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THE REGENERATION OF

Eucalyptus pauciflora Sieb. ex Spreng.

FROM SEED

by

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A thesis submitted for the degree of Doctor of Philosophy of the
Australian National University

February 1985
CHAPTER 4

BREAKING DORMANCY
CHAPTER 4

Chapter 3 has shown that the dormancy of *Eucalyptus pauciflora* seed is determined largely before the seed is dispersed, and that only extreme conditions of temperature during dispersal may have a long lasting effect on the dormancy of seeds. The environment at the soil surface is the next situation in which changes in dormancy may occur.

Water has been shown to play a major facilitating role in the induction of dormancy (Grose 1963) in *E. delegatensis* and could be similarly involved in breaking of dormancy in *E. pauciflora*. The elucidation of the exact response of seed to water is difficult and complex and may not yield results which can be applied to a field situation because of the effect of other variables such as the contact between the seed and the soil (Dowling et al. 1971). Investigation of the role of water in the breaking of dormancy of *E. pauciflora*, are described in this chapter. There aim was, firstly, to confirm that seed needs to be at high water potential for dormancy to be broken, and secondly to investigate whether the dormancy of individual seeds could be accounted for by their water content.

Climate changes markedly with altitude and these changes could be expected to have an important effect on the environment to which the seed is exposed at the soil surface. For example, it may be expected that the lower temperatures at higher altitudes would increase the chances of seed breaking dormancy prior to winter and being exposed to the risk of germination.
The investigations described in the second part of this chapter examine the influence of the temperature environment on the dormancy and germination of moist *E. pauciflora* seed and attempt to determine whether the nature and rate of the response of the seed to low temperatures varies with the altitude of seed source. In subsequent chapters laboratory studies investigate the influence of temperature on the induction of dormancy (Chapter 5) and germination of seed (Chapter 6) and eventually lead to a study of changes in dormancy and germination in the field in Chapter 7.

The influence of light on the dormancy and germination of seed was not examined since Clifford (1954) was unable to detect a light response in the germination of *E. pauciflora*. Bachelard (1967a) also found that the germination capacity of *E. pauciflora* was not significantly affected by light during germination but that there was a non-significant tendency for seed stratified in the light to have a higher germination capacity in the light than seed stratified in the dark.
4.1 Response to water during stratification

4.1.1 Change in solute potential of surrounding solution

Water availability plays an important part in the function of many plant processes and this experiment examines the breaking of dormancy in seed stratified at a range of water potentials and then set to germinate at a water potential of 0 bars and a temperature 15°C.

Methods:

A mannitol solution was diluted to provide solutions with water potentials of -2, -4, -8, -10 bars. Distilled water was used for a solution of 0 bars. Benomyl fungicide (0.2 g/l) was added to the solutions to prevent fungal infection.

Replicates (0.2 g, 43.3 viable seeds) of cleaned seed, collected from near Dicky Cooper Ck. (1740 m) in December 1980, were spread on a single layer of filter paper in 60 mm petri dishes. Five replicate dishes each received 2 ml of treatment solution and were sealed using Vaseline, placed in an airtight box and put into a refrigerator at 5°C.

At weekly intervals, the seed was rinsed, drained, dried and then weighed. During the preparation for weighing, the seed was placed on filter paper in a Buchner funnel, rinsed with distilled water and dried under suction for 10 seconds after draining was completed. After weighing the seed was returned to a petri dish with a fresh filter paper and 2 ml of treatment solution.

Following 36 days of stratification the seed was weighed, as
above, returned to a dish with distilled water, and set to germinate 15°C.

Results:

(i) Germination Capacity

There was a progressive decline in germination capacity as the water potential during stratification was reduced below -4 bars (Figure 4.1, Table 4.1) which confirms the importance of water in determining the rate of breaking of dormancy at 5°C.

![Graph showing germination capacity vs. water potential](image)

*Figure 4.1* The influence of water potential during stratification on germination capacity at 15°C.

The effect of the treatments on seed weight was determined by analysis of the weights on each weighing occasion. The results of the analysis show that, generally, there was no significant difference in the weights of the seed stratified at different water potentials. On the week when the seed weights were significantly different (week 4)
the seed in the higher water potential treatments was heavier (Figure 4.2). The analysis cannot be validly extended to investigate the effect of both time and water potential because the weights represent repeated measures of the same seed and are therefore not independent.

**TABLE 4.1**

Summary of analysis of deviance for germination capacity of seed stratified under conditions of different solute potential.

<table>
<thead>
<tr>
<th>Model terms</th>
<th>Residual d.f. deviance</th>
<th>RMD</th>
<th>Change d.f. deviance</th>
<th>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>29</td>
<td>465.2</td>
<td>16.04</td>
<td></td>
</tr>
<tr>
<td><em>ψ</em></td>
<td>25</td>
<td>37.5</td>
<td>1.50</td>
<td>4</td>
</tr>
</tbody>
</table>

*ψ* = solute potential during stratification (0, -2, -4, -8, -10 bars)

**TABLE 4.2**

Summary of the separate analyses of variance for weights of seed samples held at different solute potentials during stratification.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>d.f</th>
<th>Variance Ratio</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1 ψ</td>
<td>4</td>
<td>0.089</td>
<td>0.514</td>
</tr>
<tr>
<td>Week 2 ψ</td>
<td>4</td>
<td>2.634</td>
<td>0.058</td>
</tr>
<tr>
<td>Week 3 ψ</td>
<td>4</td>
<td>6.907</td>
<td>0.524</td>
</tr>
<tr>
<td>Week 4 ψ</td>
<td>4</td>
<td>3.703</td>
<td>0.017</td>
</tr>
<tr>
<td>Week 5 ψ</td>
<td>4</td>
<td>2.360</td>
<td>0.080</td>
</tr>
<tr>
<td>Week 6 ψ</td>
<td>4</td>
<td>0.765</td>
<td>0.560</td>
</tr>
</tbody>
</table>

*ψ* = Solute potential of solution (0, -2, -4, -8, -10 bars)

The association between such small differences in seed weight and large differences in the germination capacity shows that the influence of water on the processes of breaking dormancy in the seed operates at a more subtle level than that investigated by Grose (1960) for the
induction of dormancy in *E. delegatensis* seed. The sensitivity of the breaking of dormancy to water potential also emphasizes the difficulties of laboratory manipulation of the moisture environment of the seed in a manner which takes account of the range of response likely to occur in the field.

![Graph showing weight of seed over time](image)

*Figure 4.2* The effect of time and water potential on seed weight

(ii) Times-to-germination

The water potential of the environment of the seed during stratification has a highly significant effect on the subsequent rate of germination (Table 4.3).

The Cox model shows that the times-to-germination for seed treated at a water potential of -2 bars were slightly reduced compared to that of seed maintained at 0 bars. Treatments at lower water potentials (-4, -8, -10 bars) resulted in a delay in the day of first germination (Figure 4.3b) and increases in the times-to-germination (Figure 4.3a).
TABLE 4.3

Summary of analysis of deviance for times-to-germination of seed stratified at different water potentials.

<table>
<thead>
<tr>
<th>Model terms</th>
<th>Residual d.f. deviance</th>
<th>RMD</th>
<th>Change d.f. deviance</th>
<th>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>361 2055.0</td>
<td>5.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>346 373.5</td>
<td>1.09</td>
<td>13 1677.4</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>$+\psi$</td>
<td>344 329.0</td>
<td>0.96</td>
<td>4 49.6</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

Time = Day of observation
$\psi$ = Water potential of surrounding solution

Figure 4.3a The predicted and observed germination curves for seed stratified at different water potentials.
Figure 4.3b  The treatment constants of the Cox regression model for seed stratified at different water potentials.

Discussion:

The responses observed indicate that the processes which result in the breaking of dormancy are facilitated by high water potentials in the surrounding environment and that reducing the water potential of the environment decreases the rate at which dormancy is broken. The range of water potentials which elicit the response result in relatively small differences in the wet weight of the seed. The sensitivity of the breaking of dormancy to the availability of water places another condition on the seed which will influence the timing of germination. As the soil becomes wetter with autumn rain and the soil temperatures decline, the conditions will become increasingly favourable for the breaking of dormancy. However, the rate at which dormancy is broken will be closely associated with the availability of water.

