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

by

Douglas Graham Abrecht

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

February 1985
DECLARATION

The work presented in this thesis is my own. Specific contributions by others are acknowledged in the text and acknowledgements.

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ABSTRACT

_Eucalyptus pauciflora_ Sieb. ex Spreng. occurs over a wide range of altitudes from sea-level to treeline, but the most common occurrence of the species is in the high altitude environments of the Great Dividing Range in eastern Australia. There are gradual changes in the morphology and physiology of the species with increasing altitude. This study examined variation in the regeneration of _Eucalyptus pauciflora_ from seed in populations from a range of sub-alpine environments between 960 m and 1900 m in the Snowy Mountains of New South Wales. The influence of climatic conditions, particularly temperature, on the seasonal timing of germination was investigated using observations of dormancy, germination and emergence from seed in the field, and laboratory manipulations of dormancy.

The dormancy of _E. pauciflora_ seed varies with the origin and size of the seed. Seed from different altitudes has a similar degree of dormancy (i.e. proportion of dormant seed), with the exception of seed from treeline populations (1900 m) which has a greater degree of dormancy. The strength of dormancy increases with altitude of seed source and decreases with seed size within a population of seed. The increases in the strength of dormancy with altitude are not explained by the reduction in seed size with increasing altitude since the dormancy of seed of the same size also increases in strength with altitude of seed source.

Seasonal conditions during seed shedding are unlikely to have a large influence on the dormancy of the seed. However, exposure of capsules to temperatures of 80°C during seed shedding resulted in marked increases in the strength of dormancy of the seed. This indicates that there is a chance that the dormancy of seed shed following a fire may be greater than that of seed shed under normal conditions.

Moist seed may respond to the temperature environment in three ways; changes in dormancy, germination or death. Dormancy is broken at constant temperatures below 6°C and strengthened at temperatures above 9°C. Temperatures between 6°C and 9°C tend to break the dormancy of a portion of the seed sample and increase the strength of dormancy for the rest. The rate at which dormancy is broken declines rapidly as the availability of water declines from a water potential of -4 bars to -10 bars. The temperature at which the rate of breaking dormancy is highest (5.5°C) is similar in seed from altitudes above 1280 m. However, there is some indication that this temperature may increase in seed from lower altitudes.

As the dormancy of the seed is broken, the range of temperatures which is suitable for the germination of seed increases and the germination capacity at any temperature within the range also increases. The rate of germination shows similar increases to the breaking of dormancy. The differences in the temperature response of seed from different altitudes could be wholly attributed to differences in the strength of dormancy of the seed.

In a field test of laboratory results seed was planted in the field at 1610 m and dormancy and germination were monitored. Moist conditions and high temperatures induced dormancy in the seed in within
a week of planting and prevented the germination of a large proportion (98%) of the seed sample until late winter. Low soil temperatures and moist soil conditions led to a progressive reduction in the strength of dormancy in late autumn. Germination occurred when the soil temperatures were low (mean < 5°C) and took 30 days to complete. The first seedlings emerged around 30 days after the first germination in the field.

The relevance of differences in the strength of dormancy of seed from different altitudes was tested in a 'reciprocal' transplant experiment. Seed collected at four altitudes was planted at four altitudes between 960 m and 1740 m in autumn 1983. Very few seedlings emerged in autumn or winter. In spring there was a decrease in the number of seedlings emerging from seed from low altitudes, and an increase in the number of seedlings emerging from seed from high altitudes with increases in altitude.

The increase in the innate strength of dormancy of seed with altitude appears to be associated with a greater capacity of the environment to break dormancy at higher altitudes. Thus the timing of germination and emergence ensures that the seedling is less likely to be exposed to either the snow and needle ice as a result of premature germination during winter or drought and high temperatures associated with delaying germination until late spring or summer.
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CHAPTER 1

INTRODUCTION
CHAPTER 1

1.1 The regeneration of plants

Regeneration of plants from seed involves the passage through a series of life stages which have different sensitivities to environmental conditions. The regeneration niche is an expression of the requirements for a high chance of success in the replacement of one mature individual by a new mature individual of the next generation (Grubb 1977). The regeneration niche can be seen as a statement of the tolerance ranges for survival and the requirements for growth as seed becomes seedling and so on through the ontogeny of the plant.

Cohorts of plants generally experience their greatest mortality during the regeneration phase; therefore there is an huge potential at this stage for differential selection of individuals with attributes associated with increases in fitness. The high mortality at this stage is associated with the relative severity of the environment near the surface of the soil, and with the susceptibility to damage of plants with very limited reserves and few meristems.

The regeneration phase is important because differentiation between species in this phase has the potential to influence the species richness of a community of plants. The importance of the regeneration phase for the maintenance of species richness in a plant community, which has been discussed by Grubb (1977), is related to the enormous influence of chance at the relevant scale of environmental variation. The element of chance refers to both the presence of viable propagules of the species at the site and the microenvironmental
conditions in the vicinity of the seed and seedling.

The potential for the manipulation of community composition by modification of conditions in the regeneration phase has implications for the management of both weedy and desirable species. In order to realize this potential it is necessary to understand the environmental control of these processes and the way in which they may be manipulated.

1.2 The regeneration of Eucalyptus

Plants of the genus *Eucalyptus* are widely distributed in Australia and may be found in a wide range of environments varying in altitude from sealevel to treeline at 2000 m, and in latitude from southern Tasmania to the tropical regions north of Australia. The 440 species of the genus (Chippendale 1976) show a large degree of morphological variation ranging from the multiple-stemmed 'mallees' of drier areas and high altitude areas through the short-boled single-stemmed trees of open woodlands to the giant single-stemmed trees of the tall forests which occur in higher rainfall areas.

The regeneration niche of the eucalypts has been discussed extensively by Cremer *et al.* (1978) who discuss some of the variation in the requirements necessary for the regeneration of species from different environments and conclude that the single most important factor in the successful regeneration of many eucalypt species is fire. The sensitivity of various species to fire and the intensity of the fires to which they are exposed lead to a wide range of responses which may be characterized by the degree and mode of regeneration of individuals following the fire. The range of these responses, from the
decimation of the extant population and obligate regeneration from seed (e.g. *Eucalyptus regnans*), to facultative regeneration from seed in addition to regeneration of adults. The regeneration of adults ranges from resprouting from lignotubers (e.g. *Eucalyptus incrassata*, *Eucalyptus pauciflora*), to various levels of stem resprouting observed in species such as *Eucalyptus pilularis*, *Eucalyptus dives* and *Eucalyptus delegatensis* (Jacobs 1955). Two species in which the limitations to regeneration in the absence of fire has been studied are *E. regnans*, which grows on deep soils in high rainfall areas and is discussed by Ashton & Willis (1982) and *E. incrassata*, a mallee species growing in the semi-arid areas (Wellington 1981).

*Eucalyptus regnans* forests tend to be even aged stands in which the trees have established following fires, which may occur once every century or so. The established trees are unlikely to survive the high intensity fires when they do occur and regeneration of the forest proceeds from seed. Seed does germinate under the understorey, which is well developed in these forests, although the survival of the germinated seedlings may be limited by the presence of lipids in the soil. Seedlings which do survive establishment die within 2 years. Antagonistic microorganisms in the soil associated with the living roots of the established trees have been implicated in the death of these seedlings in a mature forest. In addition to the limitations imposed by the soil, light may also become limiting for the plant as it passes from the seedling phase, when the leaves are normal to the incident light, to the juvenile phase when the leaves become pendulous. Fire removes the limitations imposed by the biological and physical environment and permits the seedlings to establish and grow vigorously.

*Eucalyptus incrassata* populations tend to have an uneven age
distribution because many individuals are likely to survive the more frequent fires (about 20 year intervals) which occur in the mallee shrublands, and regeneration may proceed by resprouting from the lignotuber or from seed. Comparison of the water status of seedlings of *E. incrassata* at sites which differed in the time since fire showed that the seedlings in a stand burnt 4 years previously had far lower water potentials than those growing in an area which had been burnt one year previously (Wellington 1984). This observation suggests that the effect of fire is to increase the availability of water in the upper layers of the soil by temporarily removing the transpiring canopy of the adult trees. In the absence of fire seedlings germinated and emerged but then died during the first summer in the mallee.

The elements of the environment which limit the survival of seedlings in the absence of fire are replaced by other limitations to survival following a fire. It is the response of the seedling to environmental conditions during regeneration which will be examined in this thesis. The eucalypts show considerable variation in the processes involved in regeneration; specializations such as seed dormancy, differences in the optimum temperature for germination (Boland *et al* 1980) and the growth rate of seedlings (Davidson & Reid 1980, Noble 1983) have been identified in *Eucalyptus*.

*Eucalyptus pauciflora* offers the opportunity to examine the changes in the processes of regeneration in relation to altitude in the species which is distributed to the upper altitudinal limit of the eucalypts. Many environmental factors change with altitude including temperature, rainfall and the incidence of frost and snow (Costin 1954). The reduction in temperature and associated increase in the number of frosts and incidence and duration of snow cover (Slatyer *et*
results in a delay in the beginning and a hastening of the end of the growing season with increases in altitude (Costin 1954, Slatyer & Morrow 1977). In addition to a reduction in the length of the growing season the conditions during the growing season become cooler with increases in altitude which reduces the potential for growth even further as altitude increases. The changes in the environment are also associated with changes in the morphology (Green 1967) and physiology (Slatyer 1978) of the species and these changes have been interpreted as increasing the chances of survival and growth of the populations of *E. pauciflora* growing in those environments.

1.3 The regeneration of *Eucalyptus pauciflora*

*Eucalyptus pauciflora* (Sieb. ex Spreng.) is a long lived tree which has a wide distribution from sealevel to treeline (2000 m) (Holand et al. 1984); it occurs extensively at higher altitudes in the Great Dividing Range in eastern Australia. The morphological differentiation of *E. pauciflora* with altitude is such that different ecotypes were considered to be different species until Pryor (1954) suggested that the variation in morphological characters, such as tree height, canopy form, capsule and leaf shape which were being used to differentiate between the species could be considered to be continuous in response to changes in environmental factors. This proposition was supported by Green (1967, 1969a, 1969b) who demonstrated that the variation in a range of morphological and phenological characters in *E. pauciflora* was genetically controlled and varied continuously with altitude, and that the characters which had formerly been used to differentiate between the species were not stable and discrete.

Physiological processes were also found to vary in *E. pauciflora*
populations from different altitudes. Slatyer (1977) found that there was a decrease in the optimum temperature for photosynthesis and a reduction in the rate of photosynthesis at the optimum temperature with increases in the altitude of seed source. Harwood (1976) showed that the frost tolerance of this species increased with altitude of seed source.

The regeneration of a species from seed depends on some seed being in suitable place and physiological state to ensure the germination of the seed and survival and growth of a seedling to the stage of reproductive maturity. The studies in the following chapters investigate the influence of the physical environment on the process of the regeneration of *E. pauciflora* from seed. The ontogenic sequence from seed to established tree may be influenced by the environment at all stages from the production of the seed to the establishment of a seedling. The role of the environment in determining the outcome of this sequence will depend on the type and extent of the response which it elicits and the degree to which this response pre-empt the response to environmental conditions subsequently. I will now discuss the life history of *E. pauciflora* concentrating on the influences of the physical environment at various stages and the potential for that influence to control the timing of the process of regeneration.

The production of seed begins with the development of flower buds in the summer and autumn of the year prior to flowering; flowering occurs in the period from November-January (Boland *et al.*, 1980). *E. pauciflora* flowers profusely at irregular intervals but in most years some trees flower at all altitudes. Observation of the widespread and heavy flowering which occurred in the Snowy Mountains in 1982 showed that there was a delay of 2 weeks in the onset of flowering
of *E. pauciflora* from 960 m to 1850 m and that the flowering extended over a period of 4 weeks to 6 weeks. The development of the seed is completed by the summer following flowering (Cremer et al. 1978).

_Eucalyptus pauciflora_, in common with most eucalypts, does not have a large, long-lived seed bank in the soil (Howard & Ashton 1967), because almost all the seed will germinate within a year of dispersal from the capsules. The canopy seed bank has several cohorts of capsules and the timing of dispersal of seed from the capsules is the first point at which the timing of germination may be influenced. The frequency of flowering and the persistence of the capsules in the canopy makes it unlikely that there would ever be a paucity of seed for regeneration, although the amount of seed in the canopy will vary depending on the time since the last flowering.

The dispersal of seed is dependent on the local environment of the parent tree, and may occur before or after the seed is shed from the capsule. Seed dispersed inside the capsule probably has a reduced chance of becoming an established seedling because of destruction of the seed by predators or pathogens before the seed is shed from the capsule (Cremer et al. 1978). The association between the climate and the shedding of _Eucalyptus_ seed in southern Australia is well documented with a tendency for seed to be shed in late summer and autumn (*E. regnans*, Cremer 1965, Cunningham 1960; *E. delegatensis*, Grose 1963) although some seed is shed at all times of year. Fire may stimulate a large and synchronized seed fall (*E. regnans*, Cunningham 1960, Cremer 1965b; *E. diversicolor*, Christensen 1971; *E. incrassata*, Wellington 1981; *E. delegatensis*, O'Dowd & Gill 1980).

The predation of dispersed seed is likely have a critical
influence on the survival of the seed. Grose (1963) showed that the number of seedlings of *E. delegatensis* could be increased by spraying the ground with insecticide after planting and Cremer et al. (1978) noted that the number of seedlings establishing from seed could be increased by insecticide treatment of the seed. More recently the predation of seeds placed on the ground has been examined in detail in *E. regnans* forests by Ashton (1979) who showed that up to 65% of seed was removed by ants within 2 weeks of placement. Similar rates of seed removal were noted by Wellington & Noble (1985) for *E. incrassata*.

The size of the seed represents the 'capital' available for the development of the new plant and may vary within a population and between populations. The size of seed within a population had an important bearing on the size and early growth rate of the seedlings of *E. maculata* and *E. sieberi* (Grose & Zimmer 1958c), *E. delegatensis* (Grose 1960). The seed sizes of different populations also have been associated with differences in growth rate of seedlings of *E. viminalis* (Ladiges 1974). Indirect evidence for a decrease in the size of the viable seed of *E. pauciflora* with increasing altitude (Boland et al. 1980) can be obtained by comparing the mean numbers of viable seed for samples of the low altitude *E. pauciflora* ssp. *pauciflora* and higher altitude *E. pauciflora* ssp. *niphophila* form. This comparison may be confounded by a changing proportion of chaff in the seed sample, but needs to be resolved to determine whether the 'capital' is different for ecotypes growing at different altitudes.

The absence of a long-lived seed bank in the soil under *E. pauciflora* forests (Howard & Ashton 1967) suggests that all of the seed either dies or germinates within a year of dispersal. It is the processes which control the timing of germination which have a critical
role in ensuring that the establishment of the seedling occurs at a time which ensures the greatest chances of survival and growth of the seedling. The timing of germination of the seed in response to its environment can be controlled at three stages: before dispersal, between dispersal and the breaking of dormancy and during germination. The essence and impact of the interaction between the seed and its environment is markedly different in each of these stages.

A large proportion of *E. pauciflora* seed is dormant at dispersal. Dormancy is one means by which environmental conditions which are unfavourable for plant processes are avoided. The disadvantage of dormancy is that it also constitutes a time when an individual is not competing for resources (Harper 1977). The critical elements of a dormancy strategy are the timing of the induction and the breaking of dormancy in relation to conditions which are favourable for germination of the seed and emergence, survival and growth of the seedling. Increases in fitness due to dormancy are likely to be attained by minimizing the loss of competitive advantage and maximizing the survival of the individual in the prevailing conditions.

*Eucalyptus pauciflora* exhibits a variable degree of dormancy at dispersal which increases with altitude (Grose 1957) and which will determine the chances of germination when the seed reaches the ground after dispersal. The dormancy of *E. pauciflora* seed is broken by cold moist conditions (stratification) (Pryor 1954). Seed from higher altitudes may be expected to have a dormancy which requires a longer period of stratification since the chances of seed breaking dormancy before winter, due to the lower temperatures at higher altitudes, expose the seed to the possibility of germination, either during late autumn or winter which would be fatal (Slatyer pers. comm.).
The dormancy of the seed at dispersal may also be expected to vary with the conditions of seed extraction and dispersal because these conditions are a broad indicator of the conditions on the seed bed (season). The particular advantages of such a response would seem to be the modification of the response of the seed to the soil environment immediately after dispersal. Such a response could be important for seed dispersed in the spring, when conditions are suitable for establishment but the chances of prolonged periods of low temperatures for breaking dormancy are remote, or alternatively, in autumn when the chances of successful establishment are low but the chance of breaking dormancy is high.

