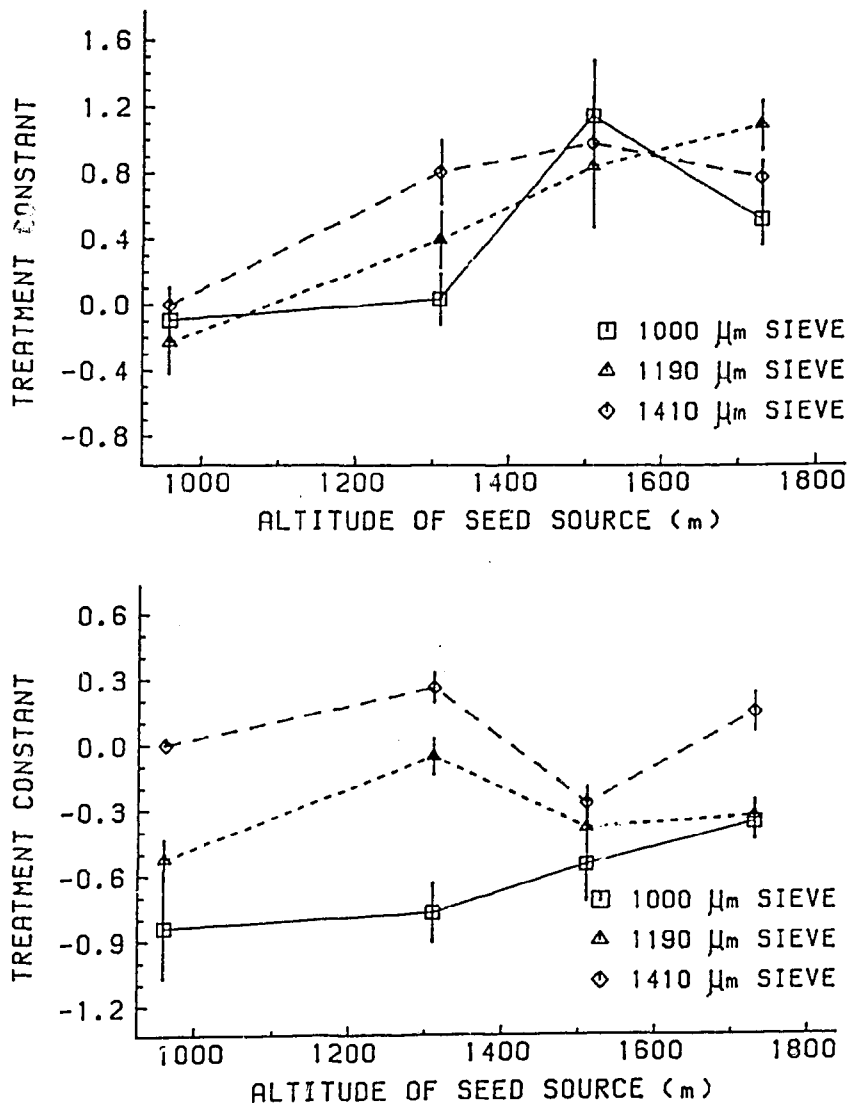


Figure 3.6 The treatment constants of the Cox models relating the times-to-germination to seed size and source for (a) 0 days and (b) 20 days of stratification.

The treatment constants can be used to derive the germination curves by calculating the values of $F_{ij}(t_m)$ (from equation 7) for the desired ij , over the range of time t_1 to t_m . The constants, γ_m , involving time-to-germination, are the coefficients of times of observation plus the constant term (time constants), resulting from the fit of the Cox model. The first step in the calculation of the predicted germination curve is to calculate the cumulative sum of the exponentials, CE_m , of the time constants for each of the times of observation (8).

$$CE_m = \exp(\gamma_1) + \exp(\gamma_2) + \dots + \exp(\gamma_m) \quad \dots(8)$$

These values are then associated with $F_{ij}(t_m)$ as follows.

$$F_{ij}(t_m) = 1 - \exp\{-\exp(\beta_{ij})[CE_m]\}$$

β_{ij} is the treatment constant associated with the ij th treatment combination.

The predicted germination curve is obtained when the values of $F_{ij}(t_m)$ are considered over the range of times from t_1 to t_m . The predicted germination curve is then compared with the observed germination curve for the treatment. The observed germination curve was derived by dividing the number of seeds which had germinated by a day by the number of seeds which had germinated at the end of the final day, for all days of the experiment.

The germination curves for the seed collected at 1310 m show that the seed which was not stratified has a lag period at the beginning of germination which is not apparent in the seed stratified for 20 days (Figure 3.7).

Information on the distribution of times-to-germination in the seed stratified for different durations may be obtained from plots of $\ln\{-\ln[1-F_{ij}(t_m)]\}$ against time (Figure 3.8) for any factor (ij) combination. It was shown in Section 2.4.2 that if $i,j=1$ then,

$$\ln\{-\ln[1-F_{ij}(t_m)]\} = \ln[\exp(\gamma_1) + \exp(\gamma_2) + \dots + \exp(\gamma_m)] \quad \dots(9)$$

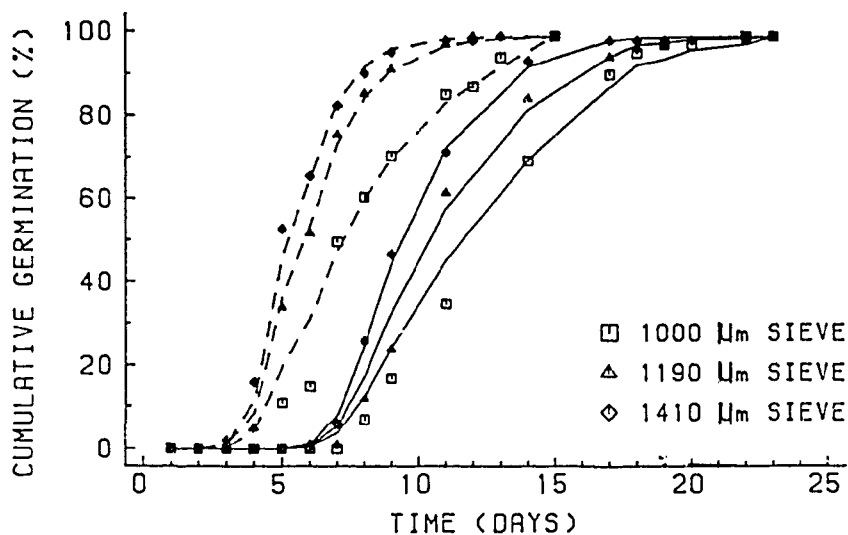


Figure 3.7 The predicted (lines) and observed (symbols) germination curves for three sizes of seed collected at 1310 metres and stratified for 0 (—) or 20 (---) days prior to germination.

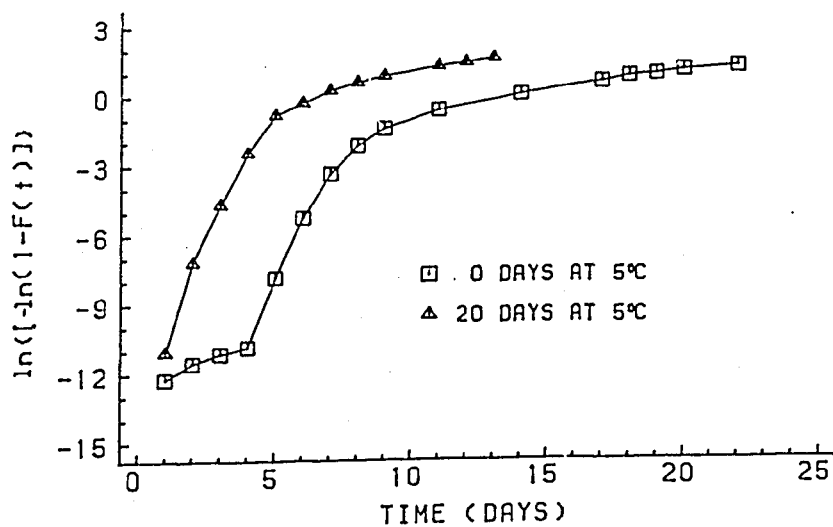


Figure 3.8 The plot of $\ln\{-\ln[1-F(t)]\}$ against time for the two stratification periods.

