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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 6

TEMPERATURE OF GERMINATION
CHAPTER 6

Temperature has been shown to be an important factor in determining whether the dormancy of Eucalyptus pauciflora seed is broken (Chapter 4) or induced (Chapter 5). This chapter describes an investigation of the influence of temperature on the germination of E. pauciflora seed.

The first section of the chapter examines the changes in the temperature response of germination as the dormancy of the seed sample is broken by stratification. The second section then examines the germination responses of seed collected at 960 m and 1910 m to temperature. The methods and results are summarized on the yellow page at the end of the chapter.
6.1 Germination of seed stratified for different periods of time.

Stratification might be expected to increase both the maximum germination capacity of the seed and the range of temperatures which are suitable for germination (Grose 1963). This section examines the changes in the temperature response of germination of seed with different histories of stratification.

Methods:

Cleaned seed (0.4 g, 80.7 viable seeds) collected at Rennix Gap (1610 m) was stratified for either 0, 10 or 20 days (seed source and stratification treatments were identical to those in Section 5.2). Following stratification two replicates of each stratification treatment were placed at each of 12 temperature levels on a gradient plate. Seeds which germinated were counted and removed from the gradient plate at daily intervals.

The seed which had not germinated after 16 days on the gradient plate was then placed in petri dishes in an incubator at 15°C. Germination was monitored every day for a further 24 days.

The temperature conditions on the gradient plate were monitored, at 30-minute intervals, using a Campbell Scientific CR21 data-logger to record the temperature of seven thermocouples, placed to span the gradient.

A diagrammatic summary of the methods and results is presented in Section 6.4 (yellow sheet) at the end of the chapter.
Results:

(1) Temperature conditions

Problems with the temperature control of the gradient plate resulted in a stepped rise in the temperatures on the gradient plate over the period of the experiment. The temperature at all levels on the gradient plate increased on the seventh and fourteenth days; however, despite the increases, the relative differences between the temperatures at various levels on the gradient remained substantially the same (Table 6.1).

<table>
<thead>
<tr>
<th>TEMPERATURE REGIME</th>
<th>TEMPERATURE FOR PERIOD</th>
<th>GERMINATION PERIOD(2) (DAYS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FIRST AND LAST DAYS</td>
<td>STRATIFICATION (DAYS)</td>
</tr>
<tr>
<td></td>
<td>1-6 7-13 14-16 Mean(^1)</td>
<td>0 10 20</td>
</tr>
<tr>
<td>1</td>
<td>3.4 5.9 6.5 5.1</td>
<td>16 16 16</td>
</tr>
<tr>
<td>2</td>
<td>5.2 7.6 8.3 6.8</td>
<td>16 16 16</td>
</tr>
<tr>
<td>3</td>
<td>7.6 9.8 10.7 9.1</td>
<td>16 16 16</td>
</tr>
<tr>
<td>4</td>
<td>10.0 12.1 13.1 11.5</td>
<td>16 16 16</td>
</tr>
<tr>
<td>5</td>
<td>12.3 14.3 15.5 13.8</td>
<td>16 16 12</td>
</tr>
<tr>
<td>6</td>
<td>14.7 16.5 17.9 16.1</td>
<td>13 10 10</td>
</tr>
<tr>
<td>7</td>
<td>17.7 19.4 20.9 19.0</td>
<td>11 8 11</td>
</tr>
<tr>
<td>8</td>
<td>20.1 21.6 23.4 21.3</td>
<td>8 7 9</td>
</tr>
<tr>
<td>9</td>
<td>22.5 23.9 25.8 23.7</td>
<td>6 9 7</td>
</tr>
<tr>
<td>10</td>
<td>24.9 26.1 28.2 26.0</td>
<td>16 8 7</td>
</tr>
<tr>
<td>11</td>
<td>27.8 28.9 31.1 28.9</td>
<td>16 16 16</td>
</tr>
<tr>
<td>12</td>
<td>30.2 31.1 33.5 31.2</td>
<td>16 16 16</td>
</tr>
</tbody>
</table>

\(^1\) Mean temperature over the 16 days of treatment

\(^2\) Period from the beginning of the time on the gradient plate to the cessation of the germination pulse in days.

The estimation of the temperature of treatment of seed placed on
the gradient plate was made difficult by the rises in temperature which occurred. Two solutions were employed depending on whether the temperature of treatment was to be used for estimating the temperature response of germination on the gradient plate or for the effect of the treatment on the gradient plate on the subsequent germination at 15°C.