A possible complicating factor associated with the method used in these experiments is that there may have been some movement of mannitol into the seeds. Movement of solute into the seed would not
per se. change the water potential treatment imposed but would increase the amount of water required to equilibrate the water potential of the contents of the seed with the surrounding solution. Mechanical restraint exerted by the seed coat on the embryo may then result in lower water potentials than expected from the water potential of the solution, because the hydrostatic potential generated when the expanding embryo fills the volume available within the seed coat may prevent further water uptake until the testa is broken.

This problem could be prevented by using a solute which does not pass into the seed, for example, Poly-ethylene glycol 20,000. This solute was not used because of potential toxic effects and the difficulties of calibrating the solutions at the water potentials required (0 to -10 bars), with the equipment available (Wescor thermocouple psychrometer: model HR-33T with C-52 sample chambers).

The possibility of carrying out more sophisticated experiments using a system to manipulate soil matric potential, such as that used by Kaufmann (1969) or McWilliam & Phillips (1971), was investigated but both methods rely on the integrity of a semi-permeable membrane between the soil and the treatment solution and the durability of the membranes available at the time was not sufficient to permit experiments of the duration (20 to 30 days) which was required.

The moisture environment of seeds could also be manipulated by placing the seed in sealed containers above solutions of known solute potential. The desired differences in water potential represent small differences in relative humidity very close to saturation and the degree of temperature control required to manipulate relative humidity in this way was not available.
4.1.2 Water uptake, seed weight and the breaking of dormancy

The previous experiment showed that the efficacy of stratification is dependent on the availability of water. This experiment, through examination of seed weight before and after stratification, tests whether the dry weight, wet weight or water content of individual seeds of *E. pauciflora* is associated with seed dormancy.

Methods:

Seed germination papers with 100 indentations were placed in 120 mm petri dishes and moistened with either 3.0, 4.0, 4.5, 5.0, 6.0 ml of distilled water. One hundred seeds from a sample of cleaned seed collected near Dicky Cooper Ck. in December 1980 were individually weighed and placed on the marked grid in each dish. The petri dishes were then sealed with a ring of Vaseline and placed in the refrigerator at 5°C.

Following 36 days of stratification the petri dishes were taken from the refrigerator and the seed was re-weighed and set to germinate at 15°C. Seed remaining after germination was spilt before the viability of the seed could be assessed. Thus the ungerminated portion of seeds includes both viable and non-viable seed.

Results:

The number of seeds which germinated increased with the amount of water applied up to 5.0 ml (Table 4.5), which confirms the importance of water in the processes of breaking of dormancy noted in Section
4.1.1. The water content and wet weight of the seed which received 5 ml of water was similar to the seed which received 4 ml of water because a faulty seal permitted sufficient water to evaporate from the petri dish to lower the wet weight of the seed without affecting the breaking of dormancy. The exclusion of this treatment from the analyses reported in Table 4.4 did not change the conclusions so it was included in the analysis presented.

### TABLE 4.4

Summary of analysis of variance for the weights of individual seeds classified by the water dose treatment and whether they germinated.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>d.f</th>
<th>Mean Square</th>
<th>Variance Ratio</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DRY WEIGHT</strong>¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>3</td>
<td>0.083</td>
<td>0.72</td>
<td>0.542</td>
</tr>
<tr>
<td>Germ</td>
<td>1</td>
<td>5.074</td>
<td>44.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Water .Germ</td>
<td>3</td>
<td>0.058</td>
<td>0.50</td>
<td>0.687</td>
</tr>
<tr>
<td>Residual</td>
<td>392</td>
<td>0.115</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>WET WEIGHT</strong>¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>3</td>
<td>1.767</td>
<td>8.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Germ</td>
<td>1</td>
<td>8.327</td>
<td>38.49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Water .Germ</td>
<td>3</td>
<td>0.065</td>
<td>0.30</td>
<td>0.825</td>
</tr>
<tr>
<td>Residual</td>
<td>392</td>
<td>0.216</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>WATER CONTENT</strong>¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>3</td>
<td>0.430</td>
<td>14.80</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Germ</td>
<td>1</td>
<td>0.099</td>
<td>3.41</td>
<td>0.062</td>
</tr>
<tr>
<td>Water .Germ</td>
<td>3</td>
<td>0.011</td>
<td>0.37</td>
<td>0.778</td>
</tr>
<tr>
<td>Residual</td>
<td>392</td>
<td>0.029</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>WET WEIGHT</strong>²</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>4</td>
<td>5.329</td>
<td>26.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Germ</td>
<td>1</td>
<td>6.383</td>
<td>31.24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Water .Germ</td>
<td>4</td>
<td>0.540</td>
<td>2.64</td>
<td>0.033</td>
</tr>
<tr>
<td>Residual</td>
<td>490</td>
<td>0.204</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Water = Amount of water
- Germ = Seed germinated or not germinated
- Includes only 4.0, 4.5, 5.0, 6.0 ml treatments
- Wet weights of all treatments (3.0, 4.0, 4.5, 5.0, 6.0 ml)
Seeds in the 3.0 ml treatment became mixed during stratification and therefore the dry and the wet weights cannot be related to one another although germination can be related to the wet weight. The first part of this discussion will be limited to those treatments which have a full record of dry weight, wet weight and germination (4.0, 4.5, 5.0, 6.0 ml water) and shows that the dormancy of seed in these treatments was not related to differences in water content but was related to both the wet and the dry weight of the seed.

The first three analyses reported in Table 4.4 show that the seeds which germinated had significantly greater dry weights (Dry weight\textsuperscript{1} [Germ], p<0.001; 1.58 mg compared to 1.29 mg), which were largely responsible for heavier wet weights (Wet weight\textsuperscript{1} [Germ], p<0.001; 2.20 mg compared to 1.84 mg) since there were no differences in the water content of seed which germinated compared with seed which did not germinate (Water content [Germ], p=0.062). The relationship between the weight of the germinated and ungerminated seed was the same, regardless of the amount of water added, since the interaction term [Water,Germ] was not significant in any of the first three analyses. The number of seeds which germinated in these treatments was uniformly high so these treatments do not permit a critical evaluation of the relationship between seed weight and dormancy because they only consider the comparison between the most dormant viable seed and the rest. The situation is further confused by the possibility that some of the 'dormant' seeds were in fact non-viable. Checks on the cleaned seed showed that there are likely to be five non-viable seeds in a sample of 100 seeds. The 3.0 ml treatment had a much larger number of dormant seeds and the results are quite different.

In contrast to Wet weight\textsuperscript{1} the final analysis in Table 4.4 shows
TABLE 4.5

The number and mean wet weight of seeds following stratification in a range of moisture environments and germination at 15°C.

<table>
<thead>
<tr>
<th>Water Dose (ml)</th>
<th>3.0</th>
<th>4.0</th>
<th>4.5</th>
<th>5.0</th>
<th>6.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt N</td>
<td>1.614</td>
<td>2.166</td>
<td>2.275</td>
<td>2.082</td>
<td>2.370</td>
</tr>
<tr>
<td>Wt N</td>
<td>41</td>
<td>73</td>
<td>80</td>
<td>85</td>
<td>83</td>
</tr>
</tbody>
</table>

Seed which germinated

<table>
<thead>
<tr>
<th>Seed which did not germinate</th>
<th>1.585</th>
<th>1.723</th>
<th>1.919</th>
<th>1.756</th>
<th>2.069</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt N</td>
<td>59</td>
<td>27</td>
<td>20</td>
<td>15</td>
<td>17</td>
</tr>
</tbody>
</table>

Wt - mean wet weight of seed (mg)

N - Number of seeds in the calculation of mean seed weight

1 from Table 4.4, WET WEIGHT

2 includes both viable and non-viable seed

For comparison between means:

within a water dose treatment \( \text{LSD}_{0.05} = 0.251 \), \( \text{LSD}_{0.01} = 0.329 \)

between germination classes \( \text{LSD}_{0.05} = 0.248 \), \( \text{LSD}_{0.01} = 0.326 \)

That the difference between the wet weights of germinated and dormant seed is influenced by the amount of water added (Wet weight, \([\text{Water.Germ}] \text{ p}=0.033\)). The relatively small difference between the weight of the seeds which germinated and those which did not in the dish receiving 3.0 ml of water is likely to be the source of this interaction (Table 4.5). Seeds in the 3.0 ml treatment became mixed during stratification and therefore the dry and the wet weights cannot be related to one another although germination can be related to the wet weight. This means that there can be no direct test of whether the dormancy and the dry weight or water content of the seed are related in this treatment, since dry weight cannot be associated with whether a seed germinated. However an indication of the response can be obtained by considering the distribution of the wet weights of the seed (Figure 4.4).
Figure 4.4 The distribution of the weights of seed which germinated (open columns) and which did not germinate (hatched) for the dishes receiving 3.0, 4.0, 4.5 ml of water prior to stratification.