The complementary log-log of $F_{ij}(t_m)$, $\ln\{-\ln[1-F_{ij}(t_m)]\}$, can be calculated readily from the time constants, γ_m , of the linear model. The differences between the germination curves of unstratified seed and

seed stratified for 20 days (Figure 3.7) are associated with differences in the distribution of times-to-germination, since Figure 3.8 shows that the seed which was not stratified has a lag time of about 3 days during which the risk of germination is low compared to the seed stratified for 20 days.

Discussion:

The size distribution of both the complete seed sample and the viable seed of E. pauciflora decreases with increasing altitude of seed source. The reduction in seed size with increasing altitude could be expected to have an impact on the number of seedlings which emerge and establish, through a reduction in the amount of reserves available for these processes. However, the experiments investigating the emergence of seedlings (discussed in Section 7.1) seem to indicate that a large proportion of the seed from all altitudes may result in established seedlings. Hence, the physiological attributes of the seed from a particular source may be the critical determinant of successful emergence rather than the size of the seed.

Comparison of the strength of dormancy of seed of the same size collected at different altitudes shows that the increase in the strength of dormancy with altitude cannot be wholly associated with the reduction in the size of the seed. Seed of the same size tends to show a similar degree of dormancy at dispersal (innate dormancy) but an increase in the strength of dormancy with altitude of seed source. The observed differences in the strength of dormancy could be an artefact of a method which uses a constant temperature to assess the strength of dormancy. Such an artefact could develop if, for example, there is a reduction in the temperature suitable for the breaking of dormancy with

altitude of seed source. This would lead to a reduction in breaking of dormancy under the standard conditions. The possibility of further differentiation in the behaviour of seed from different sources due to variation in the temperature response of the breaking of dormancy with altitude is investigated in Section 4.2.1 and this shows that seed from 1230 m to 1910 m both have similar responses to the temperature of stratification. Thus the differences in the strength of dormancy are likely to be real. Increased strength of dormancy of seed from higher altitudes imposes a requirement for a longer period of cold conditions before germination may occur. Given the increasing duration of cold conditions during winter with altitude, this may be a means of preventing germination during winter, when the chances of seedling survival are very low.

Reductions in the strength of dormancy and decreases in the times-to-germination with increasing seed size may result in earlier and more rapid germination of larger seeds compared to smaller seeds in a seed sample. Further work is required to determine the impact of these differences on the timing of germination in the field, given the possibilities for differences in the induction of dormancy and germination of seed of different sizes.

The studies in this section have investigated the relationship between the absolute size and dormancy of seed from a range of altitudes and have shown that the strength of dormancy of seed is inversely related to seed size. The dormancy of seed of the same size from different altitudes, however, became stronger as the altitude of the seed source increased. It is possible that the dormancy of a seed is related to its size, relative to the other seeds in the sample (altitude) from which it comes. This line of investigation could not

be pursued because there were no commercially available sieves which would give a greater number of seed size classes in the range of classes containing viable seeds.

3.3 Changes in dormancy with temperature of seed extraction.

The seed of E. pauciflora is released following desiccation of the capsules and is shed in a manner similar to that described for E. regnans by Cremer (1965a). Seed shedding may be stimulated by damage to branches due to the effects of fire, wind or snow, but also occurs in the course of normal canopy growth. The studies described in this section test the likely influence of the conditions prior to and during seed shedding on the dormancy of the seed reaching the ground.

Capsules with seed remaining in them often fall from the canopy and the fate of this seed depends on whether it is released. Cremer (1965b) noted that E. regnans seed arriving at the soil enclosed in capsules had little chance of surviving to germinate because of either insect predation or the action of pathogens. Grose (1960) came to similar conclusions for the seed of E. delegatensis shed in capsules although he distinguished between the fate of seed shed in capsules attached to branchlets, which was often released normally, and seed which was shed in individual capsules, which was often retained in the capsules and died.

This section investigates the influence of the environmental conditions during seed shedding on the dormancy of the seed released from the capsules.

3.3.1 Extraction of seed under ambient conditions

Different seasons have characteristic temperature regimes. The temperature regime could have an important influence on the dormancy of the seed before it reaches the soil. This section describes an experiment in which capsules were held at a range of constant temperatures until seed shedding was completed and then the degree and strength of the dormancy of the seed was assessed.

Methods:

Capsules of E. pauciflora were collected in August 1981 at Thredbo treeline (1900 m). The following day samples of the capsules (600 g) were placed at four temperatures (5,15,30,40 °C) for desiccation. Desiccation was assisted at 5, 15 and 30 °C by passing a stream of dry air, equilibrated to the temperature, through the capsules. The drying rate of the capsules and the release of seed was monitored at daily intervals by shaking the sample, separating the seed and capsules by sieving (2000µm sieve) and weighing both seed and capsules. Following extraction, the seed obtained was stored in an airtight container at the temperature of extraction. The temperature environment during seed shedding and storage was monitored daily using maximum and minimum thermometers. The experiment was repeated, without the detailed measurements of seed release, using samples of 500 g of capsules collected at Smiggins Holes in September 1981.

After 14 days, five replicates of cleaned seed (0.15 g Thredbo, 38 viable seeds; Smiggins Holes, 48 viable seeds) from each extraction temperature were stratified for either 0, 2, 4 and 6 weeks at 5°C and then set to germinate at 15°C.

Results:

(i) The temperature environment during seed shedding

The average and standard deviation of the daily maximum and minimum temperatures for each treatment show the range of conditions that the capsules were exposed to during seed shedding (Table 3.8).

TABLE 3.8

The temperature regime of seed extraction for capsules collected at Smiggins Holes (1730 m) and Thredbo (1900 m)

NOMINAL TEMP.	5°C		15°C		30°C		40°C	
	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX
Smiggins AV.	¹	¹	13.9	18.3	28.5	31.4	37.5	45.3
SD.			2.99	1.49	3.89	5.24	3.92	2.02
Thredbo AV.	1.3	5.8	11.7	15.7	30.9	32.3	38.6	44.2
SD.	1.18	0.99	0.40	1.85	1.44	1.30	1.28	2.43

Average MAXimum and MINimum temperatures based on daily records

¹ no daily records of temperature (within the range 0-6°C)

AV average (maximum or minimum) temperature (°C)

SD standard deviation

(ii) Seed shedding.

The desiccation of the capsules and release of seed was delayed by about 3 days at 5°C compared to higher temperatures. In order to ensure that the seed sample used in the experiment was similar for all extraction temperatures, the first 16 g of seed from each treatment was

used in the germination tests. The rate of seed shedding (Figure 3.9) increased with increasing temperature in the treatments where drying was assisted by the passage of air through the capsules. The slower rate of shedding of the seed at 40°C was also associated with a slower rate of desiccation of the capsules, indicating that the rate of loss of water plays an important role in seed shedding.