The rise in temperature during the experiment means that, in those regimes in which the germination period was shorter than 16 days, the temperature during germination would have been lower than the mean temperature over the whole time on the gradient plate (Table 6.1). Therefore, the temperature of germination on the gradient plate was estimated by calculating the mean temperature over the germination period in a particular treatment.

The seed which did not germinate on the gradient plate had been exposed to the full 16 days of treatment and the mean temperature for the temperature regime is used in the presentation of the incremental germination capacity after transfer to the incubator and the total germination capacity of the seed (Table 6.1).

(ii) Germination capacity of seed on the gradient plate.

The use of the mean temperature over the germination period as the basis for the comparison of the temperature responses is vindicated by the general agreement between the germination capacities of seed on the gradient plate, and seed from the same source, with the same history of stratification, placed at constant temperatures in incubators (14°C, 24°C Section 5.2) (Figure 6.1).

The differences in the temperature responses shown in Figure 6.1
Temperature of germination:

![Graph showing temperature of germination](image)

Figure 6.1  The predicted germination capacity (symbols) of seed stratified for 0, 10, 20 days and germinated in different temperature regimes. Temperature is taken as the mean temperature over the germination period (Table 6.1). Arrows show predicted germination capacities of seed from the studies of Section 5.2 and refer to seed stratified for various periods and then set to germinate at either 14°C or 24°C.

- a. 0 days at 5°C, 14°C
- b. 10 days at 5°C, 14°C
- c. 20 days at 5°C, 14°C
- d. 0 days at 5°C, 24°C
- e. 10 days at 5°C, 24°C
- f. 20 days at 5°C, 24°C

were quantified using the characteristics of the cubic spline curves fitted to the temperature responses which showed that as the duration of stratification increased, the breadth of the temperature response of germination capacity increased markedly (Table 6.2).

The analysis of germination capacity shows a significant interaction between temperature and stratification (Table 6.3), suggesting that the response to temperature is different for seed stratified for increasing periods. The significance of the interaction may, however, be the result of changes in the temperature environment during the experiment, since the seed which was stratified for longer periods germinated more rapidly, and would have been exposed to lower

TABLE 6.2

The characteristics of the cubic splines fitted to the germination capacity of seed stratified for 0, 10, 20 days and germinated at a range of temperatures.

<table>
<thead>
<tr>
<th>Duration of Stratification</th>
<th>Estimated Peak Temp °C</th>
<th>% germ.</th>
<th>Shoulder Lower</th>
<th>Upper 'Breadth'</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 days</td>
<td>18.2</td>
<td>55.1</td>
<td>15.9</td>
<td>20.5</td>
</tr>
<tr>
<td>10 days</td>
<td>19.3</td>
<td>98.7</td>
<td>16.4</td>
<td>22.7</td>
</tr>
<tr>
<td>20 days</td>
<td>19.5</td>
<td>92.7</td>
<td>13.4</td>
<td>24.2</td>
</tr>
</tbody>
</table>

Splines based on mean temperature during germination period
Shoulder: temperature at 0.75 of peak germination
'Breadth': Temperature of upper shoulder-lower shoulder
temperatures than the seed which germinated more slowly (Table 6.1).
This possibility will be considered more fully in the discussion.

TABLE 6.3

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

<table>
<thead>
<tr>
<th>Model terms</th>
<th>d.f. deviance</th>
<th>RMD</th>
<th>d.f. deviance</th>
<th>CMD</th>
<th>Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>71</td>
<td>3799.00</td>
<td>53.51</td>
<td>1</td>
<td>3511.00</td>
<td>270.08</td>
</tr>
<tr>
<td>Temp +Strat</td>
<td>58</td>
<td>288.00</td>
<td>4.97</td>
<td>13</td>
<td>3511.00</td>
<td>270.08</td>
</tr>
<tr>
<td>+Temp .Strat</td>
<td>36</td>
<td>98.38</td>
<td>2.73</td>
<td>22</td>
<td>189.62</td>
<td>8.62</td>
</tr>
</tbody>
</table>

Temp = Temperature regime on gradient plate (Table 6.1)
Strat = Duration of stratification (0, 10, 20 days)
Temperature of germination:

(iii) Times-to-germination of seed on the gradient plate

The analysis of times-to-germination was restricted to temperature levels six to eight (ca. 16-21°C) in seed which was not stratified and temperature levels five to ten (ca. 14-26°C) in the seed stratified for 10 and 20 days due to the small number of germinated seeds in the other treatments. The response of unstratified seed, over this restricted range of temperatures (ca. 16-21°C), showed similar trends to seed which had been stratified for either 10 or 20 days, therefore, only the latter analysis is discussed here.