Test for independence of germination class and seed size

<table>
<thead>
<tr>
<th></th>
<th>3.0 ml</th>
<th>4.0 ml</th>
<th>4.5 ml</th>
<th>5.0 ml</th>
<th>6.0 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X^2$</td>
<td>1.04</td>
<td>19.25</td>
<td>13.78</td>
<td>16.02</td>
<td>5.97</td>
</tr>
<tr>
<td>$p$</td>
<td>0.993</td>
<td>0.008</td>
<td>0.055</td>
<td>0.025</td>
<td>0.545</td>
</tr>
</tbody>
</table>

The overall effect of water dose during stratification on the
distribution of wet weights of germinated and dormant seed seems to be to shift from a similar frequency distribution of wet weights in the 3.0 ml treatment, through an increase in the frequency of smaller seeds in the ungerminated seed in the 4.0 ml treatment, to a similar distribution of seed weights at the higher water doses. The differences in the frequency of germinated and ungerminated seed in the various size classes were tested (using a RXC test for independence Sokal & Rohlf 1969, page 599) and the results show that there was a significant difference (p=0.008) in the distribution of seed weights of seed which germinated and dormant seed in 4.0 ml treatment (Figure 4.4).

The trend in the distribution of the wet weights of the dormant seed with the amount of water applied (Figure 4.4), may shed some light on the observations of an earlier experiment (Section 3.1.2). If the effect of a decrease in the amount of water applied retards the processes which break dormancy, and is therefore equivalent to a reduced period of stratification, then the results of the present experiment could be explained in terms of the observations in Section 3.1.2. These observations showed that the germination capacity of unstratified seed was not affected by seed size but that, following stratification for 20 days, the larger seed had a higher germination capacity than the smaller seed.

Observations in the present experiment show that the size distribution of seed which was dormant was the same as the size distribution of the seed which germinated in the 3.0 ml treatment. However, the seed which received 4.0 ml of water shows a more complete breaking of dormancy in larger seed, which leads to an decrease in the frequency of the larger seed sizes in the dormant seed.
This experiment has shown that there is no evidence for differences in the amount of water imbibed by seeds which germinated and those which remained dormant which excludes the water content of the seed as a mediator of dormancy.
4.2 The breaking of dormancy by low temperatures

Low temperatures have been shown to be effective in breaking the seed dormancy of *E. pauciflora* (Pryor 1954, Boden 1957, Grose 1957, Bachelard 1967a). It is likely that the rate at which dormancy is broken will vary with temperature since Grose (1963) showed differences in the rate of breaking of dormancy of *E. delegatensis* seed at a range of temperatures below 10°C and, more importantly, the induction of dormancy at temperatures above 10°C.

The strength of the innate dormancy of the seed of *E. pauciflora* increases with altitude although the degree of innate dormancy is increased only around treeline (Section 3.1.2). The seed was stratified at 5°C in the experiment reported in Section 3.1.2 and there is the possibility that differences attributed to the strength of dormancy may have resulted from differences in the temperature response of breaking dormancy. The first experiment in this section examines the temperature response for breaking dormancy of *E. pauciflora* seed collected at three altitudes in order to test this possibility.

Changes in the rate at which dormancy is broken as the temperature changes could have major consequences in determining the timing of germination in autumn. The second experiment examines the change in dormancy with duration of treatment at a range of temperatures.
4.2.1 Response of seed from different altitudes to temperature

Temperatures around 5°C are effective for the breaking of dormancy in a wide range of plant species requiring cold treatment (Stokes 1965). *Eucalyptus pauciflora* seed on the soil at higher altitudes tends to be exposed to cool, moist conditions for longer than seed at lower altitudes; the stronger dormancy of seed from higher altitudes has been interpreted as an adaptation to prevent premature germination. However the possibility remains that changes in the temperature response for the breaking of dormancy in seed from different altitudes could also radically alter the time when the seed breaks dormancy.

The experiment reported in this section examines the temperature response of breaking dormancy in seed collected at 1230 m, 1740 m and 1910 m.

Methods:

The temperatures required for pretreatment were obtained using a temperature gradient plate. Grose's studies with *E. delegatensis* seed indicate that the range of temperature (0-15°C) and the number of treatments within this range (1-1.5°C steps) make the definition of the temperature response of the breaking of dormancy logistically difficult using incubators because of the large number of environments which must be imposed, so an alternative method was sought. A temperature gradient plate has the advantage that a large number of temperature treatments can be imposed, which together permit the more precise definition of the responses to temperature. Therefore a more sensitive test of differences in the temperature responses of seed from different
seed sources is possible (Thompson 1970).

The temperature environment of the seed on the gradient plate was monitored by daily readings of ten thermocouples arranged to span the gradient. In addition, each thermocouple was monitored continuously for two periods of approximately 24 hours during the 20 days of treatment.

Two replicates (each 0.4 g) of cleaned seed from three sites near Saukut Ck. (1230 m, 52.7 viable seeds), Dicky Cooper Ck. (1740 m, 57.5 viable seeds) and Baker's Ck. (1910 m, 58.5 viable seeds) were placed on the gradient plate at each of thirteen temperature levels. Seeds which germinated were counted and removed.

After 20 days, the seed was removed from the plate, placed on moist filter paper in petri dishes, and set to germinate at 15°C.

Results:

(i) Temperature gradient

The daily readings of thermocouples were used to derive a relationship between the distance from the edge of the gradient plate and the temperature of treatment; this relationship was then used to estimate the mean temperature and confidence limits for the temperature at the position of the seed. The 95% confidence limits for the predicted temperatures (1.0, 2.4, 3.6, 4.8, 5.9, 7.1, 8.3, 9.4, 10.5, 11.5, 12.6 °C) were the same (±1.4°C) at all levels of the gradient. The fluctuations in temperature limit the precision of the temperature response derived, however, the correlation between the temperature
environments, at all levels on the gradient plate, leads to the imposition of treatments in which the difference in temperature between the levels of the gradient is maintained.

There was no trend in the readings of the thermocouples with time at any level on the gradient plate; either in the daily readings of temperature over the duration of the experiment, or in fifteen-minute readings over the more intensive monitoring periods.

The method is presented diagrammatically on the yellow page at the end of this chapter.

(ii) Germination capacity

Eleven seeds germinated on the gradient plate but these were restricted to the two highest temperatures and were spread amongst all the seed sources.

**TABLE 4.6**

Summary of analysis of deviance for the germination capacity of seed from three altitudes treated at a range of temperatures.

<table>
<thead>
<tr>
<th>Model terms</th>
<th>Residual d.f.</th>
<th>deviance</th>
<th>RMD</th>
<th>Change d.f.</th>
<th>deviance</th>
<th>MCD</th>
<th>Ratio</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>65</td>
<td>1354.0</td>
<td>20.83</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temp +Source</td>
<td>53</td>
<td>104.0</td>
<td>1.96</td>
<td>12</td>
<td>1250</td>
<td>104.10</td>
<td>58.19</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>+Temp .Source</td>
<td>33</td>
<td>59.1</td>
<td>1.79</td>
<td>20</td>
<td>44.89</td>
<td>2.24</td>
<td>1.25</td>
<td>0.278</td>
<td></td>
</tr>
</tbody>
</table>

Temp = Temperature of treatment on gradient plate  
Source = Seed Source: Sawpit Ck., Dicky Cooper Ck., Bakers Ck.  
1230 m  1740 m  1910 m
Germination capacity was significantly affected by both seed source and temperature (Table 4.6). However the interaction between the two factors was not significant, indicating that there was no difference in the response of the three seed sources to temperature. The lower germination capacity of seed from higher altitudes shows that the dormancy of this seed is stronger than that of seed from lower altitudes (Figure 4.5). The data for Waste Point seed in Figure 4.5 came from a similar experiment (Section 4.2.2), which was carried out under comparable conditions but at a different time.