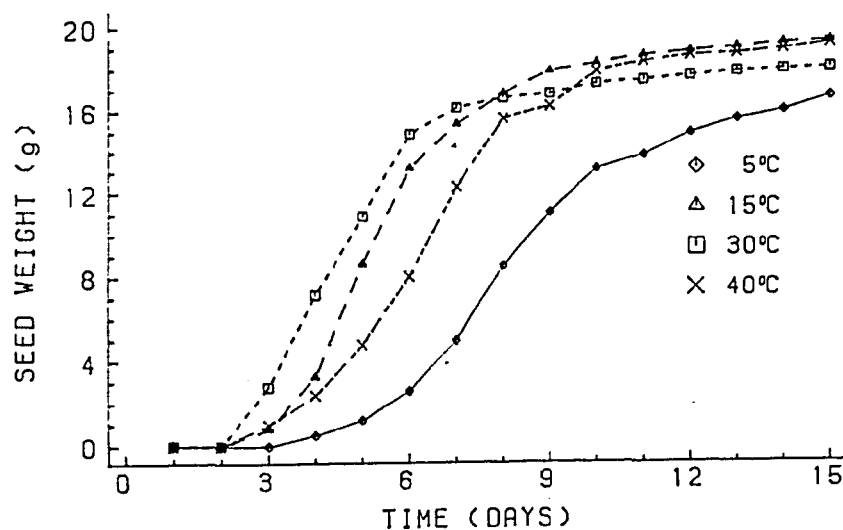


Figure 3.9 The shedding of seed during the extraction from capsules collected from Thredbo.

The relationship between the decrease in the weight of the capsules as they desiccate and the weight of the seed shed (Figure 3.10) provides further evidence of the importance of the water content of the capsules as a factor influencing seed release. Capsules at lower temperatures (5°C, 15°C) yielded slightly more seed for a given capsule weight than capsules at higher temperatures (30°C, 40°C).

The release of seed from the capsule is associated with the separation of the seed from the placenta, widening of the loculi and

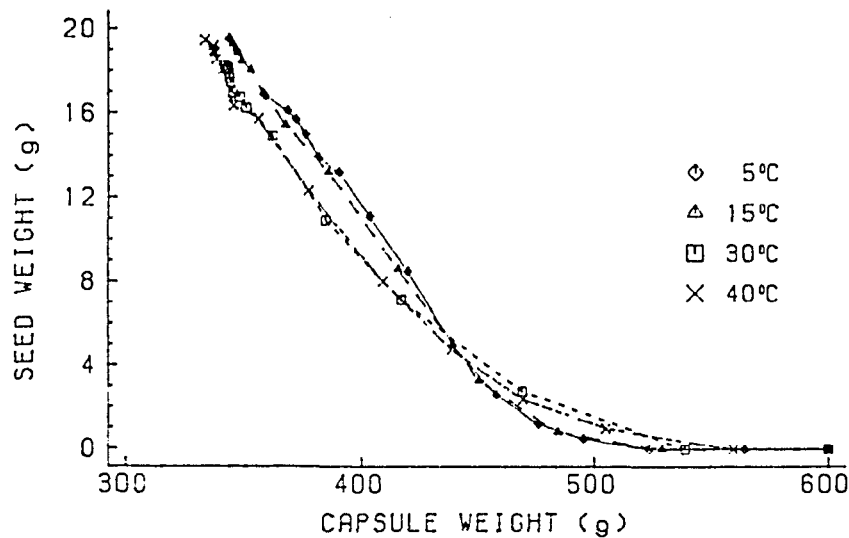


Figure 3.10 The relationship between capsule weight and the weight of seed shed from capsules collected at Thredbo and desiccated at a range of temperatures.

opening of the valves (Cremer 1965a). The latter two processes are mediated by the water content of the ovary wall which lies inside the woody tissue of the capsule, and seed release from the capsule is directly related to the water content of the ovary wall. The slower, and possibly more even drying of capsules at the lower temperatures would tend to bring the water content of the ovary wall into a closer association with the water content of the outer structures of the capsule leading to a larger amount of seed release for a given total capsule water content. At higher temperatures, the desiccation of the outer structures may occur at a greater rate than that of the ovary wall. A much more dramatic change in the association between capsule weight and seed release is seen when the capsules are rapidly desiccated under the action of high temperatures (80°C) (Section 3.3.2, Figure 3.15).

(iii) Germination capacity

The temperature of extraction from the capsule influenced the strength of dormancy of seed from both Smiggins Holes and Thredbo (Table 3.9). Although the magnitude of the effect was dependent on the seed source and the duration of the stratification treatment, seed extracted at 15°C or 30°C had a relatively weaker dormancy than seed extracted at 5°C or 40°C.

TABLE 3.9

Summary of analysis of deviance for the germination capacity of seed from two sources extracted at four temperatures and germinated after four periods of stratification.

Model terms	Residual d.f.	Residual deviance	RMD	Change d.f.	Change deviance	CMD	F Ratio	P
THREDBO								
Total	79	2159						
+Temp +Strat	73	169.2	2.32	6	1989.8	331.63	190.59	<0.001
+Temp .Strat	64	111.1	1.74	9	58.1	6.46	3.71	=0.001
SMIGGINS HOLES								
Total	79	2643	33.46					
+Temp +Strat	73	87.85	1.20	6	2555.15			<0.001
+Temp .Strat	64	73.99	1.16	9	13.86			=0.127

Temp =Temperature of extraction (5,15,30,40 °C)
 Strat =Duration of stratification (0,2,4, 6 weeks)

Seed collected at Thredbo had a stronger innate dormancy than seed from Smiggins Holes (Figure 3.11), since the germination capacity of the seed from Thredbo was lower than that from Smiggins Holes following similar stratification treatments. The effect of temperature during seed shedding on the germination capacity of the seed after