**TABLE 6.4**

Summary of analysis of deviance for the times-to-germination of seed stratified for 10 or 20 days and then set to germinate at a range of temperatures on the gradient plate.

<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>265</td>
<td>2741</td>
<td>10.3^4</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>252</td>
<td>851.4</td>
<td>3.38</td>
<td>7 1889.6  p&lt;0.001</td>
</tr>
<tr>
<td>+Temp +Strat</td>
<td>246</td>
<td>388.6</td>
<td>1.58</td>
<td>6 462.8  p&lt;0.001</td>
</tr>
<tr>
<td>+Temp .Strat</td>
<td>241</td>
<td>372.2</td>
<td>1.54</td>
<td>5 16.4  p=0.006</td>
</tr>
</tbody>
</table>

Temp = Temperature at which seed was germinated  
Strat = Duration of stratification (10, 20 days)

The response of the times-to-germination to temperature was different in seed stratified for either 10 or 20 days (Temp.Strat, p=0.006, Table 6.4). The divergence in the times-to-germination of seed stratified for 10 and 20 days at low temperatures results from a slower rate of germination of seed stratified for 10 days compared to that stratified for 20 days (Figure 6.2).

Small fluctuations in the temperature environment may have had an
Figure 6.2 The treatment constants of the Cox model for seed stratified for 10 days or for 20 days and then set to germinate at a range of temperatures.

Important influence on the differences observed in the rate of germination. For example, the observed germination curves of seed stratified for either 10 or 20 days and then exposed to temperature regime 5 (mean 13.8°C) were similar until the seventh day, when the temperature changed from 12.3°C to 14.3°C (Figure 6.3, open diamonds). Following the increase of ca. 2°C in temperature on the seventh day the seed stratified for 20 days had an increased rate of germination compared to seed stratified for only 10 days. The increase in the rate of germination of the seed stratified for 20 days when the temperature increased on the seventh day may underlie the differences in the rates of germination at low temperatures noted in Figure 6.2 and the interaction between duration of stratification and temperature noted in Table 6.4.

(iv) Incremental germination capacity after transfer to 15°C.

The degree of dormancy of the viable seed which did not germinate
The cumulative germination curves of seed stratified for 10 days (closed symbols, ----) and 20 days (open symbols, ----) and then set to germinate at a range of temperatures.

The incremental germination capacity after transfer to near optimal conditions for germination (15°C). The incremental germination capacity is defined as the number of seeds which germinated after transfer to the incubator at 15°C as a proportion of the number of viable seeds. The number of viable seeds at the time of transfer of the seed from the gradient plate to the incubator was calculated as the sum of the seeds which germinated in the incubator and those judged to be viable by scanning at the end of the experiment. The method of determining the number of viable seeds excludes those seeds which, though viable at the time of transfer, died prior to germination or squashing. The changes in the dormancy of the seed as a result of exposure to the temperatures on the gradient plate are discussed in Section 6.1(v).

The incremental germination capacity (Figure 6.4, Table 6.4) shows a marked dependence on the temperature of treatment on the gradient
The incremental germination capacity of seed transferred from the gradient plate to an incubator at 15°C.

The response may be divided into three sections based on the temperature of treatment: a high but declining incremental germination capacity as the temperature of treatment rises from 5°C to 14°C, a low incremental germination capacity between 14°C and 25°C, and an increasing incremental germination capacity from 25°C to 32°C.

**TABLE 6.4**

Summary of analysis of deviance for incremental germination following transfer to an incubator 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>71 2011</td>
<td>28.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temp +Strat</td>
<td>58 309.9</td>
<td>5.34</td>
<td>13 1701.1</td>
<td>p&lt;0.005</td>
</tr>
<tr>
<td>+Temp .Strat</td>
<td>36 48.08</td>
<td>1.34</td>
<td>22 261.2</td>
<td>p&lt;0.005</td>
</tr>
</tbody>
</table>

Temp = Temperature regime (Table 6.1)
Strat = Stratification period, 10, 20 days

The low levels of incremental germination capacity show that seed
Temperature of germination: 215.

which was exposed to temperature regimes five to ten (13.8°C to 26°C) on the gradient plate was mostly dormant at 15°C (Figure 6.4). These observations support those in Chapter 5 which showed that seed which did not germinate at temperatures of 14°C and 24°C became strongly dormant.