The changes in the dormancy of the seed following dispersal could be expected to show some sensitivity to temperature since it is known that low temperatures will break the dormancy in *E. pauciflora* and, in *E. deleatensis*, that exposure to higher temperatures will increase dormancy in fresh seed.

Grose's (1953) work with *E. deleatensis* suggests that interaction between the dormancy of the seed and the environment could be expected to continue with subsequent experiences contributing either by breaking or by enhancing dormancy until the dormancy status of the seed coincides with the threshold for germination for that seed. His studies showed that the sensitivity of the seed to the temperature environment is dependent on its moisture content, with higher moisture contents facilitating more rapid responses.

The effect of moist conditions at the higher soil temperatures of summer and autumn will be to increase the duration of dormancy breaking
conditions required to break dormancy, and so to ensure that the seed is less likely to germinate in late autumn or winter. The importance of the apparently reversible response exhibited by the breaking and induction of dormancy in moist seed is that it provides the seed with a mechanism for integrating the effects of past environmental conditions.

The proportion of seed which does not germinate, and is therefore by definition dormant, is, in many plants, closely associated with the conditions of germination (Vegis 1964). Grose (1963) has shown that the proportion of the seed germinating at temperatures between 5°C and 35°C increases in seed samples of *E. delegatensis* as the dormancy of the seed is reduced by stratification. A response of this type would lead to an increase in the range of temperatures which were suitable for germination as the stratification of the seed on the cold moist soil progressed during winter.

The response of the process of germination to environmental factors finally governs the irreversible commitment of the limited resources of the seed to the job of regeneration. The germination of the seed marks the beginning of a much closer relationship between the seed and the environment which leads to high mortalities of seedling both prior to emergence and between emergence of the cotyledons and establishment of the seedling. It is at this stage that the root and the shoot are exploring the most rapidly variable, unpredictable and extreme environments of the soil and atmosphere with few meristems and meagre reserves in the event of damage. The chances of desiccation, damage by ice heave, flooding, frosting are all enhanced in this zone and contribute to the mortality which is observed prior to establishment.
The observation and manipulation of seedlings which have emerged and established in the field gives some indication of the environments which are favourable for the first stages of regeneration. Noble (1980) investigated the relationship between Poa spp. and regenerating *E. pauciflora* following a fire near timberline in the Snowy Mountains and concluded that the size and morphology of the seedlings was influenced by the distance from a grass tussock and proposed that the grass had both a protective and competitive role in the environment of the seedlings.

Wimbush & Costin (1979) note that the regeneration of *E. pauciflora* seedlings is closely related to the physical environment at the soil surface: this determines the degree of flooding, ice heave and drought which, in turn, have important influences on the survival of seedlings. Furthermore, these authors noted that, following the removal of grazing in the subalpine environment, the available gaps were rapidly colonized by shrubby species which prevented the establishment of *E. pauciflora* for at least 20 years.

In summary the response of *E. pauciflora* to the physical environment during regeneration may be elucidated further by studies in three general areas

1. The characteristics of the seed and the timing of seed dispersal
2. The timing of germination
3. The survival and growth of seedlings

As will be seen in Section 1.3 the majority of this thesis deals with the second general area, however, aspects of the first and third topics were also investigated.
1.4 A study of the regeneration of Eucalyptus pauciflora

The present study set out to examine the regeneration niche of *E. pauciflora* by examining the influence of the environment at three critical stages in the process. The first series of studies examined the timing of seed dispersal and the differences in the seed collected at different altitudes, the second series of studies investigated the influence of the environment on the timing of germination and emergence and the third was concerned with the impact of the physical environment on the survival, growth and morphology of seedlings in the field.

The physical environment has been shown to have an important influence on the survival and growth of *E. pauciflora* (Noble 1980, Harwood 1976). Disturbance has a critical role in the regeneration of this species with seedlings rarely surviving in undisturbed situations. Fire is the most obvious and consistent disturbance and has been implicated in the even aged stands observed. However, the presence of even aged top growth does not, in itself imply synchronized regeneration from seed following fires since a large proportion of the regeneration comes from sprouting lignotubers. No fires of sufficient magnitude occurred to enable examination of the post-fire regeneration of the species during the early part of the study so an alternative approach was sought.

Noble (1980) has shown that the survival and morphology of seedlings establishing in a *Poa* grass sward could be associated with the distance from the nearest *Poa* tussock. The distance to the nearest tussock could also be viewed as defining the size of a gap and I set up experiments to investigate the influence of the size of gaps created in a mature tussock grass (*Poa* spp.) sward on the survival, growth and
Introduction

morphology of seedlings.

Differences in the microclimate with position in the gap were expected to be important also, and the effect of the position of the seedling (aspect) in the gap was also examined. Grasshoppers and frost caused overwhelming mortality of the seedlings planted in spring 1980 and drought and frost on the seedlings planted in spring 1981. Field studies on the role of nutrients, water, and shading on the growth and morphology of seedlings planted in spring 1982 are not reported in this thesis because the experimental treatments were lost in the overwhelming mortality caused by drought and frost in the autumn of 1982. These site and year specific factors could not be accounted for and these studies were not continued.

Many of the discussions in this thesis assume that successful seedlings emerge in spring and summer. The studies reported in Chapter 7 show this to be the case since seedlings which emerged in autumn died before the next spring, and no seedlings which emerged during winter survived. Slatyer (pers. comm.) has observed a similar phenomenon.
1.6 Locations of seed collection, and experimental sites

Figure 1.1 A map showing the location of seed collection and experimental sites in the Snowy Mountains area of South-eastern New South Wales.

Details of the grid reference, altitude, latitude and longitude of seed collection sites are provided in appendix 1.
USE OF THESES

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

by

Douglas Graham Abrecht

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

February 1985
CHAPTER 2

TERMS AND METHODS
CHAPTER 2

The timing of germination is dependent on the effects of environmental factors which cause dormancy to be broken and the processes of germination to commence. The four chapters which follow describe investigations of the effects of environmental factors, particularly temperature, on the dormancy and germination of *E. pauciflora*. The terms and methods used in Chapters 3, 4, 5, 6 have a common element which will be considered in this chapter to avoid unnecessary repetition.

2.1 Seed dormancy

The description of seed dormancy has become confused by the use of many terms; the definitions used in this thesis are discussed explicitly in this section. Bewley & Black (1982) note that viable seeds which do not germinate need not be dormant. They define as quiescent a seed which is prevented from germinating by conditions which do not support metabolism and growth; for example when the seed is dry. Dormancy is defined as a state in which viable seed will not germinate when exposed to conditions in which germination would ordinarily take place. Descriptions of seed dormancy are commonly qualified by categories which describe either the manner of origin of dormancy or its expression in terms of the conditions of germination.

The most commonly used categories of dormancy are based on the manner of origin of the dormancy either before (primary or innate) or following (secondary or induced) dispersal from the mother plant. Even these categories become confused in the case of seeds which become
dormant on the mother plant in response to the environmental conditions during seed production. Harper (1959) added another category to this scheme to describe seeds which, though not able to germinate under the prevailing environmental conditions, would germinate when transferred to more amenable conditions. Enforced dormancy was distinguished from induced dormancy by the persistence of the latter when the environmental conditions which prevented germination were removed. Karssen (1981) suggests that Harper's 'enforced' dormancy should be termed environmental inhibition of germination.

Turning from consideration of the origin of dormancy to the manner in which it may be expressed, another series of categories seek to describe the sensitivity of dormancy to the environmental conditions which result in germination, based on changes in germination capacity in response to temperature.

Vegis (1964), reviewing the literature, concluded that as seed became more dormant there was a progressive narrowing of the range of temperatures which would sustain germination which he termed, pre-dormancy, until germination would not occur at any temperature (true dormancy). A similar situation obtains as the seed overcomes dormancy and the range of temperatures allowing germination increases, (post-dormancy). During the periods of pre- and post- dormancy the seed exhibits partial or relative dormancy.

Two important attributes of the dormancy of a population of seeds are recognized in this thesis namely, the degree and the strength of dormancy. The degree of dormancy is defined as the proportion of viable seeds which do not germinate, and is measured as the complement of the germination capacity. The strength of dormancy is assessed by
the duration of moist, cool conditions required to break dormancy. The process by which dormancy is broken by cool, moist conditions is commonly called **stratification**.

Dormancy may also be associated with other aspects of the germination of the seed. Richter & Switzer (1982) suggest that differences in dormancy may be quantified by determining the differences in the areas under *germination curves*, defined as cumulative % germination with time. This area was estimated by integrating mathematical functions which had been fitted to the germination curves, and represents a comparison of both the rate and the extent of germination. The effect of dormancy on the times-to-germination is quantified in this thesis by Cox models which are discussed in Section 2.4.2.

### 2.2 Seed germination

The *germination* of a seed refers to the processes which culminate in the growth of the embryo; the emergence of the radicle from the testa is generally the first clear manifestation of germination (Heydecker 1972). **Seedling emergence** is the stage when the seedling emerges from the soil surface.

The *germination* of a population of seeds may be characterized by descriptions of the proportion of the seeds which germinate and the rate of germination. The proportion of the seeds which germinate is termed the *germination capacity* of a seed sample. A detailed description of the measurement and analysis of germination capacity is presented in Section 2.4.1.
The times-to-germination is the distribution of the times taken for the individual seeds in a population to germinate and is the reciprocal of the rate of germination. The rate of germination is often represented graphically as a plot of the cumulative germination (%) against time which is termed a germination curve. Two different approaches have been taken to investigations of the rate of germination; one approach leads to the creation of an index which describes the progress of germination with time and the other attempts to describe the form of the germination curve in mathematical terms.

A range of indices have been developed to describe the rate of germination. Grose (1957) used an accumulation of the proportion of the seeds germinating on the first four days of a germination period, to describe the vigor of *E. delegatensis* germination. Timson (1965) suggested an index which is mathematically identical to that of Grose (1957) but proposed that the germination period should be fixed at, for example, 10 days to facilitate comparisons between experiments. This type of index has been criticized by Heydecker (1966) because the same value of the index may be produced by widely different germination curves.

Bartlett (1937) proposed an index which was used extensively by Grose (1963) in his later studies. This index is equivalent to the Grose (1957) index divided by the germination capacity and multiplied by the number of days in the germination period. When the time intervals between observations is equal the Bartlett index is equivalent to the mean time to germination.

Kotowski (1926) devised an expression for the mean rate of germination which he termed the coefficient of velocity. This
expression was critized by Heydecker (1966) because it does not provide information on the variation in the rate of germination. Gordon (1971) proposed two indices, resistance to germination and uniformity of germination, which together describe the mean and variation in the time to germination. Germination resistance, amounts to the inverse of Kotowski's coefficient of velocity and the uniformity factor is the standard deviation of the times-to-germination.

Nichols & Heydeker (1968) suggested four methods which could be used to determine the mean time to germination and an estimate of spread about the mean. Two of their methods used moments and quartiles of the distribution of the times-to-germination and also attempted to summarize the shape of the distribution of times-to-germination by estimates of skewness. Nichols & Heydeker (1968) also used least squares regression to fit parameters to the germination curve or to the probits of the germination curve for each petri dish. The last two methods are not valid because they assume, incorrectly, that the cumulative germination (\%) at successive times is independent and that the variances of the cumulative germination at successive times are equal.

The disadvantage of using indices to describe the rate of germination is that the value of the index may not reflect some important component of the rate of germination. This problem is overcome by methods which seek to model the germination curve in mathematical terms. The techniques proposed consider the effects of treatments on the germination process as a whole whilst also permitting the derivation of parameters such as the mean time to germination and the variation about that time. A range of distributions which purport to model the distribution function of times-to-germination have been
fitted to observed cumulative germination curves.

The Normal distribution has been assumed by several workers. Janssen (1973) fitted the parameters of the Normal distribution using an unweighted least squares estimation procedure which is of doubtful statistical validity for the same reasons as have been discussed above for Nichols & Heydecker (1968). The problem of fitting a Normal distribution to germination data by least squares estimation has been overcome by Hunter et al. (1983) who used a Newton-Raphson iterative procedure to maximize the log-likelihood and estimate the mean and variance of the times-to-germination. Some of the other distribution functions which have been fitted to germination curves include the Logistic (Shimpf et al. 1977), and Weibull (Bonner & Dell 1976).

The restrictions imposed by assuming a specific function for the theoretical distribution of the probability of germination were overcome by Goodchild & Walker (1971) who characterized this relationship by fitting orthogonal polynomial regressions. However, this method also used least squares estimation procedures and is therefore not valid for the reasons outlined previously. Interpretation of the treatment effects when the order of the polynomials of best fit differ poses many difficulties because the coefficients of the regressions are not comparable.

The Cox regression models (Cox 1972) which have been used in this thesis permit the statistical comparison of the effects of treatments on the times-to-germination without the need to make assumptions about the form of the distribution of the times-to-germination. The method does require the assumption that the times-to-germination of the treatments bear a proportional relationship to some undefined, but
fitted to observed cumulative germination curves.

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common distribution of times-to-germination (proportional hazards). The details of the Cox models are presented in Section 2.4.2.

2.3 Methods used in germination experiments

2.3.1. Collection of seed

The fruit of *Eucalyptus* is a 'false fruit' developed from an inferior, multilocular ovary which is more or less adnate to and surrounded by calyx tube (Maiden 1922), and is usually called a 'capsule' (Cramer 1965a). Capsules were hand picked from at least twenty trees at any one site. Capsules were removed from all positions in the canopy although there was a bias toward those which were accessible on the lower branches. The capsules collected at a site were bulked in order to obtain the amount of seed required for experiments.

The site location and altitude of all seed used in experiments reported in this thesis are recorded in Appendix 1.

2.3.2. Extraction and storage of seed

The capsules were placed in plastic bins which were open to the air to allow desiccation under laboratory conditions of temperature (15-25°C), light (fluorescent 16 hours/day) and humidity (50-60%). The seed and chaff were separated from the dry capsules by vigorous shaking and sieving through a 2 mm mesh. Following extraction, the dry seed was stored in a refrigerator at 5°C in airtight containers.
Specific experiments were carried out on the influence of temperature of extraction on the dormancy of seed and are outlined in Section 3.3.

2.3.3. Cleaning of seed

Inspection of the seed obtained indicated that it contained a large proportion of infertile seed and chaff. In order to reduce the problems of sampling and leachates and to overcome limitations of space in the incubators it was decided to reduce the volume of the seed sample by removing this material. The seed was sorted at CSIRO Plant Introduction Section using a blower apparatus specially designed for sorting seed. The material discarded was checked for viable seed by soaking a sample in water for one day and then searching for viable embryos using the technique of Grose & Zimmer (1958a); any seed lot with more than 5 viable seeds/g in the discarded fraction was remixed, sorted and checked again (the fraction retained generally contained at least 250 seeds/g).

2.3.4. Sampling of seed for experiments

Weighed samples of seed for experiments were obtained from the well mixed, cleaned seed, by spatula. The method described by Grose (1957) of repeated division and mixing did not reduce the variance in the number of viable seeds in the replicates and it is presumed that that method was not appropriate for cleaned seed in which the proportion of viable seed is high. Seed samples were placed on a single layer of filter paper moistened with 2 ml of a solution of Benlate *R fungicide (benomyl 0.2 g/l) in water, and enclosed in a 60 mm glass petri dish.
2.3.5. Conditions of stratification

The breaking of dormancy by stratification was achieved by placing the petri dishes containing the moistened seed in plastic boxes in a refrigerator at around 5°C ± 1.5°C. The dishes were checked at weekly intervals, and distilled water was added as necessary to ensure that the seed did not dry out.

2.3.6. Conditions of germination

Boland et al. (1980) recommended two temperatures for the germination of *E. pauciflora* depending on the altitude of seed source (ssp. *pauciflora* 15°C and ssp. *niphophila* 20°C) whereas Scott (1972) recommended a single temperature of 15°C for the species. Germinations were carried out in an incubator at a standard temperature of 15°C; the temperature was monitored continuously using a thermograph and on a daily basis by readings of a maximum/minimum thermometer.