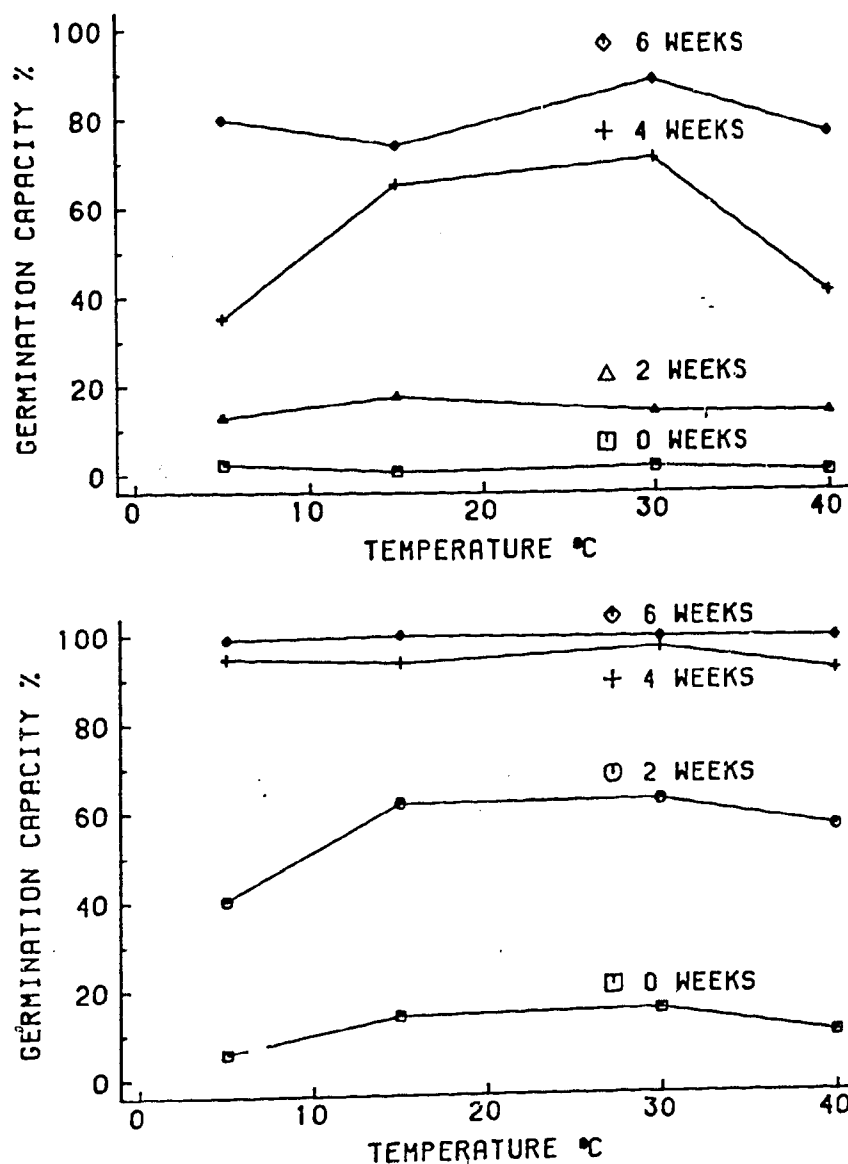


Figure 3.11 The effect of extraction temperature and stratification on the germination capacity of seed collected at (a) Thredbo and (b) Smiggins Holes

stratification reflects the differences in the strength of dormancy of the two seed sources.

The seed from Thredbo shows no effect of temperature during seed shedding following either 0 or 2 weeks of stratification. Thredbo seed had an average of 38 viable seeds per replicate and it is probable that the response to temperature during seed shedding was beyond the

resolution of the data at the low germination capacities attained by Thredbo seed after 0 or 2 weeks stratification.

The germination capacity of seed from Smiggins Holes did not exhibit the same strong interaction between the temperatures of extraction and stratification as that observed in Thredbo seed because the effect of temperature of seed shedding at 5°C and 40°C was not as marked as that in seed from Thredbo. This observation concurs with those of Gross (1953) for *E. ulmifolius* seed, in which the strength of dormancy induced by treatment was greater if the innate strength of dormancy of the seed was greater.

(iv) Times-to-germination

The effect of the temperature during seed shedding on times-to-germination was not significant ($p=0.198$) for seed from Smiggins Holes and bordered on significance ($p=0.011$) for seed collected at Thredbo (Table 3.10). Temperature of extraction has a similar effect on the times-to-germination regardless of the length of the stratification period since the [Temp.Strat] interaction is not significant in seed from either source (Table 3.10). Examination of the treatment constants for seed collected at Thredbo (Table 3.11) shows that the seed extracted at 15°C and 30°C has a faster rate of germination than seed extracted at either 5°C or 40°C.

The effect of the duration of stratification in reducing the times-to-germination is evident from a consideration of the treatment constants of the models (Table 3.11): stratification for periods of up to 4 weeks caused an increase in the rate of germination, whereas, stratification for periods longer than 4 weeks caused a decrease in the

TABLE 3.10

Summary of analysis of deviance for times-to-germination for Smiggins and Thredbo seed extracted at four temperatures and then germinated following stratification.

Model terms	Residual d.f.	Residual deviance	RMD	Change d.f.	Change deviance	CMD	F	P
THREDBO¹								
Total	671	3476						
Time	658	1007	1.53	13	2469			p<0.001
<u>+Temp+Strat</u>	653	824.6	1.26	5	182.4			p<0.001
-Temp	656	835.7	1.27	3	11.1			0.011 ²
-Strat	655	990.5	1.51	2	165.9			p<0.001 ²
<u>+Temp.Strat</u>	647	820.2	1.27	6	4.4			0.625
SMIGGINS HOLES¹								
Total	654	5820						
Time	641	2641	4.12	13	3179	244.5	103.62	<0.001
<u>+Temp+Strat</u>	636	1496	2.353	5	1145	229.0	97.03	<0.001
-Temp	639	1507	2.359	3	11	3.7	1.55	0.198 ³
-Strat	638	2636	4.132	2	1140	570.0	241.52	<0.001 ³
<u>+Temp.Strat</u>	630	1487	2.36	6	11	1.8	0.78	0.591

¹ 0 weeks stratification excluded because of low numbers of germinated seeds.

² compared with Thredbo [+Temp+Strat]

³ compared with Smiggins [+Temp+Strat]

rate of germination. The decrease in the rate of germination after longer periods of stratification indicates that there may be some deleterious effects of lengthy periods of exposure to low temperatures.

Further understanding of this effect may be gained from a more detailed examination of the germination curves (Figure 3.12). The breaking of the dormancy of a seed sample is associated with an increase in germination capacity, a shorter time to the appearance of

TABLE 3.11

The treatment constants and standard errors for temperature of extraction and stratification treatments

Temperature °C	THREDBO		SMIGGINS HOLES	
	Estimate	s.e.	Estimate	s.e.
5°C	0	0	0	0
15°C	0.179	0.077	-0.094	0.063
30°C	0.206	0.076	0.066	0.061
40°C	0.024	0.081	-0.101	0.060
Stratification				
2 weeks	0	0	0	0
4 weeks	1.110	0.096	2.024	0.069
6 weeks	0.912	0.092	1.499	0.066

the first germinated seed, and a greater rate of germination for the seed sample. The seed stratified for 6 weeks exhibited the first two characteristics but then shows a decreased rate of germination; this is consistent with a depletion of the seeds' reserves at the stratification temperature, due to respiration associated with tissue maintenance.

The germination curves predicted by the Cox model show greatest deviation from the observed germination curves (Figure 3.12) for the seed stratified for a period of 2 weeks. The lack of fit accounts for the large residual mean deviance observed in the times-to-germination of seed from Smiggins Holes (RMD=2.36, Table 3.10) and, to a lesser extent, in the seed from Thredbo (RMD=1.27, Table 3.10). In both of these cases the seed set to germinate after 2 weeks stratification showed a marked delay in time to first germination. This delay may be the cause of the lack of fit of the model, and is an indication of a