![Graph showing germination capacity vs. temperature. The graph has data points for WP Seed, SC Seed, DC Seed, and BC Seed.]  

Figure 4.5 The germination capacity of seed from four altitudes following treatment at a range of temperatures and then germination at 15°C.

The response of germination capacity to stratification at a range of temperatures shows that the germination capacity is greater for seed from lower altitudes (Figure 4.5). Cubic splines fitted to the responses (Table 4.7) confirm that seed from lower altitudes shows a 'broader' type of response, which rises to a higher maximum germination capacity.
The absence of a significant interaction (p=0.278) between seed source and temperature of treatment for the three seed sources (Sawpit Ck., Dicky Cooper Ck., Baker's Ck.) is consistent with the similarity in the shape of the responses shown in Figure 4.5. However, when the data from Waste Point are considered, there appears to be a trend for an increase in the optimum temperature for stratification with decreasing altitude. The Waste Point data cannot be included in the analysis of germination capacity because of differences in the treatment temperatures and it is, therefore not possible to quantify this trend. Investigations using seed from even lower altitudes may reveal a trend of increasing optimum temperature for stratification with decreasing altitude.

**TABLE 4.7**

The characteristics of the temperature response of stratification estimated by fitting cubic splines to the predicted germination capacity.

<table>
<thead>
<tr>
<th>Seed Source</th>
<th>Estimated Peak Temp °C</th>
<th>% germ.</th>
<th>Shoulder °C Lower</th>
<th>Upper</th>
<th>&quot;Breadth&quot; °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waste Point</td>
<td>4.6</td>
<td>92.0</td>
<td>1.4</td>
<td>7.4</td>
<td>6.0</td>
</tr>
<tr>
<td>Sawpit Ck.</td>
<td>4.2</td>
<td>77.0</td>
<td>1.7</td>
<td>7.0</td>
<td>5.3</td>
</tr>
<tr>
<td>Dicky Cooper Ck.</td>
<td>3.4</td>
<td>70.3</td>
<td>1.6</td>
<td>5.8</td>
<td>4.2</td>
</tr>
<tr>
<td>Baker's Ck.</td>
<td>3.4</td>
<td>54.7</td>
<td>1.8</td>
<td>5.4</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Shoulder: temperature at 0.75 of peak germination

(iii) Times-to-germination

Seed collected at the higher altitudes, Dicky Cooper Ck. and Baker's Ck., show similarities in the response of times-to-germination to the temperature of treatment. However the seed from Sawpit Ck. has a
much lower rate of germination despite its higher germination capacity (Figure 4.6). The analysis was restricted to temperature levels one to seven (1.0-8.3°C) because, at temperatures greater than 8.3°C, the number of germinated seeds was not large enough to ensure reliable estimation of the times-to-germination.

TABLE 4.8

Summary of analysis of deviance for the times-to-germination of seed from three altitudes treated at a range of temperatures prior to germination for 20 days at 15°C.

<table>
<thead>
<tr>
<th>Model terms</th>
<th>Residual d.f. deviance</th>
<th>Residual RMD</th>
<th>Change d.f. deviance</th>
<th>Change RMD</th>
<th>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>594</td>
<td>2695.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>577</td>
<td>814.7</td>
<td>1.41</td>
<td>17</td>
<td>1880.3</td>
</tr>
<tr>
<td>*Temp +Source</td>
<td>569</td>
<td>690.1</td>
<td>1.21</td>
<td>7</td>
<td>124.5</td>
</tr>
<tr>
<td>*Temp .Source</td>
<td>557</td>
<td>664.5</td>
<td>1.19</td>
<td>12</td>
<td>25.5</td>
</tr>
</tbody>
</table>

Time =Day of germination
Temp =Temperature of pretreatment
Source =Seed source Sawpit Ck., Dicky Cooper Ck., Baker's Ck.

The times-to-germination of the seed from the three altitudes (Figure 4.6) had a similar response to the temperature of treatment ([Temp.Source], p=0.012, Table 4.8) and therefore the treatment response can be represented by the additive model [Temp+Source]. The large number of degrees of freedom involved means that the chances of obtaining a significant interaction term by chance alone is increased. The parsimonious solution was to adopt a critical level for significance of p=0.01. The coefficients shown in Figure 4.6 indicate that the rate of germination increases at temperatures up to 6°C and then declines at higher temperatures.

The slower rate of germination of the seed from Sawpit Ck.
predicted by the treatment constants of the Cox model reflects a lag of 1 to 2 days in the germination curves of the seed from Sawpit Ck. (1240 m) compared to those of the higher altitudes (Figure 4.7). The lower rate of germination in the seed from Sawpit Ck. (1240 m) is associated with a higher germination capacity in the seed from this source and suggests that there is a change in the relationship between the times-to-germination and the germination capacity for seed from different altitudes.

![Graph of treatment constants vs temperature]

Figure 4.6 The treatment constants of the Cox model for seed from three sources treated at a range of temperatures before germination.
Figure 4.7 The predicted and observed germination curves for seed from three altitudes at (a) 3.6°C, (b) 6.0°C

(iv) Seed mortality

The effect of temperature on the number of viable seeds was not significant for any of the seed sources (Sawpit Ck., p=0.03; Dicky Cooper Ck., p=0.501; Baker's Ck., p=0.337) indicating that there is no evidence of relative mortality of seed due to the treatments.
Discussion:

Seed from higher altitudes requires a longer duration of treatment at temperatures which are suitable for breaking dormancy compared to seed from lower altitudes. Such a difference could be expected to reduce the chance of seed from higher altitudes germinating during autumn or under the snow during winter. Additionally, if the temperatures at the time when the seed first became moist were too high for dormancy to be broken, then the dormancy induced in the seed would become the primary factor determining the timing of germination, far outweighing any differences in innate dormancy due to seed source (Chapter 5).

Despite the increases in the strength of dormancy of seed from higher altitudes, there was no evidence of qualitative differences in the temperature response of breaking dormancy in seed collected at altitudes above 1240 m. However, further investigation of the temperature response of breaking dormancy of seed from lower altitudes is required before this conclusion could be applied more generally.

Differences in the strength of dormancy could be one of the factors determining whether germination and seedling emergence occurs in the field during autumn, or whether premature germination occurs during winter. This hypothesis is tested in the field in the reciprocal transplant experiments described in Chapter 7.

The more rapid germination and the lower germination capacities of the seed from higher altitudes suggest that the relationship between rate of germination and germination capacity is different in seed from different altitudes. The possibility that these observations may have
been influenced by the degree of dormancy of the seed is remote, since the findings in Section 4.2.2 show that, as the dormancy is broken by stratification, the rate of germination increases with increases in the peak germination capacity of the seed from both Waste Point (960 m) and Dicky Cooper Ck. (1740 m).

The small difference in the rate of germination of seed from the two higher altitudes compared with seed from Sawpit Ck. was also associated with similarities in the strength of dormancy (Section 3.2). The discontinuity in the strength of dormancy between seed from low altitude (960 m, 1240 m) and high altitude (1510 m, 1740 m) sources may also be reflected in the rate of germination. Unfortunately the rate of germination of seed from 1240 m, 1510 m and 1740 m cannot be compared directly with that from 960 m reported in Section 4.2.2.1 because of differences in the temperatures imposed.

A crude comparison of the day to 50% germination after 20 days treatment at an average temperature of 6°C (Figure 4.7, Figure 4.12) of the four seed sources shows that the seed from 960 m has a faster rate of germination (6.5 days) than seed from either the higher altitudes (1510 m, 1740 m) (7 days) or 1240 m (8 days). This result is contrary to the trend noted in the experiment reported in this section and may be the result of the differences in the temperature control of the treatment on the gradient plate or the germination conditions in the two experiments; further studies are required to resolve the situation.