Germinating seed was maintained under a 12 hour photoperiod using 20 W fluorescent tubes located 300 mm from the seed. The filter paper under the seeds was kept moist throughout the experiments with additions of distilled water as required. Water was added to maintain a visible meniscus at the edge of the filter paper.

2.3.7 Temperature manipulation using a gradient plate

Gradient plates have been used to provide a wide range of temperatures for the purposes of analysing the temperature responses of seed germination (e.g. Thompson 1970, Thompson & Grime 1983).
The gradient plate which was used in the present studies consisted of a rectangular aluminium plate (1.4 m X 0.7 m) which was cooled by refrigerated fluid passing through a closed channel on one long axis and heated on the opposite long axis by hot fluid passing along a similar channel. The plate was contained in an insulated box to minimize the effect of changes in ambient temperature and evaporation of water from the surface of the plate was minimized by a tight fitting lid.

The temperature gradient on the plate ranged from ca.-2.0°C to 30°C and therefore only that portion of the plate with the desired temperature range was used for experiments. The area to be used was covered with a single layer of filter paper and moistened with a solution of Benlate® fungicide (benomyl 0.2 g/l) in distilled water. The seed was placed on the filter paper with each replicate occupying an area (ca. 5 mm by 50 mm) with its long axis at right angles to the temperature gradient.

The temperature gradient was monitored by copper-constantin thermocouples which had been electrically insulated and were held on the surface of the filter paper in a configuration which spanned the gradient. The relationship between the position on the gradient and temperature was then determined by regression and the temperature of the seed treatment was estimated from this relationship.

2.3.8. Counts of germinated seeds

Seeds which had germinated were counted daily and removed from the petri dishes. Daily counts were sometimes missed and the total count
for the missed period was recorded. Germination was judged to have occurred when more than 2 mm of radicle had emerged from the testa; at this stage the hypocotyl was extended and the clinging disk (Gauba & Pryor 1958) was also generally visible. Abnormal germinations were noted and included in the total count of viable seeds but not in the germination count. The proportion of seeds which germinated abnormally did not exceed 0.5% of the viable seeds at any time.

The counts were continued until zero counts were obtained in at least half of the replicates in the slowest treatment on two consecutive days.

2.3.9. Assessing the viability of the non-germinated seed

The seeds which had not germinated were squashed with a spatula and the presence of a white embryo was taken as evidence of viability. (Grose & Zimmer 1958a). The method was tested and verified using a tetrazolium test, in which the embryos from 100 seeds which had been judged to be viable (by squashing carefully so as to split the seed coat) were soaked in a 1% solution of tetrazolium chloride for 24 hours, sectioned and examined for staining.

The mean number of viable seeds in a replicate is included in the methods section of all experiments as a guide to the number of seeds on which inferences are based. The mean number of viable seeds does not include the seeds which died during the experiment and is based on the sum of the seeds which germinated and those judged to be viable by squashing at the completion of the experiment.

The determination of viability by squashing may be questioned on
the basis that the method does not distinguish between those seeds which could germinate in the future and those which may lack something which is fundamental for germination, but which have an apparently healthy embryo. Evidence is presented in Chapter 5 which suggests that seeds which are judged to be viable by this method may germinate following further stratification.

Variation in the number of seeds shown to be viable, either through germination or squashing, is used to make assessments of the relative mortality of seed exposed to various treatments. The absolute mortality of the seed cannot be determined because there are no reliable non-destructive methods for assessing the viability of _E. pauciflora_ seed.

2.4. Statistical analysis of germination

Statistical analysis of data from germination experiments is concerned with making the correct inferences about the effects of treatments on the process of germination. The two elements of this process which are of interest are the proportion of seeds which germinated and the rate of germination of the seeds which did germinate. The extent of germination is described by the germination capacity and the rate or vigour of germination is represented by the times-to-germination.

2.4.1 Analysis of germination capacity

Statistical analyses of germination capacity are concerned with estimating the effects of treatments in Binomial data ('r' germinations
out of 'n' viable seeds). Many earlier analyses of this kind involved angular transformation of the proportions using \( \arcsin(\sqrt{r/n}) \), to stabilize the variance prior to an analysis of variance. An assumption of this analysis is that the transformed variable is Normally distributed and that the treatment effects are linear on the Normal scale. However, when the expected proportions lie outside the range 0.2 to 0.8, the \( \arcsin \) transformation fails to adequately correct for heterogeneous variance and the assumption of Normality is violated (Cunningham 1984, pers. comm.).

A statistical modelling approach has been taken to the analysis of both germination capacity and the times-to-germination. The process of statistical modelling may be divided into three steps, the specification of a plausible model to describe the behaviour of the response variable, the estimation of the parameters used in the model and finally the making of inferences and the testing of hypotheses (Dobson 1983). The approach used throughout this thesis is to model the data using Generalized Linear Models (GLMs) (Nelder & Wedderburn 1972). There are three components of a GLM,

1. The data \( y = (y_1, y_2, \ldots, y_n) \) which are assumed to be independently distributed with mean \( \mu = (\mu_1, \mu_2, \ldots, \mu_n) \).

2. The linear model which predicts

\[
y = (y_1, y_2, \ldots, y_n) = X\beta
\]

where \( \beta \) represents the parameters to be estimated and \( X \) is the matrix of explanatory variables.
3. The link function which connects $Y$ with the mean $\mu$ is

$$Y = f(\mu)$$

The analysis of Binary data, such as germination capacity, using GLMs is fully discussed by McCullagh & Nelder (1983). Generalized Linear Models require choice of a link function and an error distribution. Of the many link functions which would be suitable for the purpose McCullagh & Nelder (1983) suggest logit link function. The advantage of using a logit link function is its ease of interpretation, as the logarithm of the odds ratio. If $p$ is the probability of an event occurring then the odds ratio is defined as $(p/(1-p))$. The error distribution of Binary data is assumed to be Binomial. The necessary computing programs are provided by GENSTAT 4.03 (Alvey et al., 1980).

Analyses are summarized as tables of deviance where the deviance is analogous to the sums of squares for Normally distributed data. The observed changes in deviance are compared with the critical value of $\chi^2$ for the appropriate degrees of freedom, since the change in deviance is distributed (asymptotically) as $\chi^2$.

The value of the residual mean deviance (RMD), which is calculated by dividing the residual deviance by the residual degrees of freedom, gives an indication of the goodness of fit of the statistical model. A value of RMD=1.0 indicates that the assumption of Binomial distribution for the data is valid. The scale parameter, which is an estimate of the overall variation, is set to 1.0 for data arising from a one parameter distribution such as the Binomial distribution. Under the conditions when the residual mean deviance departs from 1.0 (say, greater than 1.5), it is not valid to use the $\chi^2$ statistic. In this
case, the F statistic, calculated as the ratio of the mean change in deviance (MCD = change in deviance divided by the change in degrees of freedom) to the residual mean deviance (RMD) of the full model, is used to test for differences between the models. The objective of the tests between statistical models is to find a parsimonious model which best describes the data. Unless otherwise stated, the level p=0.01 is taken as the critical level of significance in these models. An example with the details of the analysis is presented in Section 3.1.2. The graphs associated with the analyses show the responses predicted by the relevant model unless otherwise stated.

2.4.2. The analysis of times-to-germination

The inadequacy of the available techniques for the analysis of times-to-germination has already been discussed (Section 2.2) and assistance was sought to investigate the problem of the analysis of such data. Mr. R.B. Cunningham (A.N.U., Department of Statistics), a consultant statistician, recommended the use of a proportional hazards regression model for survival analysis, developed by Cox (1972) (subsequently referred to as a Cox model) for this purpose. Cox's (1972) technique has been adapted for grouped data, and used to analyse for the effects of wood preservatives on the longevity of timber poles (Bartlett 1978), and for the effects of treatments on the time to conception in beef heifers (Cunningham et al. 1981).

The statistical model is fully described in Cunningham et al. (1981) but the essence of the model is that if $p_{ij}$ denotes the conditional probability that a seed in a particular factor combination, i, will germinate in the time interval $(t_{j-1}, t_j)$ when it has not previously germinated, then
\[ p_{ij} = 1 - \exp\left(-\exp(\beta_i) \int_{t_j}^{\infty} \lambda_o(u) \, du \right) \]

where \( \beta_i \) are treatment constants and \( \lambda_o(u) \) is some unspecified function of time. By applying a complementary log log transformation a linear model is formed

\[ \ln[-\ln(1-p_{ij})] = \beta_i + \gamma_j \]

where \( \gamma_j \) are constants which relate to the risk of germination over each of the time intervals considered.

If the \( p_{ij} \)'s are regarded as being binomially distributed, this model belongs to the class of Generalized Linear Models which can be fitted by using the computer program GENSTAT (Alvey et al. 1980).

The analysis of deviance table provides information on the effects of treatments in a similar way to that already described for germination capacity and the contributions of the various levels within a treatment may be inferred from the coefficients, \( \beta_i \) of the model.

If the proportional hazards model of Cox (1972) is assumed, then Bartlett (1978) showed that the distribution function of times-to-germination, \( F_i(t_j) \), (the germination curve) is related to the constants \( \beta_i \) and \( \gamma_j \) as follows:

\[ F_i(t_j) = 1 - \exp\left(-\exp(\beta_i)\exp(\gamma_i)\exp(\gamma_2)\ldots\exp(\gamma_j)\right) \]

thus

\[ \ln[-\ln(1-F_i(t_j))] = \beta_i + \delta_j \]
where \( \delta_j = \ln[\exp(\gamma_1) + \exp(\gamma_2) + \ldots + \exp(\gamma_j)] \)

A plot of \( \ln[-\ln(1 - F_i(t_j))] \) against \( t_j \) for any \( i \), will provide information on the probability distribution of times-to-germination. If \( i=1 \) then \( \beta_i=0 \) so that,

\[ \ln[-\ln(1 - F_i(t_j))] = \delta_j \]

which may be readily calculated from the \( \gamma_j \) coefficients of the model. The \( \beta_i \)'s affect only the position of the curve and not the slope and so the use of any particular \( i \) will not make any difference to the conclusions about the distribution of times-to-germination.

The Cox model requires the assumption that the risk of germination for a given treatment, relative to some standard value, is constant at all times (proportional hazards), and therefore that the treatments have some common underlying distribution of times-to-germination (reflecting the standard values at each time taken over all the times of observation). The theoretical form of this distribution, however, need not be known.

The assumption of proportional hazards may be invalid when the residual mean deviance (RMD) is high and there is a discrepancy between the predicted and observed germination curves. This could result from the imposition of treatments which change the underlying distribution of times-to-germination, such as the introduction of a lag to the day of first germination. Such treatments may be identified by inspection of the observed germination curves and groups of treatments in which the form of the underlying distribution is similar may be identified.
Where there are large numbers of treatments to be compared, the Cox model is then refitted to each of these groups of treatments to derive the effects of factors and treatments within the group. Changes in the distribution of the times-to-germination can then be investigated by comparing the complementary log-log of the distribution function \( \ln(-\ln[1-F_i(t_j)]) \) plotted against time for the various groups.

The data was edited before analysis to remove treatments with an average of less than 10% of viable seeds germinating per replicate and to consider in the analysis only those viable seeds which germinated during the germination period in the experiment. The removal of the treatments with few germinating seeds is justified because such a low proportion of seed germinating (which amounts to about five seeds) over 15 to 20 days cannot provide reliable estimations of treatment effects on times-to-germination.

The form of the germination curves which are observed for *E. pauciflora* shows that the limitation of the analysis to those viable seeds which germinated during the germination period is justified. Examination of germination curves (cumulative % germination with time) showed that the germination of a sample of seed tended to asymptote within 15 to 20 days of the beginning of germination and that further seed germination was not observed over longer periods. The dormancy of the seeds which did not germinate has been accounted for in the analysis of the germination capacity. The inclusion of viable, but dormant, seed in the population of seeds to be considered in the analysis of times-to-germination would have confounded both the rate and extent of germination.

The analyses of the times-to-germination are summarized in tables.
of deviance, and the tests for the significance of the treatment effects are identical to those outlined previously for germination capacity. Within the treatments, the investigation of the contributions of the various factors is based on the treatment constants, \( \hat{b}_i \), which are presented graphically, plotted against the treatment levels. Insight into the form of the probability distribution of times-to-germination is achieved by plots of the complementary log-log of the distribution function \( \ln(-\ln(1-F_i(t_j))) \) against time. An example and discussion of the analysis using the Cox regression model is presented in Section 3.1.2.

2.5 Estimation of the parameters of the temperature response

The comparison of temperature responses is an important part of the studies in Chapters 4 and 6. Initial attempts to describe the form of these responses by either quadratic or cubic functions of temperature were not successful.

An unbiased method of estimating the peak and breadth of the response was sought to assist in the interpretation of the observed responses. An algorithm (E02BAA/F) available from NAG (1983) was used to fit cubic splines to the data by a weighted least squares estimation procedure and the temperature at the peak and at 75% of the peak value (the lower and upper shoulders) were determined. The difference between the temperatures of the lower and upper shoulders is an estimate of the 'breadth' of the temperature response around the peak.
2.6 The form of summary of methods and results (Chapters 4, 5, 6)

The subtle differences in the treatments imposed on seed in Chapters 4, 5 and 6 are important for the interpretation of the results of the experiments in these chapters. In order to assist the reader in visualizing these differences the sequence of treatments imposed on the seed and the resultant germination capacities are depicted on yellow summary sheets at the end of these chapters.

The sequence of events in an experiment is associated with a time axis and can be read from left to right across the page. The exposure of seed to a treatment is marked by an arrow pointing vertically downwards (↑). The vertical axes in the summary diagrams represent temperature; periods of constant temperatures are represented by horizontal lines and temperature changes by vertical lines in these diagrams.

The following chapters detail investigations of the response of the seed dormancy and germination of *E. pauciflora* to a range of environmental factors and develop an understanding which can be used to interpret the behaviour of the seed in the field.
2.7 Summary of definitions

Germination: The growth of the embryo resulting in the breaking of the testa and emergence of the radicle.

Germination capacity: The proportion of viable seeds which germinate under a given set of conditions.

Germination curve: The cumulative % germination of a seed sample plotted against time.

Time-to-germination: The time taken for a seed to germinate, which, when considered over a population of seeds gives a distribution of times-to-germination.

Stratification: The exposure of the seed to moist cold (ca. 5°C) conditions resulting in the breaking of dormancy.

Dormancy: A state in which hydrated, viable seed fails to germinate in conditions which normally favour metabolism and growth.

Innate dormancy: The dormancy of the seed at dispersal.

Induced dormancy: The dormancy resulting from exposure of the seed to particular environmental conditions after dispersal which can only be reversed by stratification.

Enforced dormancy: The dormancy apparent during exposure to environmental conditions which may be alleviated by a return to conditions favourable for germination.

True dorm: The seed will not germinate under any conditions i.e. the seed is dormant under all conditions which are favourable for germination.

Relative dormancy: The seed will germinate in a certain, restricted range of conditions and is dormant in all other conditions.

Degree of dormancy: The proportion of viable seeds which are dormant under a given set of conditions (complement of the germination capacity).

Strength of dormancy: The length of the period of stratification, under a given set of conditions, required to break dormancy.
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THE
REGENERATION OF
Eucalyptus pauciflora Sieb. ex Spreng.
FROM SEED

by

Douglas Graham Abrecht

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

February 1985
CHAPTER 3

INNATE DORMANCY
CHAPTER 3

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

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

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

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

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

Grose (1960) found that maternal site factors such as altitude, aspect and dominance had no apparent effect on the dormancy of the seed of *E. delegatensis* in Victoria. Grose attributed this phenomenon to the absence of any selective advantage associated with an increase in dormancy in the range of environments considered; the major cause of deaths in autumn was found to be frost damage and all of the environments experienced severe radiation frosting during autumn.
The dormancy of seed may also be influenced by the conditions under which seed is shed from the capsule. The dormancy which may be induced in seed by environmental conditions prior to, and during, dispersal may not be strictly classified as innate; however, it is convenient to include it in this chapter. Grose (1950) noted that the dormancy of *E. delegatensis* seed shed from capsules, which were dried at high temperatures on the hearth by a fire, was greater than those which were permitted to dry in the air under ambient conditions. Boland et al. (1980) also mention that high temperatures may result in increased dormancy of the seed shed from *Eucalyptus* species. These observations confirm that environmental factors have the capacity to influence the dormancy of *Eucalyptus* seed during the process of seed shedding. The potential for changes in the dormancy of dry *E. pauciflora* seed directly after shedding is shown by the increases in dormancy of *E. pauciflora* seed held under laboratory conditions for 12 weeks following extraction (Boden 1957, quoting unpublished work by Pryor).