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2 weeks	0	0	0	0
4 weeks	1.110	0.096	2.024	0.069
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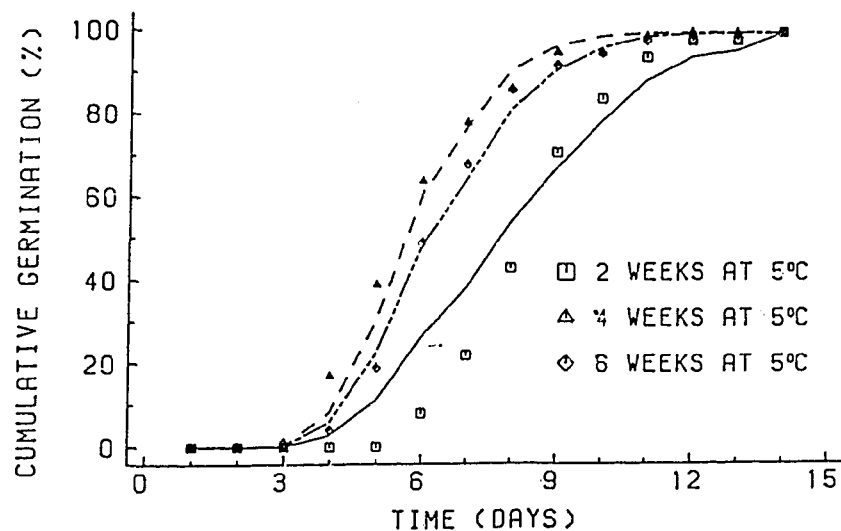
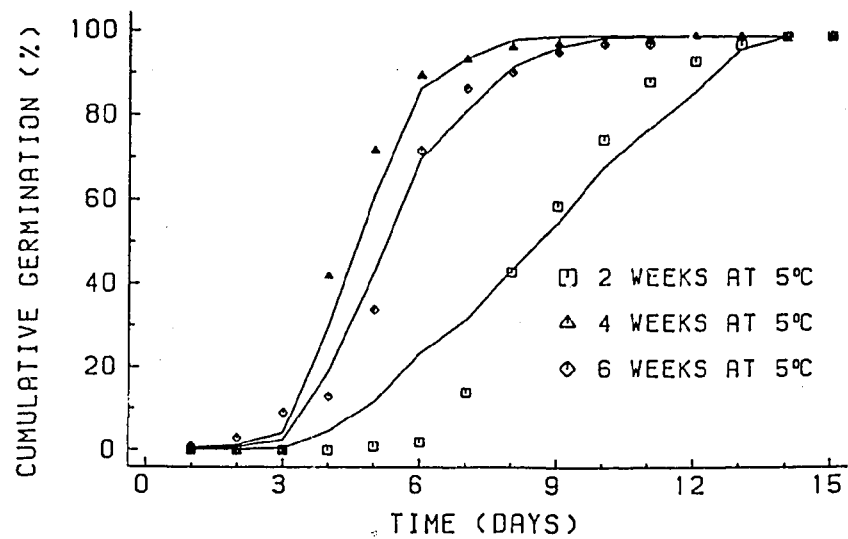


Figure 3.12 The predicted (lines) and observed (symbols) germination curves for (a) Smiggins Holes and (b) Thredbo seed.

break-down in the assumption of proportional hazards.

Analysis of variance shows that the day on which the first seed germinated is significantly affected by stratification (Thredbo and Smiggins both $p < 0.001$) but not by temperature during seed shedding (Thredbo, $p = 0.030$; Smiggins Holes $p = 0.319$). The possibility that the difference in the curves could be explained by the shift in the day to first germination was tested by re-fitting the Cox model to the data with the first day of germination predicted by the analysis of variance

as the day of first record. In both cases the mean deviance of the new model was greater than that in Table 3.10, indicating that a displacement in time could not account for the differences in the germination curves for seed stratified for 2 weeks on the one hand, and 4 and 6 weeks on the other.

Discussion:

The dormancy of seed from the two sources was influenced by the temperature during seed shedding, but the effect was small and transient in the face of the processes which break dormancy. Grose (1960) showed that the rate of response of seed to temperatures which result in the induction of dormancy depends on the water content of the seed as well as on the temperature. The seed in a desiccating capsule will be drying at a rate which is dependent on the temperature and humidity of the surrounding air. The effect of the conditions during seed shedding on the dormancy of the seed depends on the temperature but will be modified by any changes in the sensitivity of the seed to temperature as its water content changes.

The small changes in dormancy observed in these experiments show that the innate dormancy exhibited by E. pauciflora is largely determined before dispersal and is not changed markedly by the range of temperatures likely to be encountered during seed shedding. Seed dispersed under the low temperature conditions of winter or the hot conditions in summer may show a slight increase in dormancy. The relevance of these small and transient effects at dispersal will be discussed in relation to the large and more persistent changes in dormancy which may be induced by the conditions encountered in the soil (Chapters 4, 5 and 6).

Stratification was found to increase the germination capacity and to decrease the times-to-germination of seed from both Smiggins Holes and Thredbo. The influence of temperature and duration of exposure to conditions which break dormancy will be considered in more detail in Section 4.3. The strength of the dormancy of seed collected at Thredbo was greater than that of seed collected at Smiggins Holes, confirming the results of the previous section which showed that seed from sources nearer to treeline have a stronger dormancy than seed from lower altitudes.

Seed from both Thredbo and Smiggins Holes has a high degree of innate dormancy so it seems unlikely that dormancy will increase to the degree noted by Boden (1957) following storage. Boden observed a decline in the germination capacity of E. pauciflora seed from around 80% immediately after shedding to 20% after 5 weeks of storage. This agreed with Pryor's observations of a decline in germination capacity from 47% 1 week after seed shedding to 17% after 12 weeks of storage (unpublished data quoted in Boden 1957). The innate dormancy of the seed considered in this section offers little possibility of changes in dormancy of the magnitude reported by Boden.

3.3.2 The treatment of capsules at high temperatures

Since post-fire regeneration of E. pauciflora is a widespread phenomenon the seeds which have a high probability of becoming established seedlings are likely to have been exposed to a fire. The absence of a long-lived seed bank in E. pauciflora woodland (Howard & Ashton 1967), the mortality of seed in the soil during fires (Grose 1960), and the large and synchronized seed rain stimulated by intense fires (Christensen 1971, O'Dowd & Gill 1980) means that further emphasis must be placed on the canopy seed store as a source of seed for regeneration. Eucalyptus pauciflora capsules are likely to be exposed to high temperatures during intense fires and the influence of these conditions on the dormancy of the seed within the capsule may have a critical impact on the timing of germination.

The effects of fire on the subsequent germination behaviour of seeds can be tested by comparing the germination of seed collected from recently burnt areas with seed from adjacent unburnt areas. Similar comparisons can be made using the seed collected from capsules scorched over a fire.

The state of capsules observed after a wild-fire was found to vary from no visible damage to complete incineration: apparently, a large range of temperature conditions may be experienced by capsules during a fire. Grose (1960) collected seed from capsules which had sustained various degrees of damage during a slash fire in E. delegatensis and showed that there was an increase in the proportion of non-viable seed as the capsule damage increased in severity from no scorch to severe charring. Temperatures experienced by seed inside a capsule during a fire will tend to be lower than the temperature outside it, both

because of the low thermal diffusivity of the woody capsule walls and because of the short duration of exposure to high temperatures. There is, however, no information available on the absolute temperatures experienced by seed within capsules during a fire (A.M.Gill, CSIRO, Division of Plant Industry, pers. comm.).

The investigation of the response is further complicated by the critical role that the moisture content of the seed is known to play in determining seed survival at high temperatures (54°C) (Cunningham 1960).

In the experiment described below, the effect of fire on freshly picked capsules was simulated by treatment of the capsules at 80°C in an oven. The temperature and duration of treatment were chosen to include conditions likely to be more extreme than those experienced by most of the seed during a fire (A.M.Gill, CSIRO, Division of Plant Industry, pers. comm.).

Methods:

Samples (200 g) of capsules, collected at Smiggins Holes in September 1981, were spread in a layer one capsule deep on a metal tray and exposed to 80°C in an oven for 5, 10, 22, 40, 80, 160 and 260 minutes. Following treatment, the capsules were allowed to desiccate in open containers under laboratory conditions (18-22°C, 35% relative humidity).

The desiccation rate of the capsules and the release of seed were monitored daily by shaking each sample to release seed, separating the seed and capsules by sieving, and then weighing both the seed and

capsules. The separated dry seed was stored in a sealed container at 5°C until seed extraction was completed.

Following extraction, five replicates of cleaned seed (0.15 g, 38 viable seeds) from each temperature treatment were stratified for 1 week, and a further five replicates were stratified for 5 weeks, prior to germination at 15°C.