Decreases in the incremental germination capacity from temperature regimes one to five (5.1°C to 13.8°C) show that the proportion of dormant seed increased as the temperature of treatment on the gradient plate increased. Seed which was not dormant at 15°C after treatment on the gradient plate may have been non-dormant when it was placed on the gradient plate. Alternatively dormancy may have been broken during treatment on the plate. Changes in the dormancy of the seed during treatment on the gradient plate may be examined by considering the total germination capacity of the seed.

(v) The total germination capacity

The germination capacity taken over both the period on the gradient plate and the 24 days at 15°C can be used to assess the changes in the dormancy of the seed on the gradient plate. The limited duration of exposure (16 days) to temperatures on the gradient plate may have biased the observed germination capacity against samples exposed to low temperatures due to their slow rate of germination. Whilst the only way to test whether these samples will eventually germinate would be to extend the period of exposure on the gradient plate, some indication of the influence of low temperatures may be gained from a consideration of the subsequent germination of the seed when it is maintained at 15°C.
Temperature of germination: 216.

In order to determine whether there were changes in the dormancy of samples, a comparison was made between the total germination capacities of the seed and the germination capacity of a seed sample which was not placed on the gradient plate but was placed in an incubator at 15°C immediately after stratification (Section 5.2).

Seed exposed to the two lowest temperature regimes (mean temperatures 5.1°C, 6.8°C) showed an increased germination capacity at 15°C compared to that of the reference which indicates that treatment at these temperatures was successful in breaking dormancy, regardless of the duration of stratification (Figure 6.5). Regime four (mean temperature 11.5°C) resulted in an induction of dormancy in all stratification treatments since a portion of the seed population, which was able to germinate at 15°C prior to exposure on the gradient plate, did not germinate following exposure. The results with temperature regime three (mean temperature 9.1°C) are more equivocal in that dormancy was induced in seed with 0 and 10 days stratification and was broken in seed stratified for 20 days. The changes in dormancy in temperature regimes three and four is consistent with observations of the temperature response of breaking dormancy (Section 4.3) in which dormancy was broken at temperatures below around 9°C and induced at temperatures above 10°C.

The increase in the germination capacity of seed transferred from above 25°C to 15°C indicates that some seeds were exhibiting enforced dormancy at higher temperatures. The lower germination capacity of seed stratified for 10 days and 20 days, compared to the benchmark, suggests that dormancy had also been induced in some seeds. However, closer examination of the total germination capacity at the highest temperature shows that temperature regime 12 (ca. 31°C) broke dormancy.
Temperature of germination: 216.

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The increase in the germination capacity of seed transferred from above 26°C to 15°C indicates that some seeds were exhibiting enforced dormancy at higher temperatures. The lower germination capacity of seed stratified for 10 days and 20 days, compared to the benchmark, suggests that dormancy had also been induced in some seeds. However, closer examination of the total germination capacity at the highest temperature shows that temperature regime 12 (ca. 31°C) broke dormancy
Temperature of germination:

in the seed which had not been stratified whilst it induced it in seed which was stratified.

![Graphs showing germination capacity at different temperatures.](image)

Figure 6.5: The proportion of germinated seed at the end of the period on the gradient plate (solid lines) and after a further 23 days at 15°C (broken lines) for seed stratified for either (a) 0 days (b) 10 days or (c) 20 days. Arrows indicate the germination capacity of the seed placed in an incubator (Section 5.2) at 15°C directly after stratification.
(vi) Seed mortality.

Differences in the number of viable seeds between treatments gives an indication of the relative mortality of the seed exposed to the treatments. An analysis of variance showed that there was no effect of temperature of treatment on seed mortality but that stratification had a significant effect on the number of viable seeds (Table 6.5).

**TABLE 6.5**

Summary of analysis of variance for the 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>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp</td>
<td>11</td>
<td>1926.7</td>
<td>175.2</td>
<td>1.453</td>
</tr>
<tr>
<td>Strat</td>
<td>2</td>
<td>2647.0</td>
<td>1323.5</td>
<td>10.976</td>
</tr>
<tr>
<td>Temp x Strat</td>
<td>22</td>
<td>2875.3</td>
<td>130.7</td>
<td>1.084</td>
</tr>
<tr>
<td>Residual</td>
<td>36</td>
<td>4341.0</td>
<td>120.6</td>
<td></td>
</tr>
</tbody>
</table>

Temp = Temperature on gradient plate  
Strat = Duration of stratification (0, 10, 20 days)

**TABLE 6.6**

The mean number of seeds judged to be viable for the stratification treatments.