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

Methods:

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

Results and Discussion:

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

<table>
<thead>
<tr>
<th>TABLE 3.1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Summary of analysis of deviance for the germination capacity of seed collected at four altitudes and germinated at 15°C.</strong></td>
</tr>
</tbody>
</table>

<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>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>24</td>
<td>40.38</td>
<td></td>
<td>1.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source</td>
<td>20</td>
<td>21.04</td>
<td>1.05</td>
<td>19.34</td>
<td>p&lt;0.005</td>
<td></td>
</tr>
</tbody>
</table>

Source = Seed source
Innate dormancy:

![Graph showing germination capacity versus altitude]

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

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

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

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

Methods:

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

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

The dormancy of seed from the various size classes was assessed by analysis of germination capacity following either 0 or 20 days stratification. The method used for seed preparation varied from that already described (Chapter 2) in that the cleaning step was omitted and
the seed from the various size classes was divided into units depending on the amount of seed available (0.2 to 0.5 g).

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

Results and Discussion:

(i) Seed size and altitude.

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

There were equal amounts of seed from each source used in the experiment and the analysis should, therefore, have removed the main effect of seed source. However, the weighing of the seed left on the sieves had greater accuracy (±0.00005 g) than the original weighing of the replicates (±0.05 g) and this greater accuracy resulted in differences in the mean weight of seed from the different sources.
TABLE 3.2

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

<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>Size Source</td>
<td>8</td>
<td>71.675</td>
<td>8.959</td>
<td>8367.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Size Source</td>
<td>4</td>
<td>0.075</td>
<td>0.018</td>
<td>17.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Size Source</td>
<td>32</td>
<td>13.469</td>
<td>0.421</td>
<td>393.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residual</td>
<td>126</td>
<td>0.135</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>170</td>
<td>85.355</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Size = Sieve opening (2000, 1680, 1410, 1190, 1000, 841, 707, 595, 0 μm)
Source = Seed source (960 m, 1340 m, 1510 m, 1730 m, 2000 m)

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

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

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

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

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

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

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

![Graph showing germination capacity vs sieve mesh size for different altitudes and treatment lengths](image)

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

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

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

\[ \text{logit}(G) = \mu \] ....(1)

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

\[ \text{logit}(G_{ijk}) = \mu + SZ_i + SO_j + ST_k \] ....(2)

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

1000 \( \mu m \) < size\(_1\) < 1190 \( \mu m \) < size\(_2\) < 1410 \( \mu m \) < size\(_3\) < 1680 \( \mu m \) respectively were used.

The analysis of deviance differs from an analysis of variance in that the comparisons are not orthogonal, and therefore the significance of a factor or interaction between factors cannot be assessed in isolation, but rather is assessed by the change in deviance when this element is removed from the model. In practice, this means that the model (2) must be refitted with each of SZ, SO, ST removed in order to determine the change in deviance due to each of these factors.
The observed changes in deviance are usually compared with the critical value of \( \chi^2 \) associated with the change in the degrees of freedom; however, the analysis showed that this test is not appropriate because there is evidence that the data in this experiment is not Binomially distributed. When the model (4), which takes account of all the known sources of variation in the experiment is fitted to the data, the variation remaining cannot be wholly attributed to the Binomial distribution, since the residual mean deviance (RMD) is greater than 1 (RMD = 301.4/96 = 3.14) (Table 3.3). In this case, a more conservative test of significance is employed which allows for the residual variation not accounted for by the Binomial distribution. The test is similar to the F-test of analysis of variance, the F-ratio being calculated as the ratio of the mean change in deviance (MCD) to the residual mean deviance (RMD) for the full model.

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

\[
\text{logit}(G_{ijk}) = \mu + SZ_i + SO_j + ST_k + (SZ\cdot SO)_{ij} + (SZ\cdot ST)_{ik} + (SO\cdot ST)_{jk} \quad \ldots(3)
\]

Removal of each of the two-factor interactions from the model shows that only \([SZ\cdot ST]\) (p=0.004) and \([SO\cdot ST]\) (p<0.001) are significant (Table 3.3). The response to stratification was thus different for seed of different sizes and seed from different sources, but the response of germination capacity to seed size was not significantly different for the four seed sources \([SZ\cdot SO]\) (p=0.14)).
TABLE 3.3

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

<table>
<thead>
<tr>
<th>Model terms</th>
<th>Residual d.f.</th>
<th>Deviance</th>
<th>RMD d.f.</th>
<th>Deviance</th>
<th>Change d.f.</th>
<th>MCD</th>
<th>F Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>119</td>
<td>3241.0</td>
<td>27.23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main Factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+SZ +SO +ST</td>
<td>113</td>
<td>566.3</td>
<td>5.03</td>
<td>6</td>
<td>2672.7</td>
<td>445.45</td>
<td>141.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>-SZ</td>
<td>115</td>
<td>663.9</td>
<td>5.77</td>
<td>2</td>
<td>95.6</td>
<td>47.80</td>
<td>15.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>-SO</td>
<td>116</td>
<td>1700.0</td>
<td>14.65</td>
<td>3</td>
<td>1131.7</td>
<td>377.23</td>
<td>120.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>-ST</td>
<td>114</td>
<td>1984.0</td>
<td>17.40</td>
<td>1</td>
<td>1415.7</td>
<td>1415.7</td>
<td>450.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Main Factors +Interactions (2 factor)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+SZ.SO+SZ.ST+SO.ST</td>
<td>102</td>
<td>344.8</td>
<td>3.38</td>
<td>11</td>
<td>223.5</td>
<td>20.31</td>
<td>6.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>-SZ.SO</td>
<td>108</td>
<td>375.9</td>
<td>3.48</td>
<td>6</td>
<td>31.1</td>
<td>51.18</td>
<td>1.7</td>
<td>0.141</td>
</tr>
<tr>
<td>-SZ.ST</td>
<td>104</td>
<td>381.0</td>
<td>3.66</td>
<td>2</td>
<td>36.2</td>
<td>18.10</td>
<td>5.8</td>
<td>0.004</td>
</tr>
<tr>
<td>-SO.ST</td>
<td>105</td>
<td>467.8</td>
<td>4.46</td>
<td>3</td>
<td>123.0</td>
<td>41.00</td>
<td>13.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Main Factors +Interactions (2 factor +3 factor)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+SZ.SO.ST</td>
<td>96</td>
<td>301.4</td>
<td>3.14</td>
<td>6</td>
<td>43.4</td>
<td>7.23</td>
<td>2.3</td>
<td>0.040</td>
</tr>
</tbody>
</table>

SZ = Sieve opening (1000, 1190, 1410 μm)
SO = Seed source (960 m, 1310 m, 1510 m, 1730 m)
ST = Duration of stratification (0, 20 days)
1 compared with SZ+SO+ST
2 compared with SZ+SO+ST+SZ.SO+SZ.ST+SO.ST
RMD= Residual mean deviance
MCD= Mean change in deviance

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

\[
\text{logit}(G_{ijk}) = \mu + Z_i + S_j + ST_k + (SZ\cdot SO)_{ij} + (SZ\cdot ST)_{ik} + (SO\cdot ST)_{jk} + (SO\cdot SZ\cdot ST)_{ijk} \ldots (4)
\]

The three factor interaction [SZ.SO.ST] was not significant (Table 3.3, p=0.04) and therefore the most appropriate model (5) for the data
is the one including the mean, main effects and the two factor interactions which are significant (Note: except where otherwise stated p=0.01 is used as the critical level for significance).

\[
\logit(G_{ijk}) = \mu + SZ_i + SO_j + ST_k + (SZ\cdot ST)_{ik} + (SO\cdot ST)_{jk} \quad \ldots (5)
\]

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

### Table 3.4

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

<table>
<thead>
<tr>
<th></th>
<th>ESTIMATE</th>
<th>s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-0.832</td>
<td>0.087</td>
</tr>
<tr>
<td>Size_1</td>
<td>-0.264</td>
<td>0.084</td>
</tr>
<tr>
<td>Size_2</td>
<td>-0.106</td>
<td>0.121</td>
</tr>
<tr>
<td>Source_1</td>
<td>1.553</td>
<td>0.112</td>
</tr>
<tr>
<td>Source_2</td>
<td>-0.648</td>
<td>0.114</td>
</tr>
<tr>
<td>Source_3</td>
<td>-0.430</td>
<td>0.113</td>
</tr>
<tr>
<td>Source_4</td>
<td>3.745</td>
<td>0.174</td>
</tr>
<tr>
<td>Strat_1</td>
<td>1.810</td>
<td>0.218</td>
</tr>
<tr>
<td>Source_1 Strat_1</td>
<td>-1.906</td>
<td>0.193</td>
</tr>
<tr>
<td>Source_2 Strat_2</td>
<td>-1.944</td>
<td>0.193</td>
</tr>
<tr>
<td>Size_2 Strat_2</td>
<td>-0.614</td>
<td>0.118</td>
</tr>
<tr>
<td>Size_3 Strat_2</td>
<td>-0.317</td>
<td>0.163</td>
</tr>
</tbody>
</table>

The coefficients in Table 3.4 relate the covariates to the logit of germination capacity of the seed; for example, the germination capacity, \( G \), of seed of Size_2 (1190 \( \mu m < \) size < 1410 \( \mu m \)), collected at 960 m (Source_1) and stratified for 20 days (Strat_2) is given by
\[ \logit(G_{2,2}) = \mu + S_{2} + S_{1} + S_{2} + (S_{0}.S_{1})_{1,2} + (S_{Z}.S_{T})_{2,2} \]

\[ = -0.832 -0.264 + 0 + 3.745 + 0 -0.614 \]

\[ = 2.037 \]

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

Since, \[ \logit(G) = \ln \frac{G}{1-G} \]

then, \[ G_{2,2} = \frac{e^{0.0372}}{1+e^{0.0372}} \]

\( G = 0.88 \)

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

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

### TABLE 3.5

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

<table>
<thead>
<tr>
<th>STRATIFICATION</th>
<th>a. r.</th>
<th>b. n</th>
<th>c. r/n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>200</td>
<td>678</td>
<td>.29</td>
</tr>
<tr>
<td>20</td>
<td>693</td>
<td>730</td>
<td>.93</td>
</tr>
<tr>
<td>0</td>
<td>533</td>
<td>787</td>
<td>.65</td>
</tr>
<tr>
<td>20</td>
<td>817</td>
<td>868</td>
<td>.91</td>
</tr>
<tr>
<td>0</td>
<td>214</td>
<td>646</td>
<td>.17</td>
</tr>
<tr>
<td>20</td>
<td>1261</td>
<td>1195</td>
<td>.54</td>
</tr>
<tr>
<td>0</td>
<td>307</td>
<td>811</td>
<td>.19</td>
</tr>
<tr>
<td>20</td>
<td>1594</td>
<td>1684</td>
<td>.48</td>
</tr>
</tbody>
</table>

For example, in the case of the interaction between seed source and stratification [S0.ST] the predicted number of germinated seeds is determined for each source X stratification class by summing the predicted values of 'r' for each of the four seed sizes in each source X stratification class (Table 3.5a). The values of 'n' from the original data are then summed over the same treatments (Table 3.5b) and the ratios of the corresponding cells in Table 3.5a and Table 3.5b are shown in Table 3.5c.

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

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

The differences in the germination capacity of the seed in relation to seed source are broadly consistent with the other data for changes in dormancy with altitude. However, in this particular experiment care should be taken in the interpretation of the interaction between seed source and stratification because the proportion of the seed sizes in the raw seed has been changed by the sampling procedure (replicates of 0.4 g of seed regardless of seed size). The analysis of the source X stratification interaction considers the effect averaged over all the seed sizes.
Any differences in the dormancy of the seed sources (due, for example, to varying proportions of seed of different sizes, with different characteristics of dormancy) may be unreliably represented by this interaction. The high germination capacity of unstratified seed from 1310 m is unlikely to be due to changes in the representation of seed of different sizes because the germination capacity is uniformly high, regardless of seed size (Figure 3.3). It seems that the seed from this source had become less dormant in the year between the experiment reported in Section 3.1 and this experiment.

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

Figure 3.5 The predicted effect on germination capacity of seed size and duration of stratification [SZ-ST], averaged over the four altitudes of seed source.
However, the largest seed stratified for 20 days showed a higher germination capacity than the smaller seed. The germination capacity of the medium-sized seed (1190 μm < size < 1410 μm) does not conform to this trend since it had a lower germination capacity than either the smaller or the larger seed. The low germination capacity of middle-sized seed from the highest seed source (1730 m) is likely to be the cause of this inconsistency in the response of germination capacity to seed size since the seed from all other sources conformed to the trend (Figure 3.3).

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

(iii) Seed size and times-to-germination

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

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

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

The lack of coincidence of observations, which may occur when the germination periods of the treatments in an experiment are not concurrent, can be overcome in two ways, depending on whether the reduction of the data set can be achieved without disrupting the analysis. Generally, the approach taken in this thesis is to fit the Cox model to a reduced data set formed by increasing the time interval between germination counts.
The risk of germination is then considered on the days of observation which are common to the stratification treatments being compared. However, in cases such as this where the set of days of observation which are common to all stratification treatments (1, 2, 3, 4, 5, 6, 7, 8, 9, 11) was not sufficient to cover the full germination curve. The times-to-germination of each of the stratification treatments were considered in separate two-factor (Size and Source) Cox models rather than a single three factor (Size, Source, Stratification) model.

### TABLE 3.6

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

<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><strong>0 days Stratification</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>738</td>
<td>4192.0</td>
<td>5.68</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>722</td>
<td>884.5</td>
<td>1.23</td>
<td>16</td>
</tr>
<tr>
<td>+Size +Source</td>
<td>717</td>
<td>735.2</td>
<td>1.02</td>
<td>5</td>
</tr>
<tr>
<td>+Size .Source</td>
<td>711</td>
<td>705.6</td>
<td>0.99</td>
<td>6</td>
</tr>
<tr>
<td><strong>20 days Stratification</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>701</td>
<td>5943.0</td>
<td>8.48</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>689</td>
<td>1186.0</td>
<td>1.72</td>
<td>12</td>
</tr>
<tr>
<td>+Size +Source</td>
<td>684</td>
<td>1015.0</td>
<td>1.48</td>
<td>5</td>
</tr>
<tr>
<td>+Size .Source</td>
<td>678</td>
<td>981.2</td>
<td>1.45</td>
<td>6</td>
</tr>
</tbody>
</table>

The Cox model uses a complementary log-log link function to relate the probability of a seed, in factor combination \(ij\), germinating on day \(m\), \(P_{ij}(t_m)\) to a linear combination of covariates (Section 2.4.1). The selection of the model follows the procedure discussed in Section 3.1.2 (iii) in which the importance of the terms added to the model is
judged from the reduction in the residual deviance. The procedure used to fit these models is similar to that used in the analysis of germination capacity and, again, the order of fitting the covariates is important because the treatment effects are not orthogonal. Time is always fitted first and the treatments are then fitted with their interactions (Table 3.6).