Interestingly, the altitude of the discontinuity in germination behaviour coincides with the altitude at which the morphology of E. pauciflora seed source trees changed from the montane form (ssp. pauciflora) to the mallee form (ssp. niphophila).
Changes in the climatic environment of the seed on the soil with increases in altitude mean that the seed at higher altitudes is exposed to a longer period of cold, moist conditions than seed at lower altitudes. In environments where snow lies, the rise in soil temperatures in spring is dependent on the melting of the snow and the snow melt becomes later with increases in altitude. Such changes in conditions with increases in altitude amount to a narrowing of the period in which emergence is likely to be successful, because the time before the onset of high temperatures and dry conditions at the soil surface is likely to be shorter at high altitudes. The advantage of the stronger dormancy at higher altitudes has been discussed in Section 3.2, and the association between this and the more rapid germination in seed from higher altitudes, may reflect the shorter time between the moist conditions of winter and the dry conditions of summer at the higher altitudes.
4.2.2 Changes in dormancy with duration of cold treatment.

This section describes an investigation of the changes in dormancy of seed collected at two altitudes (960 m, 1740 m), exposed to a range of temperatures under moist conditions, and then set to germinate at 15°C.

4.2.2.1 Seed from Waste Point (960 m)

Methods:

A range of temperature environments between -2°C and 12°C were maintained on a temperature gradient plate. The temperature environment of the seed on the gradient plate was monitored by daily readings of ten copper-constantin thermocouples arranged to span the gradient. The temperature of all thermocouples was continuously recorded for at least 17 hours during the experiment to obtain an estimate of shorter term variations in temperature.

Capsules were collected near Waste Point (960 m) in December 1982. Twelve replicates (0.3 g, 44.5 viable seeds) of cleaned seed were placed on the gradient plate at each of ten temperature levels. Seed from two replicates of the ten temperature levels was retrieved from the gradient plate on six occasions (5, 10, 15, 20, 25, 30 days) and set to germinate in petri dishes at 15°C.

Seeds which germinated on the gradient plate were recorded and removed at each retrieval occasion. Seeds which germinated in the incubator were recorded and discarded daily. Germination counts were terminated when the replicates of seed retrieved from the gradient
plate at 30 days had ceased to germinate (17 days).

Results:

(i) Temperature gradient

The daily readings of the thermocouples were used to derive a relationship between position on the gradient plate and temperature. This regression was then used to estimate the mean temperature and confidence limits for the temperature at the position of the seed. The estimated temperatures (−1.7, −0.6, 0.6, 1.9, 2.8, 3.9, 5.0, 6.0, 7.5, 8.9, 10.2, 11.4, 12.6 °C) have 95% confidence limits of ±0.7°C for all levels of the gradient. The shorter term variations in temperature observed in the continuous monitoring of each thermocouple for 17 hours did not exceed these limits nor was there a trend in the daily readings of any of the thermocouples with time.

(ii) Germination capacity

Two seeds germinated on the gradient plate at the highest temperature and the longest duration. There was a strong influence of both temperature and duration of treatment on the germination capacity of the seed (Table 4.9). The presence of a significant interaction was probably the result of changes in the response to temperature as the duration of treatment increased.

A progressive increase in germination capacity with duration of treatment occurred at all temperatures below 9°C whereas the germination capacity decreased with duration of treatment at
temperatures above 9°C (Figure 4.7). The seed which remained on the gradient plate for 30 days showed a reduction in germination capacity at temperatures below 3°C compared to seed remaining on the plate for 25 days. The results for seed exposed to low temperatures for 30 days do not agree with other experiments in this study or with the work of Grose (1963); however, the reasons for this difference are not clear.

**TABLE 4.9**

Summary of analysis of deviance for the germination capacity of Waste Point seed treated at a range of temperatures and germinated at 15°C.

<table>
<thead>
<tr>
<th>Model terms</th>
<th>Residual d.f. deviance</th>
<th>Residual HMD</th>
<th>Change d.f. deviance</th>
<th>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>129 2190.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+Temp +Durn</td>
<td>113 512.6</td>
<td>4.53</td>
<td>16 1677.4</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>+Temp .Durn</td>
<td>85 85.24</td>
<td>1.31</td>
<td>48 427.36</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

Temp = Temperature of treatment  
Durn = Duration of treatment

The characteristics of the temperature responses shown in Figure 4.8 may be derived by fitting cubic spline curves to the data for each retrieval time. These curves permit an unbiased estimation of the optimum temperature and the breadth of the optimum (Table 4.10).

The estimation of the peak temperature (temperature of maximum germination capacity) is less precise when the germination response is 'broader', as can be seen for the curves for the 5 day and 25 day treatments (Figure 4.8). In general, there is a trend for the 'breadth' of the temperature response to increase with increasing duration of treatment. The peak temperature tends to decrease in the period from 10 to 15 days but there was little change with
longer durations of treatment. Decreases in the peak temperature were associated with a marked reduction in the temperature of the lower shoulder and a slight reduction in the temperature of the upper shoulder. The rates of increase in germination capacity at lower temperatures reflect the slower but more complete breaking of dormancy at lower temperatures which results in a widening of the temperature optimum as the duration of treatment is increased.

![Graph showing germination capacity percentage vs. temperature for different durations of treatment](image)

**Figure 4.6** The effect of temperature and duration of pretreatment on the germination capacity of *E. pauciflora* at 15°C.

These data should not be interpreted as showing that breaking of dormancy proceeds at temperatures below 0°C, since seed maintained at a mean temperature of -0.6°C still spent some time at temperatures above 0°C. Moreover, seed exposed to the west temperature treatment spent almost all the time below 0°C and showed little tendency toward the breaking of dormancy.

Whilst cubic splines can be fitted to predict the location of the peak temperature and the breadth of the temperature response, it would
be more convenient for the purpose of summarizing the temperature response of breaking dormancy to determine the mathematical form of the response. More importantly, if the mathematical form of the responses can be ascertained, then interpolation will permit prediction of the effects of other temperatures and durations of treatment on germination capacity. The data from 30 days of treatment on the gradient plate were excluded from the following analysis because of the unexplained decline in germination capacities compared to seed treated for 25 days.

**TABLE 4.10**

The characteristics of the temperature response of Waste Point seed treated at a range of temperatures for 5 to 30 days.

<table>
<thead>
<tr>
<th>Duration</th>
<th>Estimated Peak Temp °C</th>
<th>Shoulder °C</th>
<th>'Breadth' °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% germ.</td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>5 days</td>
<td>5.2</td>
<td>30.9</td>
<td>2.0</td>
</tr>
<tr>
<td>10 days</td>
<td>6.4</td>
<td>54.4</td>
<td>3.5</td>
</tr>
<tr>
<td>15 days</td>
<td>4.7</td>
<td>74.1</td>
<td>1.8</td>
</tr>
<tr>
<td>20 days</td>
<td>4.6</td>
<td>92.1</td>
<td>1.4</td>
</tr>
<tr>
<td>25 days</td>
<td>4.9</td>
<td>96.2</td>
<td>0.5</td>
</tr>
<tr>
<td>30 days</td>
<td>5.3</td>
<td>97.3</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Shoulder: temperature at 0.75 of peak germination

The mathematical form of the response of germination capacity to both temperature and duration of treatment can be investigated by replacing the factors representing temperature and duration of treatment ([Temp], [Durn]), with functions of the continuous variables temperature of treatment (°C) and duration of treatment (days) in the statistical model. The results of fitting a linear function of time in place of the factor [Durn] and either a quadratic or cubic function in place of the factor [Temp] are shown in Table 4.11.
TABLE 4.11

Summary of analysis of deviance for the replacement of the factors [Temp] and [Durn] with the variables temperature of treatment and duration of treatment.