<table>
<thead>
<tr>
<th>Stratification (days)</th>
<th>0</th>
<th>10</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viable seeds¹</td>
<td>64.5</td>
<td>75.2</td>
<td>78.7</td>
</tr>
</tbody>
</table>

¹ LSD α₀.₀₅ = 8.64

The increase in the number of viable seeds with increasing duration of stratification supports the observations in Section 5.2. It
also shows that it is likely that there are some seeds which die if they fail to germinate during their first exposure to conditions which permit germination in the non-dormant seeds. The large difference between the number of viable seeds in unstratified and stratified treatments suggests that the risk of mortality is substantially alleviated by short (10 days) periods of stratification.

Discussion:

The germination capacity of *E. pauciflora* is dependent on the temperature of germination and the duration of stratification. An important aspect of the change in the temperature response with stratification is the increasing suitability of low temperatures for germination; such a response could be expected to bring the conditions which are suitable for germination closer to those for stratification as the winter progresses.

Environmental conditions in autumn and spring are likely to have markedly different effects on the germination of the seed because of the change in the temperature response of the seed following long periods of stratification. During autumn, when the period of exposure to conditions which break dormancy is likely to be short (short period of stratification) the temperature response will tend to limit germination to very specific conditions. Seed which does not germinate will either become more strongly dormant or dormancy may be broken depending on the temperatures to which it is exposed (Section 7.1). In spring, seed which has stratified over winter will have the capacity to germinate at a wide range of temperatures and most importantly at low temperatures, at a rate which increases with temperature.
Temperature of germination: 220.

The observed response to temperature of *E. pauciflora* seed is in broad agreement with that observed for *E. delegatensis* seed by Grose (1963). He showed that as the duration of stratification was increased, both the range of temperature suitable for germination and the rate of germination were increased. Grose also observed the temperature response of *E. delegatensis* seed which had received long periods of stratification. The response of germination capacity to the duration of stratification was more fully developed at low temperatures in his studies than it was in the studies with *E. pauciflora* reported in this section (Figure 6.6). For example, Grose's observations show that the germination capacity of *E. delegatensis* seed at 10°C increases as the duration of stratification increased from 14 days to 56 days. Germination at 10°C was only observed in *E. pauciflora* seed which had received 20 days stratification at 5°C. The germination capacity of *E. pauciflora* seed might, therefore, be expected to increase further if the duration of stratification was increased beyond 20 days.

The relevance of the response of germination to low temperatures to the conditions during germination in the field is made abundantly clear in the studies of germination in Section 7.1. Several attempts were made to investigate the germination of *E. pauciflora* seed at low temperatures in the laboratory but the temperature control and reliability of refrigerators and the gradient plate over periods required to elicit the response (3 months) were not sufficient to permit detailed studies of this kind.

The influence of increases in temperature on the gradient plate has been of major concern in the interpretation of this experiment. If the seed from all stratification treatments had responded in the same way to the change in temperature then the problem would only be one of
precision of the estimate of the temperature response. However, the response of seed to the change in temperature varied depending on the amount of stratification which it had received. Consideration of the changes in the germination curves has shown that seed stratified for 20 days responded to the change in temperature on the seventh day whereas seed stratified for 10 days did not (Figure 6.3).

![Germination Capacity Chart](chart)

Figure 6.6 The germination capacity of *E. delegatensis* seed stratified for 0, 2, 4, 6, 8 weeks at 5°C and then set to germinate at a range of temperatures. Redrawn from Grose (1963), Table 3:1.

The effect of diurnal fluctuations in temperature were not investigated in this study; however, the lower germination capacity noted by Grose (1963) for *E. delegatensis* seed in oscillating temperature environments (27/5°C, 32/7°C) suggests that the periods of stratification required to permit germination would be even longer than at constant temperatures. The observed increases in the range of temperatures which are suitable for germination as the period of stratification is increased support these conclusions.
6.2 Temperature response of germination of seed from two altitudes

This experiment tests whether there is a difference in the response of germination to temperature of seed from two populations of E. pauciflora growing at 960 m and 1910 m respectively.

Methods:

Replicates of seed from Waste Point (960 m, 0.5 g, 30.3 viable seeds) and Baker's Creek (1910 m, 0.15 g, 50.4 viable seeds) were stratified for 33 days at 5°C. Following stratification two replicates of seed from each source were placed at each of seventeen temperature levels on the gradient plate.