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

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

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

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

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

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

<table>
<thead>
<tr>
<th>TERM</th>
<th>ESTIMATE</th>
<th>s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size₂</td>
<td>-0.523</td>
<td>0.089</td>
</tr>
<tr>
<td>Size₁</td>
<td>-0.839</td>
<td>0.229</td>
</tr>
<tr>
<td>Source₁</td>
<td>0.267</td>
<td>0.067</td>
</tr>
<tr>
<td>Source₂</td>
<td>-0.263</td>
<td>0.072</td>
</tr>
<tr>
<td>Source₃</td>
<td>0.158</td>
<td>0.089</td>
</tr>
<tr>
<td>Size₂, Source₁</td>
<td>0.208</td>
<td>0.120</td>
</tr>
<tr>
<td>Size₁, Source₁</td>
<td>-0.196</td>
<td>0.264</td>
</tr>
<tr>
<td>Size₂, Source₂</td>
<td>0.409</td>
<td>0.123</td>
</tr>
<tr>
<td>Size₁, Source₂</td>
<td>0.556</td>
<td>0.284</td>
</tr>
<tr>
<td>Size₂, Source₃</td>
<td>0.044</td>
<td>0.127</td>
</tr>
<tr>
<td>Size₁, Source₃</td>
<td>0.331</td>
<td>0.249</td>
</tr>
</tbody>
</table>

The treatment constants are derived by summing the relevant coefficients of the model (Table 3.7). For example, the treatment constant, \( \beta_{2,1} \), for the seed of \( \text{Size}_2 \), \( 1190 \, \mu m < \text{ size } < 1410 \, \mu m \), from \( \text{Source}_1 \), Waste point (960 m), is given by

\[
\beta_{2,1} = \text{Size}_2 + \text{Source}_1 + \text{Size}_2, \text{Source}_1 = -0.5225 + 0 + 0 = -0.5225
\]

The standard errors of the treatment constants are more difficult to determine, and the procedure depends on whether the full model is appropriate. If the full interaction term is significant, and therefore the full model is appropriate, then the treatment constants and the standard errors are derived by simply fitting all the treatment combinations as separate treatments in the model. The coefficients of the model and their standard errors are then equivalent to the treatment constants and their standard errors. In this example, the full model is appropriate and the three sizes and four sources can be
fitted as twelve treatments. If some subset of the full model was more appropriate then the standard errors would have been calculated from the variance/covariance matrix resulting from the fit of the model. The treatment constants and their standard errors (as error bars) are presented for both 0 days and 20 days stratification treatments in Figure 3.6.

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

The times-to-germination of seed stratified for 20 days have a very different response to seed size and altitude of seed source compared to unstratified seed. Generally, the larger seed exhibits more rapid germination (Figure 3.6b). There does not appear to be any trend in the times-to-germination of stratified seed with altitude, however, the response of times-to-germination to increases in seed size is different in the four seed sources. The difference between the times-to-germination of the largest and the smallest seed has a tendency to become smaller with increases in altitude. The reduction in the difference is brought about by a reduction in the rate of germination of the largest seed and an increase in the rate of germination of the smallest seed.
Innate dormancy: 63.

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

The treatment constants can be used to derive the germination curves by calculating the values of $F_{ij}(t_m)$ (from equation 7) for the desired $ij$, over the range of time $t_i$ to $t_m$. The constants, $\gamma_m$, involving time-to-germination, are the coefficients of times of observation plus the constant term (time constants), resulting from the fit of the Cox model. The first step in the calculation of the predicted germination curve is to calculate the cumulative sum of the exponentials, $CE_m$, of the time constants for each of the times of observation (8).
\[ CE_m = \exp(\gamma_1) + \exp(\gamma_2) + \ldots + \exp(\gamma_m) \]  

...(8)

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

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

\( \beta_{ij} \) is the treatment constant associated with the \( ij \)th treatment combination.

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

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

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

\[ \ln(-\ln[1-F_{ij}(t_m)]) = \ln[\exp(\gamma_1) + \exp(\gamma_2) + \ldots + \exp(\gamma_m)] \]  

...(9)
Figure 3.7 The predicted (lines) and observed (symbols) germination curves for three sizes of seed collected at 1310 metres and stratified for 0 (---) or 20 (----) days prior to germination.

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

The complementary log-log of $F_{ij}(t_m)$, $\ln(-\ln[1-F_{ij}(t_m)])$, can be calculated readily from the time constants, $\gamma_m$, of the linear model. The differences between the germination curves of unstratified seed and
seed stratified for 20 days (Figure 3.7) are associated with differences in the distribution of times-to-germination, since Figure 3.8 shows that the seed which was not stratified has a lag time of about 3 days during which the risk of germination is low compared to the seed stratified for 20 days.

Discussion:

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

Comparison of the strength of dormancy of seed of the same size collected at different altitudes shows that the increase in the strength of dormancy with altitude cannot be wholly associated with the reduction in the size of the seed. Seed of the same size tends to show a similar degree of dormancy at dispersal (innate dormancy) but an increase in the strength of dormancy with altitude of seed source. The observed differences in the strength of dormancy could be an artefact of a method which uses a constant temperature to assess the strength of dormancy. Such an artefact could develop if, for example, there is a reduction in the temperature suitable for the breaking of dormancy with
altitude of seed source. This would lead to a reduction in breaking of dormancy under the standard conditions. The possibility of further differentiation in the behaviour of seed from different sources due to variation in the temperature response of the breaking of dormancy with altitude is investigated in Section 4.2.1 and this shows that seed from 1230 m to 1910 m both have similar responses to the temperature of stratification. Thus the differences in the strength of dormancy are likely to be real. Increased strength of dormancy of seed from higher altitudes imposes a requirement for a longer period of cold conditions before germination may occur. Given the increasing duration of cold conditions during winter with altitude, this may be a means of preventing germination during winter, when the chances of seedling survival are very low.

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

The studies in this section have investigated the relationship between the absolute size and dormancy of seed from a range of altitudes and have shown that the strength of dormancy of seed is inversely related to seed size. The dormancy of seed of the same size from different altitudes, however, became stronger as the altitude of the seed source increased. It is possible that the dormancy of a seed is related to its size, relative to the other seeds in the sample (altitude) from which it comes. This line of investigation could not
be pursued because there were no commercially available sieves which would give a greater number of seed size classes in the range of classes containing viable seeds.
3.3 Changes in dormancy with temperature of seed extraction.

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

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

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

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

Methods:

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

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

(i) The temperature environment during seed shedding

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

<table>
<thead>
<tr>
<th>NOMINAL TEMP.</th>
<th>5°C</th>
<th>15°C</th>
<th>30°C</th>
<th>40°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIN</td>
<td>MAX</td>
<td>MIN</td>
<td>MAX</td>
</tr>
<tr>
<td>Smiggins AV.</td>
<td>13.9</td>
<td>18.3</td>
<td>28.5</td>
<td>31.4</td>
</tr>
<tr>
<td>SD.</td>
<td>2.99</td>
<td>1.49</td>
<td>3.89</td>
<td>5.24</td>
</tr>
<tr>
<td>Thredbo AV.</td>
<td>1.3</td>
<td>5.8</td>
<td>11.7</td>
<td>15.7</td>
</tr>
<tr>
<td>SD.</td>
<td>1.18</td>
<td>0.99</td>
<td>0.40</td>
<td>1.85</td>
</tr>
</tbody>
</table>

Average MAXimum and MINimum temperatures based on daily records
1 no daily records of temperature (within the range 0-6°C)
AV average (maximum or minimum) temperature (°C)
SD standard deviation

(ii) Seed shedding.

The desiccation of the capsules and release of seed was delayed by about 3 days at 5°C compared to higher temperatures. In order to ensure that the seed sample used in the experiment was similar for all extraction temperatures, the first 16 g of seed from each treatment was
used in the germination tests. The rate of seed shedding (Figure 3.9) increased with increasing temperature in the treatments where drying was assisted by the passage of air through the capsules. The slower rate of shedding of the seed at 40°C was also associated with a slower rate of desiccation of the capsules, indicating that the rate of loss of water plays an important role in seed shedding.

![Graph showing seed shedding over time for different temperatures](image)

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

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

The release of seed from the capsule is associated with the separation of the seed from the placenta, widening of the loculi and
The relationship between capsule weight and the weight of seed shed from capsules collected at Thredbo and desiccated at a range of temperatures.

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

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

<table>
<thead>
<tr>
<th>Table 3.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summary of analysis of deviance for the germination capacity of seed from two sources extracted at four temperatures and germinated after four periods of stratification.</td>
</tr>
</tbody>
</table>

<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>THREDBO</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>2159</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+Temp +Strat</td>
<td>73</td>
<td>169.2</td>
<td>2.32</td>
<td>6</td>
<td>1989.8</td>
<td>331.63</td>
</tr>
<tr>
<td>+Temp .Strat</td>
<td>64</td>
<td>111.1</td>
<td>1.74</td>
<td>9</td>
<td>58.1</td>
<td>6.46</td>
</tr>
<tr>
<td>SMIGGINS HOLES</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>2643</td>
<td>33.46</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+Temp +Strat</td>
<td>73</td>
<td>87.85</td>
<td>1.20</td>
<td>6</td>
<td>2555.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>+Temp .Strat</td>
<td>64</td>
<td>73.99</td>
<td>1.16</td>
<td>9</td>
<td>13.86</td>
<td>=0.127</td>
</tr>
</tbody>
</table>

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

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

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

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

The germination capacity of seed from Smiggins Holes did not exhibit the same strong interaction between the temperatures of extraction and stratification as that observed in Thredbo seed because the effect of temperature of seed shedding at 5°C and 40°C was not as marked as that in seed from Thredbo. This observation concurs with those of Gross (1985) for Eucalyptus seed, in which the strength of dormancy induced by treatment was greater if the innate strength of dormancy of the seed was greater.

(iv) Times-to-germination

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

The effect of the duration of stratification in reducing the times-to-germination is evident from a consideration of the treatment constants of the models (Table 3.11): stratification for periods of up to 4 weeks caused an increase in the rate of germination, whereas, stratification for periods longer than 4 weeks caused a decrease in the
TABLE 3.10

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

<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 CMD</th>
<th>F Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>THREDBO</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>671 3476</td>
<td>658 1007</td>
<td>1.53 13</td>
<td>2469</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+Temp+Strat</td>
<td>653 824.6</td>
<td>656 835.7</td>
<td>1.26 5</td>
<td>182.4</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>-Temp</td>
<td>655 990.5</td>
<td></td>
<td>1.51 2</td>
<td>165.9</td>
<td>0.011^2</td>
<td></td>
</tr>
<tr>
<td>-Strat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p&lt;0.001^2</td>
<td></td>
</tr>
<tr>
<td>+Temp.Strat</td>
<td>647 820.2</td>
<td></td>
<td>1.27 6</td>
<td>4.4</td>
<td>0.625</td>
<td></td>
</tr>
</tbody>
</table>

| **SMIGGINS HOLES** |                        |              |                      |            |         |       |
| Total            | 654 5820               | 641 2641     | 4.12 13              | 3179       | 244.5   | 103.62 | <0.001 |
| Time             |                        |              |                      |            |         |       |
| +Temp+Strat      | 636 1496               | 639 1507     | 2.359 5              | 1145       | 229.0   | 97.03  | <0.001 |
| -Temp            | 638 2636               |              | 4.132 2              | 1140       | 570.0   | 241.52 | <0.001 |
| -Strat           |                        |              |                      |            | 1.55    | 0.198^3|       |
| +Temp.Strat      | 630 1487               |              | 2.36 6               | 11         | 1.8     | 0.78   | 0.591  |

1 0 weeks stratification excluded because of low numbers of germinated seeds.
2 compared with Thredbo [+Temp+Strat]
3 compared with Smiggins [+Temp+Strat]

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

Further understanding of this effect may be gained from a more detailed examination of the germination curves (Figure 3.12). The breaking of the dormancy of a seed sample is associated with an increase in germination capacity, a shorter time to the appearance of
the first germinated seed, and a greater rate of germination for the seed sample. The seed stratified for 6 weeks exhibited the first two characteristics but then shows a decreased rate of germination; this is consistent with a depletion of the seeds' reserves at the stratification temperature, due to respiration associated with tissue maintenance.

The germination curves predicted by the Cox model show greatest deviation from the observed germination curves (Figure 3.12) for the seed stratified for a period of 2 weeks. The lack of fit accounts for the large residual mean deviance observed in the times-to-germination of seed from Smiggins Holes (RMD=2.35, Table 3.10) and, to a lesser extent, in the seed from Thredbo (RMD=1.27, Table 3.10). In both of these cases the seed set to germinate after 2 weeks stratification showed a marked delay in time to first germination. This delay may be the cause of the lack of fit of the model, and is an indication of a
TABLE 3.11

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

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>THREDBO</th>
<th>\text{Estimate}</th>
<th>\text{s.e.}</th>
<th>\text{SMIGGINS HOLES}</th>
<th>\text{Estimate}</th>
<th>\text{s.e.}</th>
</tr>
</thead>
<tbody>
<tr>
<td>5°C</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>15°C</td>
<td>0.179</td>
<td>0.077</td>
<td></td>
<td>-0.094</td>
<td>0.063</td>
<td></td>
</tr>
<tr>
<td>30°C</td>
<td>0.206</td>
<td>0.076</td>
<td></td>
<td>0.066</td>
<td>0.061</td>
<td></td>
</tr>
<tr>
<td>40°C</td>
<td>0.024</td>
<td>0.081</td>
<td></td>
<td>-0.101</td>
<td>0.060</td>
<td></td>
</tr>
</tbody>
</table>

Stratification

| 2 weeks  | 0       | 0               |             | 0                     | 0               |             |
| 4 weeks  | 1.110   | 0.096           |             | 2.024                 | 0.069           |             |
| 6 weeks  | 0.912   | 0.092           |             | 1.499                 | 0.066           |             |

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

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

break-down in the assumption of proportional hazards.

Analysis of variance shows that the day on which the first seed germinated is significantly affected by stratification (Thredbo and Smiggins both p<0.001) but not by temperature during seed shedding (Thredbo, p=0.030; Smiggins Holes p=0.319). The possibility that the difference in the curves could be explained by the shift in the day to first germination was tested by re-fitting the Cox model to the data with the first day of germination predicted by the analysis of variance
as the day of first record. In both cases the mean deviation of the new model was greater than that in Table 3.10, indicating that a displacement in time could not account for the differences in the germination curves for seed stratified for 2 weeks on the one hand, and 4 and 6 weeks on the other.

Discussion:

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

The small changes in dormancy observed in these experiments show that the innate dormancy exhibited by *E. pauciflora* is largely determined before dispersal and is not changed markedly by the range of temperatures likely to be encountered during seed shedding. Seed dispersed under the low temperature conditions of winter or the hot conditions in summer may show a slight increase in dormancy. The relevance of these small and transient effects at dispersal will be discussed in relation to the large and more persistent changes in dormancy which may be induced by the conditions encountered in the soil (Chapters 4, 5 and 6).
Stratification was found to increase the germination capacity and to decrease the time-to-germination of seed from both Smiggins Holes and Thredbo. The influence of temperature and duration of exposure to conditions which break dormancy will be considered in more detail in Section 4.3. The strength of the dormancy of seed collected at Thredbo was greater than that of seed collected at Smiggins Holes, confirming the results of the previous section which showed that seed from sources nearer to treeline have a stronger dormancy than seed from lower altitudes.

Seed from both Thredbo and Smiggins Holes has a high degree of innate dormancy so it seems unlikely that dormancy will increase to the degree noted by Boden (1957) following storage. Boden observed a decline in the germination capacity of _E. pauciflora_ seed from around 50% immediately after shedding to 20% after 5 weeks of storage. This agreed with Pryor's observations of a decline in germination capacity from 47% 1 week after seed shedding to 17% after 12 weeks of storage (unpublished data quoted in Boden 1957). The innate dormancy of the seed considered in this section offers little possibility of changes in dormancy of the magnitude reported by Boden.
3.3.2 The treatment of capsules at high temperatures

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

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

The state of capsules observed after a wild-fire was found to vary from no visible damage to complete incineration; apparently, a large range of temperature conditions may be experienced by capsules during a fire. Grose (1960) collected seed from capsules which had sustained various degrees of damage during a slash fire in *E. delegatensis* and showed that there was an increase in the proportion of non-viable seed as the capsule damage increased in severity from no scorch to severe charring. Temperatures experienced by seed inside a capsule during a fire will tend to be lower than the temperature outside it, both
because of the low thermal diffusivity of the woody capsule walls and because of the short duration of exposure to high temperatures. There is, however, no information available on the absolute temperatures experienced by seed within capsules during a fire (A.M. Gill, CSIRO, Division of Plant Industry, pers. comm.).

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

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

Methods:

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

The desiccation rate of the capsules and the release of seed were monitored daily by shaking each sample to release seed, separating the seed and capsules by sieving, and then weighing both the seed and
capsules. The separated dry seed was stored in a sealed container at 5°C until seed extraction was completed.