<table>
<thead>
<tr>
<th>Model terms</th>
<th>Residual d.f.</th>
<th>Residual deviance</th>
<th>Change d.f.</th>
<th>Change deviance</th>
<th>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp *Durn(^1)</td>
<td>65</td>
<td>85.24</td>
<td>1.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temp *D</td>
<td>104</td>
<td>138.6</td>
<td>1.33</td>
<td>39</td>
<td>53.35</td>
</tr>
<tr>
<td>(T-TSQ) *Durn</td>
<td>115</td>
<td>189.2</td>
<td>1.65</td>
<td>50</td>
<td>103.96</td>
</tr>
<tr>
<td>(T+TSQ+TCU) *Durn</td>
<td>110</td>
<td>184.5</td>
<td>1.68</td>
<td>45</td>
<td>99.26</td>
</tr>
</tbody>
</table>

Factors:  
Temp = Temperature of treatment  
Durn = Duration of treatment

Variables:  
T = Temperature of treatment; TSQ=T\(^2\); TCU=T\(^3\)  
D = Duration of treatment (days)

\(^1\) The full model, [Temp*Durn]=\(\mu + Temp*Durn + Temp.Durn\)
\(^2\) Compared with [Temp*Durn]

The statistical models [Temp*Durn] and [Temp*D] are not significantly different (p=0.063) suggesting that the factor [Durn] (duration of treatment) may be replaced by a variable, D, the duration of pretreatment in days. The linear model (1) can therefore replace the full model of Table 4.11.

\[
\text{Logit}(G) = \mu + \text{Temp} + D + \text{Temp.D} \\
\text{...(1)}
\]

This model can be further reduced to a term which is dependent on temperature (T) alone (\(\beta_0(T)=\mu + \text{Temp}\)) and a term which is dependent on the temperature and the duration of pretreatment (\(\beta_1(T)=D + \text{Temp.D}\)). The term \(\beta_1\) is an expression of the rate of change of logit(G), the log of the odds ratio, with duration of treatment. Figure 4.9 shows that
Figure 4.9 The coefficients of the model relating temperature and duration of treatment to germination capacity (G)

Model: \( \text{logit}(G) = \beta_0(T) + \beta_1(T) \cdot D \)

where \( D \) is the duration of treatment in days.

The rate of change increases to a temperature of around 5.5°C, the optimum temperature for stratification at constant temperatures.

The temperature response was investigated in a similar way, but neither quadratic nor cubic functions of the treatment temperature could replace the factor [Temp] in the model and no attempt was made to apply more complex models to the data (Table 4.11). The criterion for the 'adequacy' of the empirical model is that the amount of variation explained is not significantly different from that explained by the factor [Temp]. This criterion may be far too strict for some purposes, if the major concern is the large and significant proportion of the variation which is explained by either quadratic or cubic and not the small, though significant portion which not explained by these functions. Unfortunately, at this stage the response of the seed to the temperature and duration of pretreatment cannot be fully summarized in terms of the variables temperature and duration of treatment. This
limits the usefulness of the model in the prediction of the response of seed to other regimes.

(iii) Times-to-germination

The times-to-germination are influenced by temperature in a very different way to the germination capacity; the rate of germination increases with temperature at all temperatures up to 25 days of treatment and higher temperatures only begin to show a slower rate of germination after 25 days of treatment (Figure 4.10). The effect of duration of treatment on times-to-germination was investigated by consideration of the overall response of the times-to-germination to temperature. Then the response to temperature at each retrieval time was examined in detail.

The Cox model requires that the treatments have a common time course of observations so that the risk of germination at a certain time may be expressed in terms of a hazard function and an effect of treatment (Cunningham et. al. 1981). The retrieval of seed at intervals of 5 days from the gradient plate meant that a day on which the germination count was missed was 5 days out of phase in the course of germination in seed retrieved from the gradient plate at successive times. There were 6 days on which the observations of germination coincided in all of the retrieval treatments.

The threshold number of germinating seeds for including treatments in the analysis of times-to-germination was raised from five to ten in this analysis because of the limited (2) replication. This condition led to the exclusion of all of the data for seed treated for 5 days, and the two lowest (−1.7, −0.6°C) and three highest (10.2, 11.4, 12.6°C)
limits the usefulness of the model in the prediction of the response of seed to other regimes.

(iii) Times-to-germination

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The threshold number of germinating seeds for including treatments in the analysis of times-to-germination was raised from five to ten in this analysis because of the limited (2) replication. This condition led to the exclusion of all of the data for seed treated for 5 days, and the two lowest (-1.7, -0.6°C) and three highest (10.2, 11.4, 12.6°C)
temperature treatments, from the analysis.

TABLE 4.12

Summary of analysis of deviance for the effect of temperature and duration of stratification on the times-to-germination at 15°C.

<table>
<thead>
<tr>
<th>Model terms</th>
<th>Residual d.f. deviance</th>
<th>RMD</th>
<th>Change d.f. deviance</th>
<th>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>384 3726.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>350 879.3</td>
<td>2.31</td>
<td>4</td>
<td>2846.7</td>
</tr>
<tr>
<td>+Temp +Durn</td>
<td>359 502.5</td>
<td>1.36</td>
<td>11</td>
<td>376.8</td>
</tr>
<tr>
<td>+Temp .Durn</td>
<td>341 418.0</td>
<td>1.23</td>
<td>28</td>
<td>88.5</td>
</tr>
</tbody>
</table>

Time = Day of observation (1, 6, 7, 10, 11, 15)
Temp = Temperature of stratification (0.6°C to 8.9°C)
Durn = Duration of stratification (5, 10, 15, 20, 25, 30 days)

Figure 4.10 The treatment constants of the Cox model fitted to the times-to-germination for the days of common observations, summary of analysis in Table 4.12.

The times-to-germination show that the response to temperature
changes, depending on the duration of treatment (Table 4.12). The rate of germination increases as a result of treatment at any temperature on the gradient plate, but the increase in the rate of germination with duration of treatment is most marked at those temperatures which have been found to be optimal for breaking the dormancy of seed. The response of times-to-germination to temperature of treatment was not significant for seed treated for 10 days in the subsequent analysis (Table 4.13, see discussion below). However, the increase in the rate of germination with increases in temperature, evident in seed treated for 15 days, changes into a response which shows a maximum at around 4.5°C after 30 days of treatment (Figure 4.10).

The temperature response will now be considered in more detail by analysing the times-to-germination on each retrieval occasion separately; this avoids the severe reduction of the data set necessary in the previous analysis and permits a more detailed investigation of the responses and comparison of the predicted and observed germination curves.

The times-to-germination of seed show a significant response to temperature of treatment on the gradient plate for all durations greater than 10 days (Table 4.13). The form of the temperature response can be represented by the treatment constants of the Cox model (Figure 4.11) which show that the trend of increased rate of germination with temperature at 15 days turns to a response showing a maximum at 25 days which becomes further developed after 30 days of treatment.

Predicted and observed germination curves have been grouped to permit comparison of the effects of different durations of treatment.
TABLE 4.13

Summary of analysis of deviance for the effect of stratification temperature on the times-to-germination at 15°C.

<table>
<thead>
<tr>
<th>Model terms</th>
<th>Residual d.f. deviance</th>
<th>Residual RMD</th>
<th>Change d.f. deviance</th>
<th>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>10 DAYS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>187</td>
<td>201.0</td>
<td>1.06</td>
<td>7</td>
</tr>
<tr>
<td>Temp</td>
<td>180</td>
<td>191.2</td>
<td>1.06</td>
<td></td>
</tr>
<tr>
<td><strong>15 DAYS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>224</td>
<td>266.9</td>
<td>1.06</td>
<td>8</td>
</tr>
<tr>
<td>Temp</td>
<td>215</td>
<td>228.7</td>
<td>1.06</td>
<td></td>
</tr>
<tr>
<td><strong>20 DAYS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>186</td>
<td>311.7</td>
<td>1.50</td>
<td>7</td>
</tr>
<tr>
<td>Temp</td>
<td>179</td>
<td>268.1</td>
<td>1.50</td>
<td></td>
</tr>
<tr>
<td><strong>25 DAYS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>238</td>
<td>478.3</td>
<td>1.50</td>
<td>8</td>
</tr>
<tr>
<td>Temp</td>
<td>230</td>
<td>344.9</td>
<td>1.50</td>
<td></td>
</tr>
<tr>
<td><strong>30 DAYS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>215</td>
<td>431.1</td>
<td>1.53</td>
<td>7</td>
</tr>
<tr>
<td>Temp</td>
<td>208</td>
<td>317.4</td>
<td>1.53</td>
<td></td>
</tr>
</tbody>
</table>

Time = Day of observation  
Temp = Temperature of treatment  
10, 15 days: 0.6°C-8.9°C  
20, 25 days: -0.6°C-8.9°C  
30 days: 0.6°C-8.9°C

The predicted curves in Figure 4.12 are derived from the treatment constants presented in Figure 4.11. The germination curves presented show that the rate of germination increases with temperature at 1.9°C, 3.9°C and 6.0°C and shows very little change with increasing duration of treatment at 8.9°C. The germination curves of seed treated at 8.9°C were similar to the curves of seed which had been treated at the lower temperatures for shorter durations. It is suggested that this similarity in rate of germination is associated with the dormancy of the seed and represents the minimum rate at which the processes which lead to the germination of seed can operate at a temperature of 15°C.
Figure 4.11 The treatment constants of Cox models fitted to the times-to-germination of seed from each retrieval time separately.
Figure 4.12  The predicted (lines) and observed (symbols) germination curves for seed at 4 temperatures for either 15, 20, 25 or 30 days.
The germination curves of seed stratified at 1.9°C and 8.9°C both have lower rates of germination compared to seed placed at intermediate temperatures; however, the rate of germination of the seed at the lower temperature is gradually increasing with time. Treatment at temperatures as low as 1.9°C for short durations (15 days) also seems to retard the processes of germination since seed exposed to this treatment germinated more slowly than seed exposed to any of the other treatments.