The temperature gradient was monitored by daily readings of thermocouples placed to span the gradient. Seeds which germinated were counted and removed from the plate at daily intervals for the first 15 days and then less frequently. After 40 days on the gradient plate, seed from the five lowest temperature levels was placed in petri dishes and moved to an incubator at 15°C and germination was observed for a further 20 days.

Results:

(1) Temperature conditions

The estimated temperatures for the seed positioned on the gradient plate were 3.6, 5.2, 7.0, 8.7, 10.4, 12.1, 13.9, 15.6, 17.4, 19.8, 21.6, 23.5, 25.3, 27.2, 29.9, 31.0, 32.9°C.
(ii) Germination capacity on the gradient plate

Seed at the three highest temperatures was found to have rotted within 5 days of being placed on the gradient plate and as a result these treatments were excluded from the analyses. The germination capacity of seed on the gradient plate showed a response to temperature which is similar to that noted in Section 6.1, except that the optimum temperature was lower and the germination capacity was greater at lower temperatures (compare Figure 6.7 with Figure 6.1).

![Graph showing germination capacity percentage vs. temperature](image)

**Figure 6.7** The germination capacity of Waste Point and Baker's Creek seed stratified for 33 days and germinated at a range of temperatures (lines are cubic splines fitted to the observed (symbols) data).

Seed from Baker's Ck. (910 m) had a lower germination capacity at all temperatures compared to seed from Waste Point (960 m); this observation is consistent with those in Section 4.2.1 which showed that the germination capacity of seed from Waste Point was greater than seed from Baker's Ck after stratification for 20 days. The cubic splines (Table 6.7, Figure 6.7) show that the peak temperature and the
'breath' of the temperature response were also greater for Waste Point seed.

### TABLE 6.7
Summary of analysis of deviance for the germination capacities of two seed sources germinated at a range of temperatures.

<table>
<thead>
<tr>
<th>Model terms</th>
<th>Residual d.f. deviance</th>
<th>RMD</th>
<th>Change d.f. deviance</th>
<th>CMD</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>55</td>
<td>1037.0</td>
<td>18.85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source +Temp</td>
<td>41</td>
<td>98.7</td>
<td>2.41</td>
<td>14</td>
<td>938.28</td>
<td>67.02</td>
</tr>
<tr>
<td>-Temp</td>
<td>54</td>
<td>788.5</td>
<td>14.60</td>
<td>13</td>
<td>689.90</td>
<td>53.07</td>
</tr>
<tr>
<td>Source .Temp</td>
<td>28</td>
<td>53.18</td>
<td>1.90</td>
<td>13</td>
<td>45.54</td>
<td>3.50</td>
</tr>
</tbody>
</table>

Source = Seed source (Waste Point, Baker's Creek)
Temp = Temperature of germination

### TABLE 6.8
The characteristics of the cubic splines fitted to the response of germination capacity to temperature for Waste Point and Baker's Creek seed after 16 and 40 days on the gradient plate.

<table>
<thead>
<tr>
<th>Seed Source</th>
<th>Estimated Peak</th>
<th>Shoulder</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temp °C</td>
<td>% germ.</td>
</tr>
<tr>
<td>Waste Point</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 days</td>
<td>16.6</td>
<td>81.5</td>
</tr>
<tr>
<td>40 days</td>
<td>17.2</td>
<td>84.7</td>
</tr>
<tr>
<td>Baker's Creek</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 days</td>
<td>16.0</td>
<td>58.0</td>
</tr>
<tr>
<td>40 days</td>
<td>14.9</td>
<td>57.0</td>
</tr>
</tbody>
</table>

Shoulder: temperature at 0.75 of peak germination

Comparison of the characteristics of the response of germination capacity to temperature of germination shows that the estimated
Temperature of germination: 225.

temperature at the peak germination capacity was low in the present experiment (Table 6.8) compared to the previous experiment (see Table 6.2). The longer period of germination (40 days compared to 16 days) in this experiment led to an increase in the proportion of seeds which germinated at the lower temperatures, particularly in the seed from Waste Point (Figure 6.8) and this may have resulted in the observed reduction in the temperature response. This hypothesis was tested by comparing cubic splines fitted to the data based on the germination capacity after the first 16 days on the gradient plate with those fitted to the data from the longer period (Table 6.8).