Results and Discussion:

(i) Seed shedding from the capsules

The effect of the treatments ranged from desiccation of the capsules and release of the seed over a period of 10 days, to almost complete desiccation and substantial seed release during the period of exposure to 80°C. Rates of desiccation of capsules and rate of seed shedding from the two extremes of treatment is shown in Figure 3.13. The increased rate of seed shedding which occurred at longer durations of treatment at high temperatures is consistent with the more rapid desiccation of the capsules noted in Section 3.3.1.

The yield of seed for a given reduction in the weight of capsules declined as the temperature at which fresh capsules were desiccated was increased from 15°C to 40°C (Section 3.3.1). The trend for lower yields of seed for a given capsule weight is also apparent in the seed shed from capsules exposed to 80°C for increasing times (Figure 3.14). The explanation is likely to be similar, with the longer duration of treatment at 80°C resulting in more rapid desiccation of the woody part of the capsule compared to the ovary wall. Since it is the desiccation of the ovary wall which determines the shedding of the

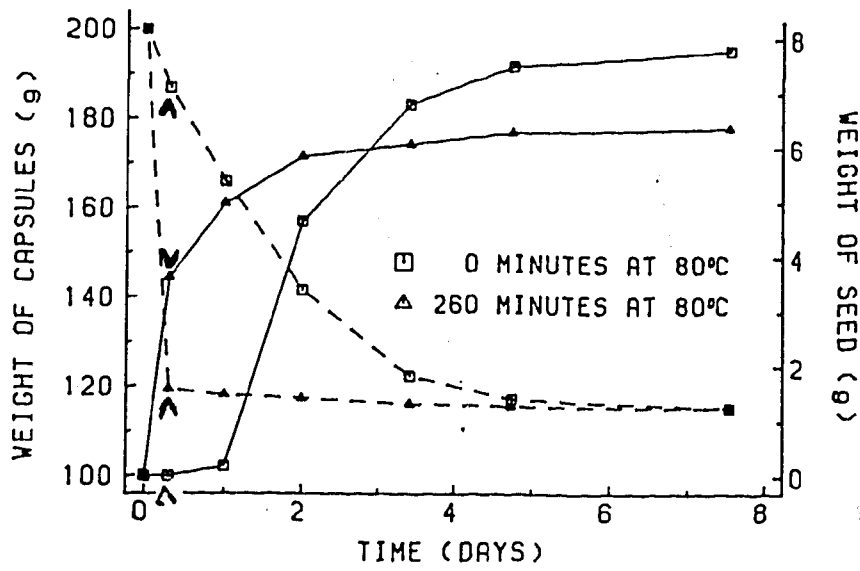


Figure 3.13 The shedding of seed (—) and desiccation of capsules (---) treated at 80°C for either 0 or 260 minutes before extraction under laboratory conditions. Arrows show the state at the end of treatment at 80°C

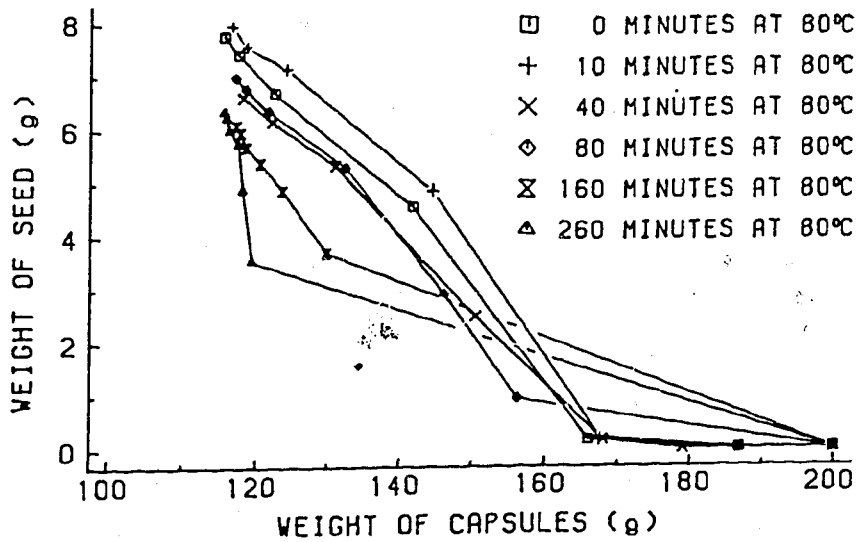


Figure 3.14 The relationship between the weight of the capsules and the weight of the seed released following exposure to 80°C for a range of times.

seed, any change in the relative rate of drying of the ovary wall and the woody structures of the capsule will cause a change in the relationship between the total water content of the capsule and seed release. This situation would be particularly applicable to the capsules exposed to 80°C for more than 40 minutes which showed considerable desiccation during the period of treatment at 80°C.

(ii) Germination capacity

The dormancy of seed increased markedly with the duration of exposure to 80°C (Table 3.12, Figure 3.15). The small increases in the germination of seed stratified for 5 weeks indicates either that the dormancy imposed by the high temperatures was very strong or that mortality was high. The condition of the embryos after both stratification and germination indicated that they were still healthy suggesting that the seed had become strongly dormant as a result of treatment. A further experiment with a much longer duration of stratification would be required to confirm that seed exposed to 80°C for long periods can survive stratification and germinate successfully.

The temperatures experienced inside capsules during a fire are likely to be highly variable. This experiment was designed to provide an estimate of the changes which are likely to occur over a wide range of conditions. The maximum temperature experienced by the majority of the capsules in the canopy of a tree, during a high intensity fire, are unlikely to exceed 80°C for more than 10 minutes (A.M.Gill CSIRO, Division of Plant Industry pers. comm.). The temperatures experienced by seed inside the capsules are unknown, but if they do not exceed the temperatures experienced by the seed in the capsules held at 80°C for 10 minutes they are unlikely to have a marked influence on the

TABLE 3.12

Summary of analysis of deviance for the germination capacity of seed extracted from capsules exposed to 80°C.

Model terms	Residual d.f.	Residual deviance	RMD	Change d.f.	Change deviance	Significance Level
Total	79	2336	29.57			
Durn +Strat	71	89.94	1.27	8	2246.06	p<0.001
Durn .Strat	64	65.38	1.02	7	24.56	p<0.001

Durn. =Duration of treatment at 80°C
 Strat =Duration of stratification (1,5 weeks)

dormancy of the seed. The time required to elicit a response in the dormancy of the seed as likely to be less than 22 minutes and close enough to Gill's estimate of the temperature regime experienced by capsules during a fire to stimulate further investigation of this aspect of the response to fire. Specifically the dormancy of seed dispersed from a burnt stand should be compared with the dormancy of the seed from unburnt trees nearby.