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

Results and Discussion:

(i) Seed shedding from the capsules

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

The yield of seed for a given reduction in the weight of capsules declined as the temperature at which fresh capsules were desiccated was increased from 15°C to 40°C (Section 3.3.1). The trend for lower yields of seed for a given capsule weight is also apparent in the seed shed from capsules exposed to 80°C for increasing times (Figure 3.14). The explanation is likely to be similar, with the longer duration of treatment at 80°C resulting in more rapid desiccation of the woody part of the capsule compared to the ovary wall. Since it is the desiccation of the ovary wall which determines the shedding of the
Figure 3.13  
The shedding of seed (---) and desiccation of capsules (----) treated at 80°C for either 0 or 260 minutes before extraction under laboratory conditions. Arrows show the state at the end of treatment at 80°C.

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

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

(ii) Germination capacity

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

The temperatures experienced inside capsules during a fire are likely to be highly variable. This experiment was designed to provide an estimate of the changes which are likely to occur over a wide range of conditions. The maximum temperature experienced by the majority of the capsules in the canopy of a tree, during a high intensity fire, are unlikely to exceed 80°C for more than 10 minutes (A.M. Gill CSIRO, Division of Plant Industry pers. comm.). The temperatures experienced by seed inside the capsules are unknown, but if they do not exceed the temperatures experienced by the seed in the capsules held at 80°C for 10 minutes they are unlikely to have a marked influence on the
TABLE 3.12

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

<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>Total</td>
<td>79 2336</td>
<td>29.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Durn +Strat</td>
<td>71 89.94</td>
<td>1.27</td>
<td>8 2246.06</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Durn .Strat</td>
<td>64 65.38</td>
<td>1.02</td>
<td>7 24.56</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

Durn. = Duration of treatment at 80°C
Strat = Duration of stratification (1.5 weeks)

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

![Graph](image)

**Figure 3.15** The germination capacity of seed extracted from capsules exposed to treatment at 80°C
(iii) Times-to-germination

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

TABLE 3.13

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

<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>Time</td>
<td>211</td>
<td>307.1</td>
<td>1.46</td>
<td></td>
</tr>
<tr>
<td>Time +Durn</td>
<td>207</td>
<td>252.4</td>
<td>1.22</td>
<td>54.70</td>
</tr>
</tbody>
</table>

Time = Day of observation
Durn = Duration of treatment at 80°C

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

The germination curves confirm that the effect of heating the capsules for 22 minutes or more prior to seed shedding results in an increase in the times-to-germination even after 5 weeks stratification (Figure 3.17).
Figure 3.16 The treatment constants of the Cox model for times-to-germination of seed extracted from capsules following exposure to 80°C for a range of times.

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

(iii) Seed mortality

The experimental design would not permit an assessment of the mortality of seed associated with increasing duration of treatment at
80°C. Samples of equal weight were drawn from seed cleaned after the high temperature treatment; an analysis of the total numbers of viable seed in the various treatments could, therefore, reflect either a real effect of the treatment, or an artefact of seed cleaning and sampling caused by differences in seed characteristics other than viability (for example, seed water content or seed weight).
3.4 Treatment of dry seed at elevated temperatures

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

Methods:

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

Results:

(1) Germination capacity

The initial analysis showed that there was a significant effect of high temperature on germination capacity. However, further investigation showed that this result was biased by a single replicate
In which the number of germinated seeds was much lower than in the other replicates. There was no apparent reason for the low germination. When this replicate was excluded from the analysis the effect of treatment at 80°C on germination capacity was not significant (Table 3.14, p=0.558) and showed no trend with duration of treatment (85%, 78%, 83%, 81% for 0, 30, 60, 120 minutes respectively).

**TABLE 3.14**

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

<table>
<thead>
<tr>
<th>Model terms</th>
<th>Residual d.f. deviance</th>
<th>RMD</th>
<th>Change d.f. deviance</th>
<th>CHD</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>18</td>
<td>54.75</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time at 80°C</td>
<td>15</td>
<td>47.84</td>
<td>3.19</td>
<td>3</td>
<td>6.91</td>
<td>2.30</td>
</tr>
</tbody>
</table>

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

The sensitivity of both the induction of dormancy and mortality of the seed to the water content has been shown by several authors. Grose (1963) also found that the rate at which dormancy was induced in *E. delegatensis* seed was dependent on the water content of the seed at temperatures of 27°C and 35°C. Cunningham (1960) showed that the mortality of *E. regnans* seed at high temperatures depended on the water content of the seed.
The water content of the seed contained in freshly-picked capsules has not been investigated. The water content of the capsule is high and it could be expected that the seed would be held in a moist environment within the capsule which may prevent the desiccation of the seed.

(ii) Times-to-germination

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

| TABLE 3.15 |
| Summary of analysis of deviance for times-to-germination of seed following treatment at 80°C. |

<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>Time</td>
<td>209</td>
<td>292.6</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Time + Durn</td>
<td>206</td>
<td>271.9</td>
<td>1.3</td>
<td>20.70</td>
</tr>
</tbody>
</table>

Time = Day of observation
Durn = Duration of treatment at 80°C

Increasing the duration of exposure of dry seed to 80°C caused a consistent and significant increase in the times-to-germination which was expressed as a reduction in the rate of germination (Figure 3.18, Table 3.15). The sensitivity of the Cox model in the analysis of times-to-germination is well demonstrated in this experiment in which the differences between the germination curves of the various treatments is small (Figure 3.19).
Figure 3.18 The treatment constants of the Cox model for the times-to-germination of seed treated at 80°C, stratified and then germinated at 15°C.

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

(iii) Seed mortality

Seed mortality was assessed in this experiment by analysing the total numbers of viable seeds. All treatments started with similar
numbers of seeds and, therefore, any variation in the numbers of viable seeds can be attributed to the heat treatment. There was no evidence of mortality in dry seed exposed to temperatures of 80°C for periods up to two hours (Table 3.16).

**TABLE 3.16**

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

<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>Time at 80°C</td>
<td>3</td>
<td>118.8</td>
<td>39.6</td>
<td>0.202</td>
<td>p=0.894</td>
</tr>
<tr>
<td>Residual</td>
<td>16</td>
<td>3130.0</td>
<td>96.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>3248.8</td>
<td>171.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion:

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

Cunningham (1960) demonstrated the importance of the seed moisture content on the observed response of *E. regnans* to high temperatures and noted that mortality of seed in the field could be expected when high soil temperatures follow rain in summer.
The effect of sub-lethal treatments at high temperatures was investigated by Grose (1960), who found that the proportion of dormant seed, and the strength of dormancy both increased as a result of treatment of *E. delegatensis* seed at 27°C and 32°C and that the rate of induction of dormancy was decreased with reductions in the water content of the seed from 40% to 20%.
3.5 Conclusions

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

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

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

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

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

6. The dormancy of dry seed is not increased by exposure to high (80°C) temperatures although the rate of germination is reduced.
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THE
REGENERATION OF
Eucalyptus pauciflora Sieb. ex Spreng.
FROM SEED

by

Douglas Graham Abrecht

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. Response to water during stratification

#### 4.1 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>+ψ</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](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 2056.0</td>
<td></td>
<td>5.69</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>348 375.6</td>
<td>1.09</td>
<td>13 1677.4</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>+ψ</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
ψ = 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></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></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></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></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

1 Includes only 4.0, 4.5, 5.0, 6.0 ml treatments
2 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></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed which</td>
<td>1.614</td>
<td>2.166</td>
<td>2.275</td>
<td>2.082</td>
<td>2.370</td>
</tr>
<tr>
<td>germinated</td>
<td>41</td>
<td>73</td>
<td>80</td>
<td>85</td>
<td>83</td>
</tr>
<tr>
<td>Seed which</td>
<td>1.585</td>
<td>1.723</td>
<td>1.919</td>
<td>1.756</td>
<td>2.069</td>
</tr>
<tr>
<td>not germinate²</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 weight², [Water.Germ] \( p=0.033 \)). 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).
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>Water Dose (ml)</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>( \chi^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 (1953) 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 Saupit 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. deviance</th>
<th>Change d.f. deviance</th>
<th>F Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>65 1354.0 20.83</td>
<td>12 1250 104.10 58.19</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Temp +Source</td>
<td>53 104.0 1.96</td>
<td>12 1250 104.10 58.19</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Temp .Source</td>
<td>33 59.1 1.79</td>
<td>20 44.89 2.24 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 Watson 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.

![Germination capacity graph](image)

**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</th>
<th>Shoulder</th>
<th>'Breadth'</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temp °C</td>
<td>% germ.</td>
<td>°C Lower</td>
</tr>
<tr>
<td>Waste Point</td>
<td>4.6</td>
<td>92.0</td>
<td>1.4</td>
</tr>
<tr>
<td>Sawpit Ck.</td>
<td>4.2</td>
<td>77.0</td>
<td>1.7</td>
</tr>
<tr>
<td>Dicky Cooper Ck.</td>
<td>3.4</td>
<td>70.3</td>
<td>1.6</td>
</tr>
<tr>
<td>Baker's Ck.</td>
<td>3.4</td>
<td>54.7</td>
<td>1.8</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>Change d.f. deviance</th>
<th>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>594 2695.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>577 814.7 1.41</td>
<td>17 1880.3</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><em>Temp</em> +<em>Source</em></td>
<td>569 690.1 1.21</td>
<td>7 124.6</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><em>Temp</em> .<em>Source</em></td>
<td>557 664.5 1.19</td>
<td>12 25.6</td>
<td>p=0.012</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 showing treatment constants vs. temperature](image)

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>HMD</th>
<th>Change d.f. deviance</th>
<th>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>129</td>
<td>2190.0</td>
<td>4.53</td>
<td>16</td>
</tr>
<tr>
<td>+Temp +Durn</td>
<td>113</td>
<td>512.6</td>
<td>1.31</td>
<td>48</td>
</tr>
<tr>
<td>+Temp .Durn</td>
<td>65</td>
<td>85.24</td>
<td>1.31</td>
<td>48</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
'breath' of the temperature response to increase with increasing
duration of treatment. The peak temperature tends to decrease in the
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](image)

**Figure 4.8** The effect of temperature and duration of pretreatment on the germination capacity of *F. 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>% germ.</th>
<th>Shoulder °C Lower</th>
<th>Shoulder °C Upper</th>
<th>'Breadth' °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 days</td>
<td>5.2</td>
<td>30.9</td>
<td>2.0</td>
<td>9.9</td>
<td>7.9</td>
</tr>
<tr>
<td>10 days</td>
<td>6.4</td>
<td>54.4</td>
<td>3.5</td>
<td>8.6</td>
<td>5.1</td>
</tr>
<tr>
<td>15 days</td>
<td>4.7</td>
<td>74.1</td>
<td>1.8</td>
<td>7.5</td>
<td>5.7</td>
</tr>
<tr>
<td>20 days</td>
<td>4.6</td>
<td>92.1</td>
<td>1.4</td>
<td>7.4</td>
<td>6.0</td>
</tr>
<tr>
<td>25 days</td>
<td>4.9</td>
<td>96.2</td>
<td>0.5</td>
<td>7.6</td>
<td>7.1</td>
</tr>
<tr>
<td>30 days</td>
<td>5.3</td>
<td>97.3</td>
<td>1.5</td>
<td>7.7</td>
<td>6.2</td>
</tr>
</tbody>
</table>

Should: 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>Deviance</th>
<th>RMD</th>
<th>Change d.f.</th>
<th>Deviance</th>
<th>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp *Durn</td>
<td>65</td>
<td>85.24</td>
<td>1.31</td>
<td></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>
<td>p=0.063²</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>
<td>p&lt;0.005²</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>
<td>p&lt;0.005²</td>
</tr>
</tbody>
</table>

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

Variables:
T = Temperature of treatment; TSQ = T²; TCU = T³
D = Duration of treatment (days)

The full model, [Temp*Durn] = \(\mu + \text{Temp*Durn*Temp.Durn}\)
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.

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

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 \(\text{logit}(G)\), 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: logit(G) = \beta_0(T) + \beta_1(T)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 is 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 \((-1.7, -0.6^\circ C)\) and three highest \((10.2, 11.4, 12.6^\circ C)\)
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</td>
<td>3726.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>350</td>
<td>879.3</td>
<td>2.31</td>
<td>4</td>
</tr>
<tr>
<td>+Temp +Durn</td>
<td>359</td>
<td>502.5</td>
<td>1.36</td>
<td>11</td>
</tr>
<tr>
<td>+Temp .Durn</td>
<td>341</td>
<td>418.0</td>
<td>1.23</td>
<td>28</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>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></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>216</td>
<td>228.7</td>
<td></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></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></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></td>
<td></td>
</tr>
</tbody>
</table>

Time = Day of observation
Temp = Temperature of treatment 10, 20, 30 days 0.6°C-8.9°C
15, 25 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 A.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 4.14

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>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 lie 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
(±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 Wattle 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_url)

**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 the 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>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>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  516.5</td>
</tr>
<tr>
<td>+Temp .Durn</td>
<td>33</td>
<td>34.03</td>
<td>1.03</td>
<td>20  151.67</td>
</tr>
<tr>
<td>Temp *D(^1)</td>
<td>44</td>
<td>45.69</td>
<td>1.04</td>
<td>11  11.66(^2)</td>
</tr>
</tbody>
</table>

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

Variable:
- \( D \) = Duration of treatment (days)

\(^1\) Full model \([\text{Temp} \times D] = \mu + \text{Temp} \times D\)

\(^2\) Compared to \([\text{Temp} + \text{Durn} + \text{Temp} \times \text{Durn}]\)
Figure 4.14 The coefficients of the model relating temperature and duration of treatment to germination capacity (G).

Model: Logit(G) = q(T) + q(T).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. deviance</th>
<th>RMD</th>
<th>Change d.f. 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>
</tr>
<tr>
<td>Time</td>
<td>672</td>
<td>792.8</td>
<td>1.18</td>
<td>17 1378.2 p&lt;0.001</td>
</tr>
<tr>
<td>*Temp +Durn</td>
<td>663</td>
<td>709.6</td>
<td>1.07</td>
<td>9 83.2 p&lt;0.001</td>
</tr>
<tr>
<td>*Temp .Durn</td>
<td>649</td>
<td>688.2</td>
<td>1.06</td>
<td>14 21.4 p&lt;0.091</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.

![Graph showing treatment constants for three durations of treatment at different temperatures.]

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>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>Durn</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 Durn</td>
<td>20</td>
<td>2737.5</td>
<td>136.9</td>
<td>0.703</td>
<td>p=0.714</td>
</tr>
<tr>
<td>Residual</td>
<td>33</td>
<td>5770.0</td>
<td>174.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Temp = Temperature of treatment
Durn = 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.
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.

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°C.

![Graph showing slope vs temperature for waste point and dicky cooper](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: \( \text{logit}(G) = \beta_0(T) + \beta_1(T) \cdot D \)

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°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
USE OF THESES

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

by

Douglas Graham Abrecht

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

February 1985
CHAPTER 5

INDUCTION OF DORMANCY
CHAPTER 5

The studies in Chapter 4 showed that the dormancy of *Eucalyptus pauciflora* seed was broken at temperatures below about 9°C and was induced in moist seed exposed to temperatures above 9°C. Induction of dormancy is associated with an increase in the period of stratification required to permit the germination of the seed and is a means whereby germination may be delayed in response to environmental conditions. The induction of dormancy may be measured either as a reduction in the germination capacity of the seed which is not dormant, or as an increase in the duration of stratification required to permit germination in seed which is dormant.

Grose (1963) examined the induction of dormancy in the seed of *E. delegatensis* and found that the germination capacity of seed which had been stratified was reduced by exposure to temperatures of 27°C and 35°C for periods of 4 to 8 hours prior to germination at 17°C (Grose 1963). Increasing the temperature of treatment or decreasing the duration of stratification prior to treatment increased the degree of dormancy induced. Grose's (1963) studies, and those of Section 4.2.1, focused on changes in the degree of dormancy following treatment at higher temperatures. As the degree of dormancy (the proportion of seed which does not germinate) increases, the delays imposed by the changes in the strength of dormancy (the length of the stratification period required to break dormancy) of that seed will play an increasingly important role in the timing of germination.