The cubic splines fitted to the response of germination capacity to temperature after 16 days on the gradient plate (Table 6.8) show that increasing the length of the germination period from 16 to 40 days does not account for the lower temperature at the peak germination observed in this experiment compared to that in Section 6.1.

(iii) Incremental germination capacity after transfer to 15°C.

The transfer of ungerminated seed from the five lowest temperatures to 15°C after 40 days resulted in the germination of more seed. The incremental germination capacity is shown in Figure 6.9 and the analysis indicates that the seed from the two seed sources behaved differently (Table 6.9, Temp. Source, p<0.001).

The seed incubated at 15°C following 40 days on the gradient plate showed a decrease in incremental germination with increasing temperature which was similar to that noted in Section 6.1 (iv) (compare Figure 6.4 and Figure 6.9). In particular the incremental germination capacity of seed which was less dormant when it was placed
Figure 6.8 The germination capacity of Waste Point (top) and Baker's Ck. seed after 16 days (solid lines) or 40 days (dashed lines) on the gradient plate.

on the gradient plate (Section 6.1, seed stratified for 20 days, Section 6.2, seed from Waste Point) was higher than that of the seed with a greater degree of dormancy. The relative importance of changes in dormancy on the gradient plate and the dormancy of the seed when it was placed on the gradient plate can be assessed by consideration of the total germination capacity.
Temperature of germination: 227.

![Graph showing germination capacity vs temperature.]

Figure 6.9  The incremental germination capacity of seed collected from two sources, stratified for 40 days, treated on the gradient plate at a range of temperatures for 40 days and then transferred to an incubator at 15°C.

### Table 6.9

Summary of analysis of deviance for the incremental germination after transfer of seed from the gradient plate to 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>19</td>
<td>496.0</td>
<td>26.11</td>
<td></td>
</tr>
<tr>
<td>Temp +Source</td>
<td>14</td>
<td>73.92</td>
<td>5.28</td>
<td>422.08</td>
</tr>
<tr>
<td>+Temp .Source</td>
<td>10</td>
<td>11.97</td>
<td>1.20</td>
<td>61.95</td>
</tr>
</tbody>
</table>

Temp = Gradient plate temperature
Source = Seed source (Waste Point, Baker's Creek)

(iv) The total germination capacity

The changes in the dormancy of the seed as a result of treatment on the gradient plate were assessed from a consideration of the total germination capacity over the whole experiment in relation to the germination capacity of the stratified seed placed at 15°C directly after stratification. The temperature response depicted in Figure 6.10.
shows that the seed from Waste Point had a high germination capacity at a temperature of 15°C when it was placed on the gradient plate (shown by the arrow). The total germination capacity of the seed from the five lowest temperature levels suggests that treatment at temperatures below 10°C on the gradient plate resulted in the breaking of dormancy.

Figure 6.10 The total germination capacity of Waste Point (top) and Baker’s Ck. seed in relation to the germination capacity on the gradient plate.

The seed from Baker’s Creek had a lower germination capacity than seed from Waste Point, when it was placed on the gradient plate at a
Temperature of germination: 229.

temperature of 15°C. The sharp rise in the total germination capacity of the seed from Baker's Ck. as the temperature of treatment on the gradient plate declined shows that dormancy of the seed from Baker's Ck. was broken at temperatures below 8°C on the gradient plate. This response is consistent with the observations of the changes in dormancy in Sections 6.1 and 4.2.1.

(v) Seed mortality

The mortality of all of the seeds placed at the three highest temperatures (29.9°C, 31.0°C, 32.9°C) may not have been due to the direct effect of the temperature conditions on the seed since there was also a prolific growth of a laboratory mould at those temperatures. If temperature was the primary cause of the mortality of the seed then some evidence of mortality at temperatures approaching 29.9°C may be expected. However, there is no evidence of differential mortality of seed set to germinate at a range of temperatures (3.6°C to 27.2°C) since analysis of variance showed that there were no significant differences in the numbers of viable seed in samples of seed exposed to that range of temperatures (Waste Point, p=0.87; Baker's Ck., p=0.389).
Temperature of germination:

Discussion:

Seed from Waste Point (960 m) and Baker's Ck. (1910 m) which had been stratified for the same period showed differences in the temperature response to germination (Figure 6.7). The broader response of germination capacity to temperature in seed from Waste Point may reflect a real difference in the response of seed germination to temperature or may be a consequence of the weaker dormancy exhibited by the seed from Waste Point compared to the seed from Baker's Ck.