The Cox model fitted to the individual retrievals has confirmed the general trend noted previously. After longer periods, the rate of germination of seed placed at low temperatures begins to rise whilst the rate at high temperatures remains constant and a response develops which shows an optimum at about 5.5°C.

(iv) Seed mortality

Analysis of variance showed that there were no significant differences in the number of viable seeds with either temperature or duration of treatment (Table 4.14). Since the number of viable seeds showed no trend with either duration or temperature of treatment in this experiment there is no evidence of differential mortality as a result of the treatments.
<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>d.f</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>Variance Ratio</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp</td>
<td>12</td>
<td>1207.5</td>
<td>100.63</td>
<td>1.992</td>
<td>p = 0.036</td>
</tr>
<tr>
<td>Durn</td>
<td>5</td>
<td>657.4</td>
<td>131.49</td>
<td>2.603</td>
<td>p = 0.031</td>
</tr>
<tr>
<td>Temp * Durn</td>
<td>60</td>
<td>4091.5</td>
<td>68.19</td>
<td>1.350</td>
<td>p = 0.106</td>
</tr>
<tr>
<td>Residual</td>
<td>78</td>
<td>3940.5</td>
<td>50.52</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Temp = Temperature of treatment  
Durn = Duration of treatment

Discussion:

The rate at which the dormancy of *E. pauciflora* seed is broken by cold treatment depends on both the temperature and duration of treatment. Generally, the rate at which dormancy is broken increases with increasing temperature from 0°C to about 5.5°C. The reductions in germination capacity with increasing durations of treatment at temperatures above 9°C show that dormancy has been induced in seeds which were initially able to germinate. Induction of dormancy may well extend to temperatures lower than 9°C in the sense that some seeds would never germinate, regardless of the duration of treatment at temperatures between 5.5°C and 9°C. Following treatment at these temperatures these seeds would require longer periods of cold treatment than seeds which had been treated at lower temperatures. Increases in the strength of dormancy by exposure to higher temperatures (15°C) are discussed in Sections 5.1 and 5.2.

Soil temperatures under the snow generally vary between -0.5°C and 0.5°C so it is likely that the dormancy of the seed will change very
slowly during the period of snow cover at higher altitudes. As the snow melts, the temperatures will rise and the dormancy will be broken more rapidly, leading to germination and emergence. The snow becomes shallower and the duration of snow lies declines with decreases in altitude until at altitudes of 960 m the snow rarely lies for more than a week. The shallow depth of snow at low altitudes would also be less effective in reducing the variation in soil temperatures which is apparent at high altitudes under snow. The seed on the soil at lower altitudes will, therefore, be subjected to a greater variation in temperature and moisture conditions during autumn and winter than seed at higher altitudes. The impact of this variation on the breaking of dormancy is not known but it could be expected to lead to an enhancement of the differences in the dormancy of seed within a seed sample. The enhancement of the variation in the dormancy of the seed sample results from the increased possibility of germination as soon as the seed becomes non-dormant, when the temperature oscillates. In contrast, seed on the soil at higher altitudes is likely to become substantially non-dormant before any of the seed has the opportunity to germinate because of the long period of consistently low temperatures in winter. The higher temperatures at lower altitudes would permit stratification throughout the winter period and may be important in promoting the longer period of emergence which is observed at lower altitudes (Section 7.2).
4.2.2.2 Seed from Dicky Cooper Ck. (1740 m)

A second experiment looks at the influence of the temperature of stratification on the subsequent germination of seed collected at an higher altitude with the objective of comparing the response to that for seed from Waste Point.

Methods:

The major difference in the methods between this and the last experiment is that the durations of treatment were generated by sequential placement of the seed on the gradient plate in this experiment whereas the seed was removed from the gradient plate after various intervals in the previous experiment.

Time and resources did not permit the range of treatments which were used in the previous experiment so cleaned seed collected near Dicky Cooper Ck. (0.4 g, 57.5 viable seeds) was exposed to three durations of treatment (10, 15, 20 days) at eleven temperature levels. There were two replicates of each treatment combination.

Results:

(1) The temperature gradient

This experiment was run at the same time as the investigation of the temperature response of seed from different altitudes (Section 4.2.1) and was exposed to the same temperature regime. Re-iterating, the 95% confidence limits for the predicted temperatures (1.0, 2.4, 3.6, 4.8, 5.9, 7.1, 8.3, 9.4, 10.5, 11.5, 12.6 °C) were the same.
(11.4°C) at all levels of the gradient.

(ii) Germination capacity

Eight seeds, from the higher temperature treatments, germinated on the gradient plate between 10 and 20 days of treatment. The results of the germination on the gradient plate show that there was a gradual increase in germination capacity with duration of treatment at all temperatures below 7°C (Figure 4.13). Seed treated at temperatures above about 8°C became more dormant with increasing durations of treatment. Unfortunately, poor temperature control makes the exact determination of the discontinuity in response impossible. The response seems to be similar to the seed from Waste Point although the temperature at which the response turns from a nett breaking to a nett induction of dormancy is lower.

![Graph showing germination capacity](image)

**Figure 4.13** The germination capacity of seed from Dicky Cooper Ck. treated at a range of temperatures for 10, 15, 20 days.

Cubic splines were fitted to the responses to determine the
characteristics of the curves (Table 4.15). There was a decrease in the temperature of peak germination with increasing duration of treatment which is associated with lower temperatures at both the upper and lower shoulders. The change in the shape of the response from 10 to 20 days in seed from Dicky Cooper Ck. is similar to that observed with Waste Point seed from 5 to 15 days and is consistent with a slower but more complete breaking of dormancy at lower temperatures.

TABLE 4.15

The characteristics of the cubic splines fitted to the temperature response of seed from Dicky Cooper Ck. treated at a range of temperatures for 10, 15 and 20 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Estimated Peak Temp °C</th>
<th>% germ.</th>
<th>Shoulder Lower °C</th>
<th>Shoulder Upper °C</th>
<th>'Breadth' °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 days</td>
<td>6.5</td>
<td>29.2</td>
<td>3.8</td>
<td>8.8</td>
<td>5.0</td>
</tr>
<tr>
<td>15 days</td>
<td>5.2</td>
<td>39.6</td>
<td>2.5</td>
<td>7.8</td>
<td>5.3</td>
</tr>
<tr>
<td>20 days</td>
<td>3.4</td>
<td>70.3</td>
<td>1.6</td>
<td>5.6</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Shoulder: temperature at 0.75 of peak germination

The analysis of deviance (Table 4.16) confirms that the response to duration of treatment varies with the temperature of treatment and that the effect of duration of treatment may be replaced by a linear function of time in a similar manner to that noted for seed from Waste Point.

The coefficients of the model [Temp*D] indicate that the rate of increase of germination capacity increases with duration of treatment up to a temperature of around 40°C and then declines (Figure 4.14). These coefficients are not as soundly based as those for Waste Point because they were derived from three compared to five durations of treatment, and the temperature control was not as good as in the
previous experiment; however they also indicate that the logarithm of the odds ratio of germination capacity changes in a predictable manner with temperature during the breaking of dormancy.

**TABLE 4.16**

Summary of the analysis of deviance for the germination capacity of seed treated for three durations at a range of temperatures.