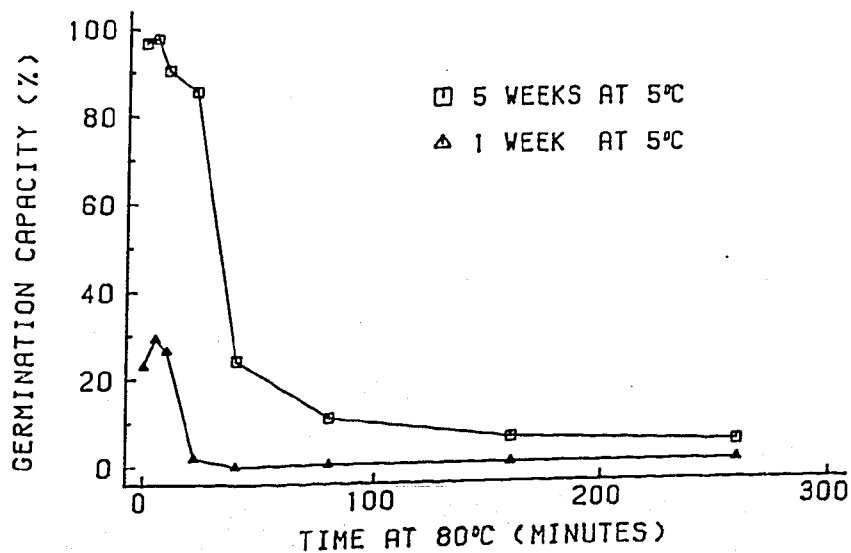


Figure 3.15 The germination capacity of seed extracted from capsules exposed to treatment at 80°C

(iii) Times-to-germination

The Cox model could only be fitted to a limited number of the treatments (5 weeks stratification, 0,5,10,22,40 minutes at 80°C) due to the low number of seeds which germinated in many of the treatments.

TABLE 3.13

Summary of analysis of deviance for the times-to-germination of seed extracted from capsules treated at 80°C.

Model terms	d.f.	Residual deviance	RMD	Change d.f.	Change deviance	Significance Level
Time	211	307.1	1.46			
Time +Durn	207	252.4	1.22	4	54.70	p<0.001

Time =Day of observation

Durn =Duration of treatment at 80°C

The results of the Cox model show that the exposure of capsules to temperatures of 80°C had a significant effect on the times-to-germination (Table 3.13). Figure 3.16 shows that exposure to high temperatures for periods of up to 10 minutes had no impact on the times-to-germination of samples which had been stratified for 5 weeks. However, for periods of exposure to high temperatures of 22 minutes or longer, both the rate of germination and the germination capacity were reduced, even following 5 weeks stratification.

The germination curves confirm that the effect of heating the capsules for 22 minutes or more prior to seed shedding results in an increase in the times-to-germination even after 5 weeks stratification (Figure 3.17).

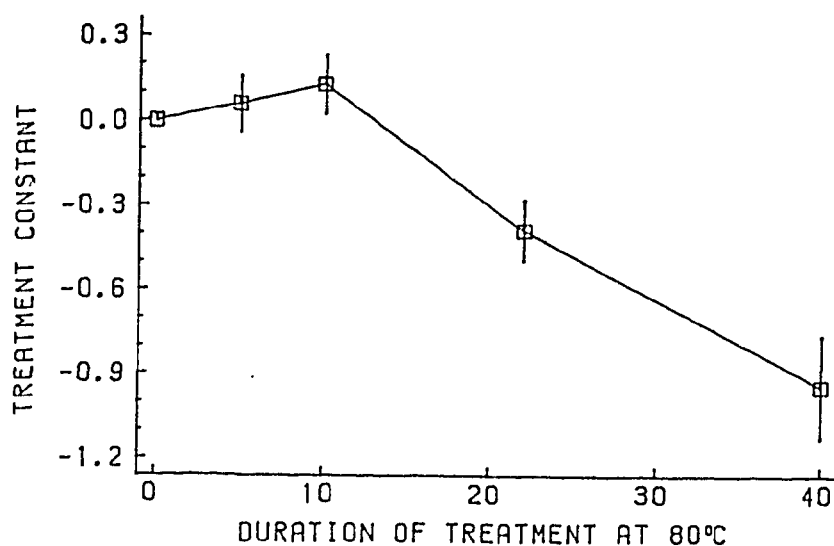


Figure 3.16 The treatment constants of the Cox model for times-to-germination of seed extracted from capsules following exposure to 80°C for a range of times.

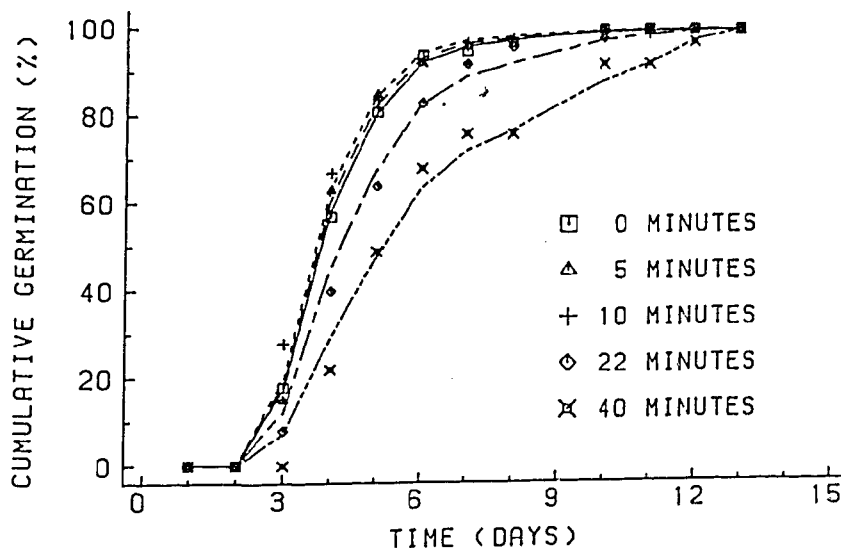


Figure 3.17 The predicted (lines) and observed (symbols) germination curves for seed extracted from capsules treated at 80°C for various times.

(iii) Seed mortality

The experimental design would not permit an assessment of the mortality of seed associated with increasing duration of treatment at

80°C. Samples of equal weight were drawn from seed cleaned after the high temperature treatment; an analysis of the total numbers of viable seed in the various treatments could, therefore, reflect either a real effect of the treatment, or an artefact of seed cleaning and sampling caused by differences in seed characteristics other than viability (for example, seed water content or seed weight).

3.4 Treatment of dry seed at elevated temperatures

The response of the seed to high temperatures is important because it has the potential to place further controls on the timing of germination. Changes in dormancy were observed in seed contained in capsules exposed to 80°C for periods of 22 minutes or longer (Section 3.3.2). Field measurements under dry conditions show that the temperature near the soil surface may reach 80°C on a summer's day; seed falling to the soil surface in summer is also likely to experience these temperatures. An experiment was set up to test whether exposure of dry seed to temperatures of 80°C results in changes in the germination behaviour or mortality of the seed.

Methods:

Replicates (0.4 g, 81.2 viable seeds) of cleaned seed collected at Rennix Gap in March 1953 were placed on dry filter paper in petri dishes and put into an oven at 80°C. Five dishes were removed from the oven and allowed to cool at room temperature (22°C) after 30, 60, 120 minutes of treatment. These and a further five replicates which had received no heat treatment were then stratified for 30 days at 5°C and set to germinate at 15°C.

Results:

(i) Germination capacity

The initial analysis showed that there was a significant effect of high temperature on germination capacity. However, further investigation showed that this result was biased by a single replicate

in which the number of germinated seeds was much lower than in the other replicates. There was no apparent reason for the low germination. When this replicate was excluded from the analysis the effect of treatment at 80°C on germination capacity was not significant (Table 3.14, $p=0.558$) and showed no trend with duration of treatment (85%, 78%, 83%, 81% for 0, 30, 60, 120 minutes respectively).

TABLE 3.14

Summary of analysis of deviance for the germination capacity of seed treated at 80°C.

Model terms	Residual d.f.	Residual deviance	RMD	Change d.f.	Change deviance	CMD	F Ratio	P
Total	18	54.75						
Time at 80°C	15	47.84	3.19	3	6.91	2.30	0.72	0.558

The lack of response of dry seed to high temperatures presents an interesting problem for the interpretation of the marked effect that high temperatures had on the seed prior to its extraction from the capsule. One possible reason for this anomaly may be that the seed inside the capsule has a higher water content which would make it more sensitive to the higher temperatures (Cunningham 1960).