The experiments reported in Section 4.2.1 showed that, after stratification for 20 days, the germination capacity of seed from Waste Point was greater than the germination capacity of seed from Baker's Ck. (Figure 4.4). The stronger dormancy of seed from Baker's Ck. was also apparent in the present experiment since the germination capacity of this seed was lower than that of the seed from Waste Point after stratification for 33 days, regardless of the temperature of germination (Figure 6.7). The following discussion compares the trends in the response of germination capacity to temperature as dormancy was broken by stratification with the differences in the response of the germination capacity to temperature in seed from Waste Point and Baker's Ck..

The differences in the temperature responses of seed from Waste Point and Baker's Ck. are consistent with the differences expected in seed samples with different strengths of dormancy. As the dormancy of seed from Rennix Gap (1610 m) was broken the temperature of maximum germination remained reasonably constant and the breadth of the response increased (Figure 6.1, Table 6.2); this change in response was associated with an increase in the range of temperatures which were
Temperature of germination: 231.

suitable for germination, both above and below the temperature at which maximum germination capacity was observed. The greater germination capacity and broader temperature response of the seed from Waste Point which has a weaker dormancy than the seed from Baker's Ck. (Section 4.2.1) is consistent with the trend as the strength of dormancy is reduced by stratification in the seed from Rennix Gap. Further work examining the temperature response of seed from a range of altitudes after a range of stratification times would be required to determine whether the degree of dormancy is the sole influence on the form of the temperature response of seed from different altitudes.

The conditions in the field at Rennix Gap broke dormancy to the extent that a large proportion of the seed sample germinated in spring when the soil temperatures are low (Section 6.1). The germination of seed at low temperatures means that the strength of the dormancy of the seed will be critical in the timing of germination since the processes of germination will begin as soon as the dormancy of the seed is broken.

As the dormancy of the seed becomes weaker compared to the capacity of the environment to break dormancy the proportion of the seed sample which germinates in winter is likely to increase and the risk of post-germination mortality due to frost and mechanical damage by needle ice is also likely to increase. As the dormancy of the seed becomes stronger compared to the capacity of the environment to break dormancy the germination of the seed will be delayed and a proportion of the seed may not break dormancy before the temperature environment becomes unsuitable for either the breaking of dormancy or germination. The delay in the germination of the seed may also increase the risk of mortality due to exposure of the preemergent and newly emergent
Temperature of germination: 232.

seedling to the hotter and drier conditions at the soil surface later in spring. Increases in the strength of dormancy of seed with altitude are a critical element in the interpretation of the reciprocal transplant experiments reported in Section 7.1.

With the exception of reports concerning temperature optima for germination (Boland et al. 1980, Green 1969b) there has been little published work on the temperature response of germination of *E. pauciflora* from different altitudes. Green (1969b) reported an investigation of the temperature response of germination of unstratified seed collected at a range of altitudes. Unfortunately he presented the results as the mean number of seeds which germinated in three replicates of 0.2 g from each seed source; this had the effect of confounding the number of viable seeds and the germination capacity of the seed samples.

Boland et al. (1980) recommend two temperatures for the germination of *E. pauciflora* (E. pauciflora ssp. pauciflora 15°C and *E. pauciflora* ssp. niphophila 20°C). The studies in this section do not support a higher optimum temperature for seed from higher altitude (*E. pauciflora* ssp. niphophila) and provide evidence that the optimum temperature of this seed may be slightly lower (15°C) than seed from lower altitudes (17°C). The difficulties in estimating the optimum temperature in seed with different strengths of dormancy is apparent from these studies since it appears that seed from the higher altitudes is likely to have a much more peaked temperature response than seed from lower altitudes after the same duration of stratification. The difference in the breadth of the response would lead to different sensitivities to the temperature at which germination was assessed particularly if the tests were carried out at a limited number of
Temperature of germination:

temperatures.
6.3 Conclusions

1. The temperature of germination influences both the germination capacity and the rate of germination of *E. pauciflora* seed (Figure 6.1, Figure 6.2).

2. As dormancy is broken by stratification the maximum germination capacity increases and the range of temperatures which will sustain germination increases (Figure 6.1).

3. The change in the temperature response of seed with increasing altitudes may be explained by increases in the strength of dormancy. The increase in the strength of dormancy with altitude means that a given period of stratification will result in a lower maximum germination capacity and a narrower temperature response in seed from higher altitudes.
6.4 Summary of methods and results

A. Section 6.1
B. Section 6.2