<table>
<thead>
<tr>
<th>Model terms</th>
<th>d.f. deviance</th>
<th>RMD</th>
<th>Change d.f. deviance</th>
<th>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>65</td>
<td>702.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temp +Durn</td>
<td>53</td>
<td>185.7</td>
<td>3.50</td>
<td>12</td>
</tr>
<tr>
<td>+Temp .Durn</td>
<td>33</td>
<td>34.03</td>
<td>1.03</td>
<td>20</td>
</tr>
<tr>
<td>Temp *D¹</td>
<td>44</td>
<td>15.69</td>
<td>1.04</td>
<td>11</td>
</tr>
</tbody>
</table>

Factors:
- Temp = Temperature of treatment
- Durn = Duration of treatment

Variable:
- D = Duration of treatment (days)

¹ Full model [Temp*D]=μ+Temp + Temp.D
² Compared to [Temp+Durn+Temp.Durn]
Figure 4.14 The coefficients of the model relating temperature and duration of treatment to germination capacity (G).

Model: Logit(G) = \( \beta_0(T) + \beta_1(T) \cdot D \)
where D is the duration of treatment in days

(iii). Times-to-germination

The analysis was restricted to the first eight temperature levels (1.0°C to 9.4°C) because there were not enough germinated seeds at the higher temperatures to permit a reliable analysis. The interaction between the temperature and the duration of treatment is not significant (Table 4.17, [Temp.Durn] p=0.091) in the times-to-germination of Dicky Cooper seed treated at these temperatures. The treatment constants in Figure 4.15 show that there is a general rise in the times-to-germination with increasing temperature of treatment.

The treatment constants in Figure 4.15 do not show the same trend as Waste Point seed (Figure 4.10), in which the times-to-germination initially increase with increasing temperature and then begin to show...
the development of an optimum at longer durations of treatment. The optimum is not as well developed in this experiment because the experiment did not extend for long enough to permit the full development of the temperature response. The poor temperature control in this experiment may also have dissipated any strongly temperature specific response and caused a mediation of the responses noted in the previous section and considerable divergence between the predicted and observed germination curves.

**TABLE 4.17**

Summary of analysis of deviance for times-to-germination of seed treated for three durations at a range of temperatures.

<table>
<thead>
<tr>
<th>Model terms</th>
<th>Residual d.f.</th>
<th>deviance</th>
<th>Change d.f.</th>
<th>deviance</th>
<th>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>689</td>
<td>2171.0</td>
<td>3.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>672</td>
<td>792.8</td>
<td>1.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Temp +Durn</td>
<td>663</td>
<td>709.6</td>
<td>1.07</td>
<td>9</td>
<td>83.2</td>
</tr>
<tr>
<td>*Temp .Durn</td>
<td>649</td>
<td>688.2</td>
<td>1.06</td>
<td>14</td>
<td>21.4</td>
</tr>
</tbody>
</table>

Time = Day of observation  
Temp = Temperature of treatment  
Durn = Duration of treatment

The analysis of the times-to-germination of each duration separately showed that temperature had no significant effect on the times-to-germination of seed treated for 10 days (p=0.267), was almost significant after 15 days (p=0.011) and became significant after 20 days (p<0.001) of treatment. When the Cox model is fitted to the times-to-germination of seed treated for 20 days alone the treatment constants follow a similar pattern to those in Figure 4.15, when the Cox model is fitted to the three durations of treatment together, so they are not presented. The lack of significance of the temperature
of treatment on times-to-germination after 10 and 15 days of treatment suggests that the response was developing more slowly in seed from Dicky Cooper Ck. than in seed from Waste Point. An experiment of longer duration is required to fully examine the differences between the seed sources.

Figure 4.15 The treatment constants of the Cox model (Table 4.17, [Time*Temp+Burn]) for three durations of treatment at a range of temperatures prior to germination at 15°C.

(iv) Seed mortality

There was no significant effect of temperature or duration of treatment on the number of viable seeds (Table 4.18) and no trend in the mean number of viable seeds with either temperature or duration of treatment.
### TABLE 4.18

Summary of analysis of variance for number of viable seeds.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>d.f</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>Variance Ratio</th>
<th>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp</td>
<td>10</td>
<td>1665.7</td>
<td>166.6</td>
<td>0.953</td>
<td>p=0.501</td>
</tr>
<tr>
<td>Durm</td>
<td>2</td>
<td>305.2</td>
<td>152.6</td>
<td>0.873</td>
<td>p=0.570</td>
</tr>
<tr>
<td>Temp x Durm</td>
<td>20</td>
<td>2737.5</td>
<td>136.9</td>
<td>0.703</td>
<td>p=0.714</td>
</tr>
<tr>
<td>Residu.</td>
<td>33</td>
<td>5770.0</td>
<td>174.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Temp = Temperature of treatment  
Durm = Duration of treatment

Discussion:

The nature of the temperature response of the breaking of dormancy depends on the duration of treatment; short durations of treatment show a reduction in the optimum temperature and a 'broadening' of the response of germination capacity to temperature. Grose noted a similar response in *E. delegatensis* and the response of this species and *E. pauciflora* show a remarkable similarity (Figure 4.16).

The reduction of the germination capacity by treatment at temperatures above 8°C in these studies has occurred at a much lower temperature than in *E. delegatensis* in which Grose (1963) found induction of dormancy at 10°C after 4 weeks of treatment and at 13°C or only 2 weeks. The higher threshold temperature for the induction of dormancy observed by Grose (1963) may also have been due to the higher final germination temperatures (21°C) which he used. The studies reported in Chapter 6 show that seed set to germinate at a temperature of 21°C is more likely to germinate than at 15°C regardless of the amount of stratification the seed has received.
Breaking Dormancy:

The rate at which dormancy was broken in seed from both Waste Point and Dicky Cooper Ck. was such that the factor [Durn] (the duration of stratification) could be replaced by a variable, 'D', the duration of treatment in days, without a significant increase in the residual deviance. The rate of change of germination capacity varied with temperature and the response shows similarities (Figure 4.17) in the seed from the two sources. Similarities in the general form of the relationship would indicate that there is some common factor which underlies the breaking of dormancy in seed from a range of altitudes and causes the logarithm of the odds ratio of germination to vary in a linear fashion with temperature. Further experiments are required to test whether the divergence in the curves at temperatures greater than 5°C is real or an artefact of poor temperature control and a short duration of treatment in the experiment with the seed from Dicky Cooper Ck.

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**Figure 4.16** The temperature response for the breaking of dormancy in *E. delegatensis*. (redrawn from the Grose (1963), Table 3.5)
The curves in Figure 4.17 show that the seed of *E. pauciflora* has a dormancy which may be either broken or induced depending on the temperature of moist treatment to which it is exposed. The extension of these observations beyond the first time that the seed becomes moist have not been attempted. However, experiments in the next chapter show that the dormancy which can be induced in this first encounter is very strong compared to the dormancy of the fresh seed and that it may be broken at temperatures of $5^\circ C$.

![Figure 4.17](image)

**Figure 4.17** A comparison of the coefficients of the model relating temperature and duration of treatment to germination capacity ($g$) for seed from Waste Point and Dicky Cooper Ck.

Model: $\logit(G) = \theta_1(T) + \theta_2(T)D$

where $D$ is the duration of treatment in days.

The response of the times-to-germination to temperature and to duration of moist treatment is very different to the response of germination capacity. The rate of germination increases with increases in the temperature of moist treatment up to a temperature of about $9^\circ C$, and the rate of increase is greatest at those temperatures which are suitable for the breaking of dormancy. The change in the rate of
germination is associated with an earlier day of first germination and a greater rate of germination subsequently. This indicates that the germination of the seeds will become more rapid and synchronized as the dormancy of a sample of seed is broken.
4.3 Conclusions

1. The moisture environment has a facilitatory role in the breaking of dormancy; decreases in the water potential of the water surrounding the seed lead to a slower breaking of dormancy and a slower rate of germination.

2. The water content of those seeds which germinate and those which remain dormant is the same under a range of moisture conditions indicating that seed water content is not related to the chances of germination.

3. The dormancy of the seed is broken at an increasing rate by temperatures from 0°C to around 5°C. Dormancy is induced in seed exposed to constant temperatures greater than around 9°C.

4. The dormancy of seed from higher altitudes is stronger than the dormancy of seed from lower altitudes.

5. The times-to-germination decrease with increasing temperature of stratification: the rate at which the times-to-germination decrease is greatest at temperatures around 5°C.
4.4 Summary of methods and results

A. Section 4.1.1
B. Section 4.2.1
C. Section 4.2.2.1
D. Section 4.2.2.2