A large proportion of the seed is likely to fall to the ground in summer and autumn. Following rainfall, this seed would be in a moist
condition and could be exposed to temperatures greater than 9°C near the surface of the soil. The changes in the dormancy of moist seed under these conditions will be a major determinant of the proportion of seed which germinates in autumn, and which will subsequently be exposed to the rigours of winter as seedlings. The long period of exposure to cold, moist conditions during winter means that most of the seed in the soil is likely to be non-dormant at the onset of spring. Field observations suggest that, depending on the distribution of rainfall, the surface soil may dry out soon after snow melt and before seedling emergence in spring. The change in dormancy of seed exposed to drying following stratification was investigated.
5.1 Induction of dormancy

The studies which follow are concerned with the changes in the strength of dormancy of moist seed as a result of exposure to temperatures of 15°C. Section 5.1.1 examines the effect of short periods (1, 3 days) of exposure to 15°C on the strength of dormancy of moist seed and leads to the investigation of longer periods (5, 10, 15 days) of exposure at 15°C in Section 5.1.2.

5.1.1 Dormancy after treatment at 15°C for 0, 1, 3 days

Methods:

Replicates of cleaned seed (0.2 g, 30.3 viable seeds) collected near Dicky Cooper Ck. were moistened and exposed to 15°C for either 0, 1 or 3 days. Five replicates of each treatment were stratified for periods of 14, 28, 42, 56 days at 5°C and set to germinate at 15°C. The method used and the results obtained are presented diagramatically on the yellow summary sheet at the end of the chapter.

Results:

(i) Germination capacity

The large effect of increasing durations of stratification dominated the small but significant effect (p=0.010) of the duration of treatment on germination capacity. (Table 5.1, Figure 5.1). The dormancy induced in the seed treated at 15°C for 3 days was strong enough to reduce the germination capacity of the seed following stratification for both 14 days and 28 days (Figure 5.1), but the
effect was not apparent after longer periods of stratification. The rise in the germination capacity of seed which had been treated at 15°C for 1 day, following stratification for 28 days, is not significant.

### Table 5.1

Summary of analysis of deviance for germination capacity of seed following treatment at 15°C and stratification at 5°C.

<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>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>59</td>
<td>529.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treat + Strat</td>
<td>54</td>
<td>58.76</td>
<td>1.09</td>
<td>5</td>
<td>471.04</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Treat - Strat</td>
<td>56</td>
<td>67.98</td>
<td>1.21</td>
<td>2</td>
<td>9.22</td>
<td>p=0.010</td>
</tr>
<tr>
<td>-Strat</td>
<td>57</td>
<td>523.30</td>
<td>9.18</td>
<td>1</td>
<td>464.54</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>+Treat + Strat</td>
<td>48</td>
<td>42.60</td>
<td>0.89</td>
<td>6</td>
<td>16.16</td>
<td>p=0.013</td>
</tr>
</tbody>
</table>

Treat = Duration of treatment at 15°C (0, 1, 3 days)
Strat = Duration of stratification at 5°C (14, 28, 42, 56 days)

![Figure 5.1](image-url)  
**Figure 5.1** The germination capacity of *E. pauciflora* seed following treatment at 15°C for 0, 1, 3 days and stratification at 5°C.
(ii) Times-to-germination

A lack of synchrony in the days of missed observations between the four stratification periods meant that the whole data set could not be treated in the one analysis without the loss of a large amount of information. In addition there were not enough days of observation during the germination period for the seed germinated after 56 days stratification to permit a reliable fit of the Cox model and so this treatment was excluded from the analysis.

**TABLE 5.2**

Summary of analysis of deviance for the times-to-germination of seed treated at 15°C for 0,1,3 days and then stratified for 14,28,42 days before germination.

<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><strong>14 days</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>178</td>
<td>147.0</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>Time +Treat</td>
<td>176</td>
<td>143.7</td>
<td>0.82</td>
<td>2</td>
</tr>
<tr>
<td><strong>28 days</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>169</td>
<td>201.0</td>
<td>1.18</td>
<td></td>
</tr>
<tr>
<td>Time +Treat</td>
<td>167</td>
<td>183.6</td>
<td>1.10</td>
<td>2</td>
</tr>
<tr>
<td><strong>42 days</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>153</td>
<td>181.8</td>
<td>1.19</td>
<td></td>
</tr>
<tr>
<td>Time +Treat</td>
<td>151</td>
<td>175.0</td>
<td>1.16</td>
<td>2</td>
</tr>
</tbody>
</table>

Time = Time since beginning of germination period (days)
Treat = Duration of treatment at 15°C (days)

The analysis of times-to-germination shows that the differences between the treatments were not significant after 14 days and 42 days stratification but were significant after 28 days stratification (Table 5.2). Comparison of the treatment constants (Table 5.3) shows that there was a tendency for a decrease in times-to-germination with
increasing periods of treatment at 15°C; this effect was strongly evident in the seed stratified for 28 days but was less evident after stratification for 42 days.

TABLE 5.3

The treatment constants of the Cox model for the times-to-germination of seed treated for 0, 1, 3 days, stratified for 28 and 42 days and then set to germinate at 15°C.

<table>
<thead>
<tr>
<th>Stratification</th>
<th>28 days</th>
<th>42 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ESTIMATE s.e</td>
<td>ESTIMATE s.e</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 days</td>
<td>0.000 -</td>
<td>0.000 -</td>
</tr>
<tr>
<td>1 day</td>
<td>0.302 0.130</td>
<td>0.298 0.119</td>
</tr>
<tr>
<td>3 days</td>
<td>0.596 0.144</td>
<td>0.204 0.114</td>
</tr>
</tbody>
</table>

Figure 5.2 The predicted (lines) and observed (symbols) germination curves for seed soaked for 0, 1, 3 days at 15°C prior to stratification for 28 days and germination at 15°C.

The reduction in the difference in the rate of germination of the treatments at 15°C after 42 days of stratification was a result of an
increase in the rate of germination due to stratification. The observed
difference between the rate of germination of the seed which received
moist treatment at $15^\circ C$ and that which did not seems to be
characteristic of short durations of treatment at $15^\circ C$ since no such
behaviour was observed following the longer periods of treatment at
$15^\circ C$ in the next experiment.

A similar phenomenon occurred in *E. delegatensis* seed which showed
an increased rate of germination after 8 hours of treatment at $27^\circ C$,
followed by germination at $17^\circ C$, compared to no treatment at $27^\circ C$ and
also treatment for longer periods (Grose 1963). This response was
observed in seed which had been stratified for 0, 2, 3, 4 weeks prior to
treatment, suggesting that the effect is not restricted to the
imbibition phase. The enhancement of the rate of germination by short
periods of exposure to high temperatures is likely to be important in
the field environment where temperature fluctuates diurnally.
Unfortunately this effect could not be investigated in this thesis
because of delays in commissioning a temperature gradient plate capable
of imposing a diurnally varying temperature environment.
5.1.2 Dormancy after treatment for 0,5,10,15 days at 15°C

Treatment of seed for 3 days at 15°C leads to an increase in the strength of dormancy and also an increase in the rate of germination following stratification (Section 5.1.1). This section investigates the induction of dormancy by longer periods of treatment at 15°C.

Methods:

Replicates of cleaned seed (0.4 g, 75.4% viable seeds), collected at Rennix Gap in March 1983, were placed on moist filter paper in petri dishes and treated at 15°C for 0,5,10,15 days. Five replicates of each treatment were then stratified at 5°C for either 10,20,40 days before germination at 15°C.

Results:

(i) Seed germination during treatment at 15°C and stratification

Daily records of germination were not kept for this part of the experiment, but the proportion of the seeds which had germinated at the end of the treatment at 15°C and at the end of stratification was recorded. Since both showed similar trends, only the changes in germination capacity after treatment at 15°C followed by stratification is discussed.

Although there was a tendency for a few seeds to germinate during the longer periods of stratification, the analysis of germination capacity in Table 5.4 shows that stratification did not have a significant effect on germination capacity (Strat, p=0.064).
Induction of dormancy:

The variation in germination capacity can be explained by the duration of treatment at 15°C (Treat, p<0.001). The absence of a significant effect of stratification means that the proportion of seeds which germinated during treatment at 15°C are the same for all durations of stratification. These were 0%, 0%, 5.2%, 8.4% for the 0, 5, 10, 15 days of treatment, respectively.

**TABLE 5.4**

Summary of analysis of deviance for the proportion of seed which germinated during treatment at 15°C and stratification.

<table>
<thead>
<tr>
<th>Model terms</th>
<th>Residual d.f. deviance</th>
<th>Change d.f. deviance</th>
<th>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>59 285.80 4.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treat +Strat</td>
<td>54 49.13 0.91</td>
<td>5 237.67 p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>-Treat</td>
<td>57 282.90 4.96</td>
<td>3 233.77 p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>-Strat</td>
<td>56 54.58 0.97</td>
<td>2 5.45 p=0.064</td>
<td></td>
</tr>
<tr>
<td>Treat .Strat</td>
<td>48 46.12 0.96</td>
<td>6 3.01 p&lt;0.009</td>
<td></td>
</tr>
</tbody>
</table>

Treat =Duration of treatment at 15°C (0, 5, 10, 15 days)
Strat =Duration of stratification (10, 20, 40 days)

(ii) Germination capacity following stratification

The germination capacity of seed following either 20 or 40 days of stratification was reduced progressively as the duration of treatment at 15°C increased (Figure 5.3, Table 5.5). The reduction in the proportion of seeds which germinated as the duration of treatment at 15°C was increased indicates that dormancy was induced by the treatment. Table 5.5 shows that stratification had a similar effect on germination capacity in all treatments ([Treat,Strat], p=0.222) and the appropriate model for the data is therefore [Treat+Strat].
TABLE 5.5

Summary of analysis of deviance for the germination capacity following treatment at 15°C and stratification treatments.

<table>
<thead>
<tr>
<th>Model terms</th>
<th>Residual d.f.</th>
<th>deviance</th>
<th>RMD</th>
<th>d.f. deviance</th>
<th>MCD</th>
<th>104</th>
<th>0</th>
<th>&lt;0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>59</td>
<td>2785</td>
<td>16.85</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treat *Strat</td>
<td>54</td>
<td>133.8</td>
<td>2.48</td>
<td>5</td>
<td>2551.2</td>
<td>245.45</td>
<td>104.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>-Treat</td>
<td>57</td>
<td>2199.0</td>
<td>38.59</td>
<td>3</td>
<td>2065.2</td>
<td>688.40</td>
<td>204.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>-Strat</td>
<td>56</td>
<td>943.5</td>
<td>16.85</td>
<td>2</td>
<td>809.7</td>
<td>404.85</td>
<td>120.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>+Treat .Strat</td>
<td>48</td>
<td>113.5</td>
<td>3.36</td>
<td>6</td>
<td>20.29</td>
<td>3.38</td>
<td>1.43</td>
<td>0.222</td>
</tr>
</tbody>
</table>

The duration of stratification required to reverse the dormancy which has been induced can be determined from Figure 5.3. If the germination capacity of the seed which has been stratified for 10 days without prior treatment at 15°C is used as a reference point, then, a further 10 days of stratification was sufficient to overcome the dormancy induced by 2.5 days of treatment at 15°C. Similarly, a further 30 days of stratification was required to reverse the dormancy induced by 6.0 days treatment at 15°C. The induction of dormancy at 15°C is apparently much more rapid than the breaking of dormancy at 5°C. The probable impact of this response in an alternating temperature environment will be discussed in Section 6.1.

The analysis to this point has examined the effects on germination capacity of stratification and treatment at 15°C. The investigation was extended by considering the empirical relationship between germination capacity, the duration of treatment at 15°C, and stratification (see Section 5.6). If an empirical model can be found
Induction of dormancy:

to replace the statistical model of the data then the empirical model can be used for determining the effects of periods of treatment at 15°C and stratification of intermediate duration. In order to fit such an empirical model to this data, the treatment factors [Strat] and [Treat] were replaced by continuous variables in the model. Statistical analysis showed that both factors could be replaced by variables in a model which was not significantly different (p=0.032) from the statistical model [Treat+Strat]. However, much closer agreement would need to be obtained before this model could replace [Treat+Strat]. The factor [Strat] can, however, be replaced by a linear function of duration of stratification (p=0.168) with a greater degree of confidence. The derivation and explanation of the fitting of the empirical model described above is presented in Section 5.6.

![Graph showing germination capacity over time for different treatments](image)

Figure 5.3 The germination capacity of moist seed treated at 15°C and then stratified prior to germination at 15°C.
(iii) Times-to-germination

The number of seeds which germinated in the shorter stratification treatments (10, 20 days) following 5, 10 or 15 days of exposure to 15°C were too low to ensure reliable fitting of the Cox model. The following analysis was restricted to examining the effect of treatment at 15°C on times-to-germination of seed which had been stratified for 40 days.

<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>F ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0,5,10,15 days at 15°C</td>
<td>202</td>
<td>653.5</td>
<td>3.235</td>
<td>3</td>
<td>275.2</td>
<td>91.77</td>
<td>48.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time</td>
<td>199</td>
<td>378.3</td>
<td>1.901</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time +Treat^1</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>275.2</td>
<td>91.77</td>
<td>48.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>5,10,15 days at 15°C</td>
<td>147</td>
<td>127.3</td>
<td>0.870</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>145</td>
<td>119.7</td>
<td>0.825</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time +Treat^2</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>7.6</td>
<td></td>
<td></td>
<td>0.022</td>
</tr>
<tr>
<td>0,5,10,15 days at 15°C aligned on predicted day 1</td>
<td>165</td>
<td>470.9</td>
<td>2.85</td>
<td>3</td>
<td>7.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>162</td>
<td>358.4</td>
<td>2.212</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time +Treat^2</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>112.5</td>
<td>37.50</td>
<td>16.95</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Time =day during the germination period
Treat =duration of treatment at 15°C (0.5,10,15 days)

Examination of the germination curves (Figure 5.4) shows that the seed which was not exposed to moist conditions at 15°C had a shorter lag and higher rate of germination than the rest of the treatments (Figure 5.4). This difference in the germination curves is indicative of a difference in the distribution of times-to-germination and may be the cause of the high residual mean deviance (RMD=1.901) of the model [Time+Treat^1] (Table 5.6). In view of the differences in
times-to-germination of seed which had been exposed to moist treatment at 15°C, the model was refitted to these treatments alone.

The Cox model fitted to the treatments 5, 10 and 15 days at 15°C alone (Table 5.6, [Time+Treat²]) showed a superior fit to the data (RMD=0.825) and indicated that moist seed treated at 15°C for 5 days germinates at a significantly faster rate than seed treated for either 10 or 15 days (Figure 5.5).

![Cumulative Germination Graph](image)

**Figure 5.4** The predicted (lines) and observed (symbols) germination curves for seed treated at 15°C, stratified for 40 days and germinated at 15°C ([Time+Treat¹], Table 5.6)

The lack of fit of the first model could have been due to a time lag to the first day of germination (Figure 5.4, symbols). The time to the day on which the first seed germinated can be viewed as a period during which there is no risk of germination. Analysis of variance showed that the day on which the first seed germinated in a treatment became significantly later with increasing duration of treatment at 15°C (p<0.001). Fitting a Cox model to the data aligned on the predicted day of first germination as day 1 (Table 5.5, [Time+Treat¹])
resulted in a worse fit to the data (RMD=2.212) which indicates that the lack of fit in the original model is not due to a simple time lag which could be represented by the day of first germination.

![Graph showing cumulative germination over time for different durations of treatment at 15°C.]

Figure 5.5 The predicted (lines) and observed (symbols) germination curves for seed treated at 15°C, stratified for 40 days and germinated at 15°C ([Time*Treat²], Table 5.6).

Examination of the times-to-germination has shown that the effects of the treatment at 15°C may be observed even after a long period of stratification and may include a reduction in the germination capacity and a reduction in the rate at which germination occurs. Interestingly, the rate of germination was reduced by treatment at 15°C for 5 days or more, whereas there was a stimulation of the rate of germination by up to 3 days of treatment at 15°C (Section 5.1.1).

(iv) Seed mortality during treatment at 15°C and stratification.

The relative mortality of seed due to the treatment at 15°C can be assessed from the differences in the number of seeds which had been shown to be viable by the end of the experiment. The number of viable seeds is determined by summing the number of seeds which germinated
Induction of dormancy: (during treatment at 15°C and following stratification), and the number of viable but ungerminated seeds (assessed by squashing the seed remaining after germination).