The sensitivity of both the induction of dormancy and mortality of the seed to the water content has been shown by several authors. Grose (1963) also found that the rate at which dormancy was induced in E. delegatensis seed was dependent on the water content of the seed at temperatures of 27°C and 35°C. Cunningham (1960) showed that the mortality of E. regnans seed at high temperatures depended on the water content of the seed.

The water content of the seed contained in freshly-picked capsules has not been investigated. The water content of the capsule is high and it could be expected that the seed would be held in a moist environment within the capsule which may prevent the desiccation of the seed.

(ii) Times-to-germination

The replicate which was excluded from the analysis of germination capacity was also excluded from the analysis of the times-to-germination.

TABLE 3.15

Summary of analysis of deviance for times-to-germination of seed following treatment at 80°C.

Model terms	Residual d.f.	Residual deviance	RMD	Change d.f.	Change deviance	Significance Level
Time	209	292.6	1.4			
Time +Durn	206	271.9	1.3	3	20.70	p<0.005

Time =Day of observation

Durn =Duration of treatment at 80°C

Increasing the duration of exposure of dry seed to 80°C caused a consistent and significant increase in the times-to-germination which was expressed as a reduction in the rate of germination (Figure 3.18, Table 3.15). The sensitivity of the Cox model in the analysis of times-to-germination is well demonstrated in this experiment in which the differences between the germination curves of the various treatments is small (Figure 3.19).

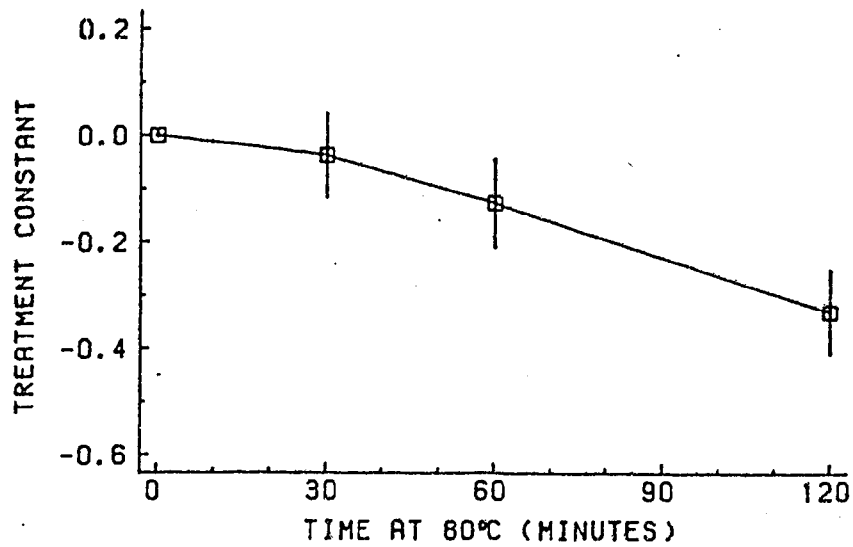


Figure 3.18 The treatment constants of the Cox model for the times-to-germination of seed treated at 80°C, stratified and then germinated at 15°C.

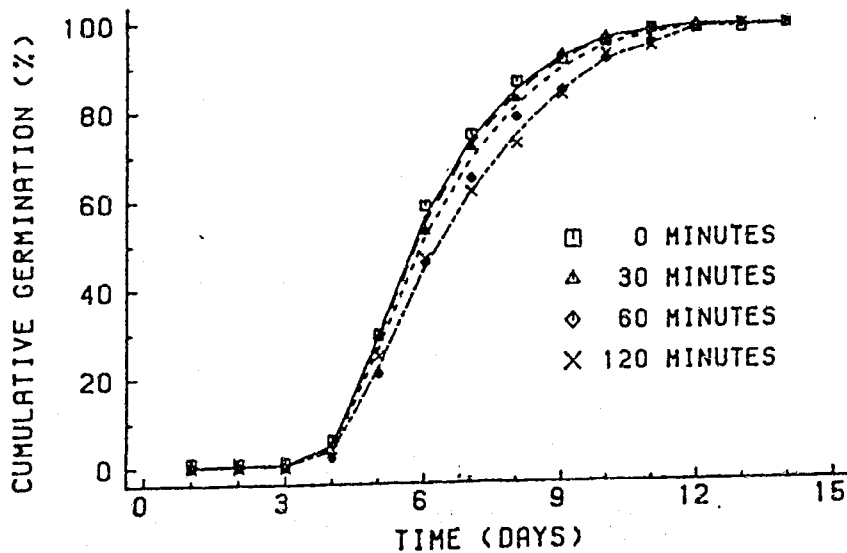


Figure 3.19 The predicted and observed cumulative germination (%) of seed heated at 80°C for 0, 0.5, 1.0, 2.0 hours

(iii) Seed mortality

Seed mortality was assessed in this experiment by analysing the total numbers of viable seeds. All treatments started with similar

numbers of seeds and, therefore, any variation in the numbers of viable seeds can be attributed to the heat treatment. There was no evidence of mortality in dry seed exposed to temperatures of 80°C for periods up to two hours (Table 3.16).

TABLE 3.16

Summary of analysis of variance for the numbers of viable seeds in weighed samples of seed following treatment at 80°C.

Source of Variation	d.f	Sum of Squares	Mean Square	Variance Ratio	Significance level
Time at 80°C	3	118.8	39.6	0.202	p=0.894
Residual	16	3130.0	96.3		
Total	19	3248.8	171.0		

Discussion:

Knowledge of the response of seed to high temperatures is important because of the frequency with which high temperature conditions occur at the soil surface. The mortality of Eucalyptus seed exposed to high temperature conditions has been shown, by several workers, to depend on the temperature and the moisture content of the seed during exposure (Boden 1957, Cunningham 1960, Grose 1960). Boden (1957) found that the viability of dry E. pauciflora seed was not reduced by exposure to temperatures of 96°C for one hour, whereas moistened seed was killed by temperatures above 75°C.

Cunningham (1960) demonstrated the importance of the seed moisture content on the observed response of E. regnans to high temperatures and noted that mortality of seed in the field could be expected when high soil temperatures follow rain in summer.

The effect of sub-lethal treatments at high temperatures was investigated by Grose (1960), who found that the proportion of dormant seed, and the strength of dormancy both increased as a result of treatment of E. delegatensis seed at 27°C and 32°C and that the rate of induction of dormancy was decreased with reductions in the water content of the seed from 40% to 20%.

3.5 Conclusions

1. There is no evidence for a consistent change in the degree of dormancy of seed with increasing altitude of seed source although seed from tree-line populations shows a larger proportion of dormant seed.

2. There is no consistent change in the degree of dormancy of seed of different sizes from the same lot of seed although the strength of dormancy decreases with increases in seed size. Seeds of the same size from different altitudes do not appear to have the same dormancy.

3. There is a reduction in the size the seed and chaff, and in the size of viable seed, as the altitude of the seed source increases.

4. Seed shed from capsules at high (40°C) and low (5°C) temperatures has a tendency to be more strongly dormant than seed shed at lower (15°C, 30°C) temperatures.

5. Exposing the capsules to high temperatures before seed extraction can increase the dormancy of the seed; the duration and temperature of treatment required to elicit changes in dormancy are slightly higher than those likely to be experienced by most capsules during a fire.

6. The dormancy of dry seed is not increased by exposure to high (80°C) temperatures although the rate of germination is reduced.