Analysis of variance showed that there was a significant effect of duration of treatment at 15°C (p=0.001) on seed mortality whereas neither stratification (p=0.234), nor the interaction between treatment at 15°C and stratification (p=0.763), had a significant effect (Table 5.7). This result is important because it shows that the differences in seed mortality are primarily associated with the duration of treatment at 15°C and bear no relationship to the duration of stratification.

**TABLE 5.7**

Summary of analysis of variance of the number of viable seeds.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>d.f</th>
<th>Sum of Squares</th>
<th>Mean Variance</th>
<th>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treat</td>
<td>3</td>
<td>2274.4</td>
<td>758.1</td>
<td>p=0.001</td>
</tr>
<tr>
<td>Strat</td>
<td>2</td>
<td>316.0</td>
<td>158.0</td>
<td>p=0.234</td>
</tr>
<tr>
<td>Treat . Strat</td>
<td>6</td>
<td>354.5</td>
<td>59.1</td>
<td>p=0.763</td>
</tr>
<tr>
<td>Residual</td>
<td>48</td>
<td>5083.2</td>
<td>105.9</td>
<td></td>
</tr>
</tbody>
</table>

Treat = Duration of treatment at 15°C
Strat = Duration of stratification

The number of viable seeds declined as the duration of treatment at 15°C was increased, indicating that seeds had become non-viable during the period of treatment at 15°C and stratification (Table 5.8). The mortality of moist seed exposed to higher temperatures may be considerable (from Table 5.8, 15 days at 15°C, 82-67/82-18%).

The conclusions of the analyses of germination capacity and
times-to-germination were not confounded by the mortality of seeds during treatment: the number of viable seeds in the analyses was calculated from the sum of the seeds which germinated and the viable seeds assessed by the squash technique.

**TABLE 5.8**

The mean number of viable seeds in each of the treatment at 15°C.

<table>
<thead>
<tr>
<th>Duration of treatment at 15°C</th>
<th>5 days</th>
<th>10 days</th>
<th>15 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viable seeds</td>
<td>82.5</td>
<td>80.3</td>
<td>71.1</td>
</tr>
</tbody>
</table>

LSD_{0.05} = 7.56  
LSD_{0.01} = 10.09

**Discussion:**

The studies in this section show that the reduction in germination capacity of seed exposed to temperatures greater than 9°C is likely to be accompanied by an increase in the strength of the dormancy of the fraction of the seed which does not germinate. The dormancy induced by exposure to high temperatures was far stronger than the innate dormancy of the seed. The response to high temperatures can be regarded as constituting a mechanism for reducing the risk of germination for some considerable time.

The strength of dormancy increases as the duration of treatment at temperatures suitable for the induction of dormancy is increased since the strength of dormancy increased with increasing duration of exposure to 15°C from 3 to 15 days (Section 5.1), and there was a reduction in the germination capacity of seed exposed to temperatures above 9°C for 30 days in Section 4.3.1. These observations suggest that the
induction of dormancy requires exposure to high temperatures on a time scale of several days to weeks to be effective in changing the timing of germination.

Induction of dormancy is likely to occur in summer and autumn when the seed is moistened by rain and the soil temperatures are above 9°C. The advantage of the induction of dormancy is likely to be the minimization of germination during autumn, since any seeds which germinate during autumn are likely to die during winter. However, the cost of the induction of dormancy seems to be mortality of some of the seed.

The method used to assess changes in the strength of dormancy in these experiments may be open to criticism because of the possibility that the transfer of the seed to the lower temperatures for stratification, rather than exposure to 15°C, may have contributed to the increased dormancy. However, this effect could also be expected to apply to seed in the field. The investigation of the origin of the dormancy would require a method which would permit the measurement of seed dormancy which was independent of the conditions which break dormancy.
5.2 The induction of dormancy in seed set to germinate.

The induction of dormancy in newly-shed seed exposed to temperatures of 15°C reduced the likelihood of germination for a considerable time (Section 5.1). The seeds which do not germinate during an experience of higher temperature (15°C), regardless of whether the seed has been stratified, would seem to be in an analogous situation to that created in the previous experiment in which dormancy was induced in freshly shed seed.

Grose (1963) investigated the effect of a single, short period (0 to 48 hours) of exposure to 27°C on the induction of dormancy in previously stratified E. delegatensis. He found that the degree of dormancy, assessed by germination at 17°C following high temperature treatment, increased as the duration of treatment was increased. Grose also found that the effect of the exposure to high temperatures was reduced in seed stratified for longer periods prior to treatment. The reduction in susceptibility to induction of dormancy in seed which has been stratified for longer periods is probably related to the weaker dormancy of the seed at the time of exposure to high temperatures. This phenomenon may be important for the survival of the seed in field situation because it would ensure that dormancy was easily induced in autumn, when germination is futile, and was less easily induced in spring, when the induction of dormancy may result in the seed missing an opportunity to germinate and establish.

Seed which germinates in autumn is unlikely to survive winter. However, the conditions during late autumn are sufficiently cold and moist to begin to break the dormancy of some of the seed and result in germination (Section 7.1). The induction of dormancy by exposure to
high temperatures in seed which has been partially stratified may act to reduce the risk of further germination in the seed which remains. The studies in this section describe a test of the effect of prior stratification on the induction of dormancy.

Methods:

Cleaned seed (0.4 g, 80.7 viable seeds) collected at Rennix Gap (1610 m) in March 1983 was used in this experiment. The experiment was divided into two parts, the first imposed a series of pretreatments and the second examined the strength of the dormancy induced in the seeds which did not germinate during pretreatment.

The six pretreatments were imposed by taking seed stratified for three periods (0, 10, 20 days at 5°C) and setting it to germinate at two temperatures (14°C, 24°C) in the dark. The germination of seed was then monitored daily for a period of 16 days.

The strength of the dormancy of the seed which had not germinated during pretreatment was then assessed from the germination capacity of the seed following another period of stratification. Five randomly chosen dishes from each pretreatment were allocated to each of six periods of restratification (0, 10, 20, 40, 60 days) at 5°C and then set to germinate at 15°C.

During the pretreatment, seeds were maintained under dark conditions except for exposure to 10 minutes of light daily when germinated seeds were counted and removed. All other methods used have been described previously (Chapter 2). The methods are presented diagrammatically on the yellow sheet at the end of the chapter.
Results:

(i) Germination capacity during pretreatment

The proportion of the seed which germinated during the various pretreatments showed a marked response to both duration of stratification and germination temperature (Figure 5.6). The strong interaction apparent in the figure is also borne out in the analysis (Table 5.9).

![Graph showing germination capacity over days of stratification at 5°C and 24°C](image)

Figure 5.6 Germination capacity of seed germinated at either 14°C or 24°C plotted against the duration of stratification.

The interaction between the duration of stratification and germination temperature is considered in a broader context in Section 6.1. In brief, Section 6.1 confirms that the temperatures chosen for pretreatment lie on either side of the optimum temperature for germination, and shows that the effect of the temperature of germination on germination capacity is likely to be highly dependent on
the temperatures chosen for germination.

TABLE 5.9

Summary of analysis of deviance for the germination capacity of stratification and temperature treatments.

<table>
<thead>
<tr>
<th>Model terms</th>
<th>Residual d.f. deviance</th>
<th>Residual MVD</th>
<th>Change d.f. deviance</th>
<th>Change MCD</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>149</td>
<td>5019</td>
<td>33.68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temp +Strat</td>
<td>146</td>
<td>673.0</td>
<td>4.61</td>
<td>3</td>
<td>4346</td>
<td>1488.67</td>
</tr>
<tr>
<td>+Temp .Strat</td>
<td>144</td>
<td>313.2</td>
<td>2.18</td>
<td>2</td>
<td>359.8</td>
<td>179.90</td>
</tr>
</tbody>
</table>

Temp =Temperature of germination (14°C, 24°C)
Strat =Duration of stratification at 5°C (0,10,20 days)

(ii) Times-to-germination during pretreatment

None of the unstratified seed which was set to germinate at 24°C was found to germinate, and as a result, this treatment was not considered in this analysis.

The outcome of the analysis for the remaining treatments indicates that the Cox model was not describing the data adequately (Table 5.10, [Time+Treat]) mean deviance=1.725). Consideration of the observed germination curves (Figure 5.7, symbols) shows that the time until the seed began to germinate was much greater in seed germinated at 14°C than in seed germinated at 24°C.

Two possible explanations for the lack of fit observed in the full model were tested. Firstly, that the discrepancy resulted from differences in the day of first germination, and secondly, that there was an underlying difference in the distribution of times-to-germination of seed germinated at 14°C and 24°C.
TABLE 5.10

Summary of analysis of deviance for the times-to-germination of seed stratified for either 0,10,20 days and germinated at 14°C or 24°C.

<table>
<thead>
<tr>
<th>Model terms</th>
<th>Residual df</th>
<th>deviance RMD</th>
<th>Change df</th>
<th>deviance CMD</th>
<th>F</th>
<th>Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germination at 14°C and 24°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1468</td>
<td>9148.0</td>
<td>6.23</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time* Treat¹</td>
<td>1451</td>
<td>2503.0</td>
<td>1.73</td>
<td>17</td>
<td>6645</td>
<td>390.9</td>
<td>225.9</td>
</tr>
<tr>
<td>Aligned on day of first germinated seed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1143</td>
<td>4784.0</td>
<td>4.19</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time* Treat²</td>
<td>1125</td>
<td>1704.0</td>
<td>1.51</td>
<td>18</td>
<td>3080</td>
<td>171.1</td>
<td>113.3</td>
</tr>
<tr>
<td>Germination at 14°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>951</td>
<td>1716.0</td>
<td>1.80</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time +Strat³</td>
<td>949</td>
<td>1127.0</td>
<td>1.19</td>
<td>2</td>
<td>589</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germination at 24°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>490</td>
<td>646.7</td>
<td>1.32</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time +Strat⁴</td>
<td>489</td>
<td>557.9</td>
<td>1.16</td>
<td>1</td>
<td>78.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Time = Day of observation
Strat = Stratification treatment (0,10,20 days)
Treat = Stratification (1,10,20 days), Temperature (14°C, 24°C)

An analysis of variance showed that the day on which the first seed germinated became earlier with increasing temperature of germination and increasing durations of stratification. The Cox model (Table 5.10) fitted to the data aligned on the predicted day on which the first seed germinated for each treatment had a lower residual mean deviance (RMD=1.51) than the original model. Although some of this lack of fit of the model can be attributed to a simple time lag to the day of first germination, there is still evidence of a substantial lack
of fit of the model due to other causes.

Figure 5.7 The observed (symbols) and predicted (lines) germination curves for seed stratified for 0, 10, 20 days and then germinated at either 14°C or 24°C.

Predicted germination curves from analyses in Table 5.10
14°C from [Time+Strat]
24°C from [Time+Strat]

The presence of an underlying difference in the distribution of times-to-germination in seed germinated at either 14°C or 24°C can be tested by fitting the Cox model to each temperature treatment separately and then comparing the distributions of the times-to-germination. The result is a good fit of the model to each of the temperature treatments (Table 5.10, Figure 5.7), which shows that increasing the duration of stratification significantly increased the rate of germination at either temperature (Table 5.11).

The major difference in the distribution of times-to-germination of the two temperature treatments was a period of low risk of germination for the first 3 days of the germination period at 14°C (Figure 5.8).
Induction of dormancy:

TABLE 5.11

The treatment constants for the Cox models applied to seed germinated at either 14°C or 24°C.

<table>
<thead>
<tr>
<th>Pretreatment Temp</th>
<th>Strat</th>
<th>Estimate</th>
<th>s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>14°C</td>
<td>0 days</td>
<td>0.0000</td>
<td>-</td>
</tr>
<tr>
<td>14°C</td>
<td>10 days</td>
<td>0.7599</td>
<td>0.0741</td>
</tr>
<tr>
<td>14°C</td>
<td>20 days</td>
<td>1.3945</td>
<td>0.0678</td>
</tr>
<tr>
<td>24°C</td>
<td>10 days</td>
<td>0.0000</td>
<td>-</td>
</tr>
<tr>
<td>24°C</td>
<td>20 days</td>
<td>0.3758</td>
<td>0.0431</td>
</tr>
</tbody>
</table>

Figure 5.8 The plots of \( \ln(-\ln[1-F(t)]) \) against time for the Cox models fitted to seed set to germinate at either 14°C or 24°C.

The results obtained by fitting the Cox model confirm the observation that increasing both the duration of stratification and the temperature of germination decreases the times-to-germination. This
result shows that the seed entering the second phase of the experiment had very different past histories when assessed from the response of the seeds that germinated in the pretreatments. The impact of the combined effects of temperature and dormancy (stratification) on germination capacity is discussed more fully in Section 6.1.

(iii) Germination capacity after restratification

The incremental germination capacity, defined as the number of seeds which germinated following restratification as a proportion of the number of viable seeds present at the end of the restratification period, forms the basis of this analysis. An analysis of incremental germination capacity permits the comparison of the effects of pretreatment on the seeds which germinated after restratification since it excludes those seeds which germinated during pretreatment. Analysis of the germination capacity over the whole experiment would be of little use in investigating the strength of the dormancy of the seed which did not germinate during pretreatment because the germination capacity during pretreatment and the germination capacity following restratification would be confounded.

The incremental germination capacity followed the trends predicted by the experiments described in Section 5.1.2. Seed which did not germinate became strongly dormant and although the dormancy could be reversed by further stratification it was much stronger than the dormancy of the seed at dispersal.

The incremental germination capacity following restratification showed a marked response to the pretreatments and the period of restratification (Figure 5.9); this result confirms that the strength
of the dormancy induced was different for the various pretreatments.

An analysis of deviance (Table 5.12) showed that the three factor interaction was not significant \((p=0.666)\). There were, however, significant two-factor interactions to be considered (Table 5.12). The relative importance of the three two-factor interactions can be assessed by comparing the deviance when each of the interactions is excluded from the model with the deviance obtained by fitting the main effects and the three two-factor interactions at the same time.

![Germination capacity following restratification for duration of stratification and temperature of germination pretreatments](image)

**Figure 5.9**  Germination capacity following restratification for duration of stratification and temperature of germination pretreatments (---) \(14\degree C\), (----) \(24\degree C\).

The interaction between the pretreatment temperature and duration of stratification, \([T.S]\), accounted for most of the variation in the complete two factor model, since there was no significant difference \((p=0.336)\) between the residual deviances of the models \([T+S+R+T.S]\) and \([T+S+R+T.S+T.R+S.R]\). A smaller portion of the variation was described by the interaction between pretreatment temperature and duration of restratification, \([T.R]\), and virtually none was described by the
interaction between the duration of stratification and restratification, [S.R].

The interaction between the temperature of germination and the duration of stratification, [T.S], may be investigated further by examining the predicted effect of the interacting factors on germination capacity, averaged over all of the levels of the factor [R] (in a manner similar to that described in Section 3.1.2). A large portion of the [T.S] interaction can be attributed to the apparent sensitivity of the response at 24°C to the dormancy of the seed. Figure 5.10 shows that, whereas seed placed at 14°C during the pretreatment germination period showed an increase in germination capacity (and therefore a decrease in the strength of the dormancy induced) with increasing duration of pretreatment stratification, the seed set to germinate at 24°C exhibited a more complex response. The dormancy induced in unstratified seed set to germinate at 24°C was substantially weaker than that induced in the seed stratified for 10 days before germination and was similar to that of seed stratified for 20 days.

The lack of significance of both the [T.R] and [S.R] interactions is important because it shows that the breaking of dormancy during restratification was similar, regardless of the pretreatment conditions. The variation in the germination capacities of the pretreatments means that the portion of the seed sample entering the second part of the experiment also varied enormously. The absence of interactions between the pretreatment factors and the duration of restratification shows that the rate at which dormancy was broken was similar in the seed remaining after all pretreatments.
Induction of dormancy:

### TABLE 5.12

Summary of analysis of deviance of incremental germination capacity classified by pretreatment and duration of re-stratification.

<table>
<thead>
<tr>
<th>Model terms</th>
<th>Residual d.f.</th>
<th>Residual deviance</th>
<th>RMD d.f.</th>
<th>Change d.f. deviance</th>
<th>MCD</th>
<th>F Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>149</td>
<td>3396.2</td>
<td>22.79</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T+S+R</td>
<td>142</td>
<td>337.7</td>
<td>2.38</td>
<td>7</td>
<td>3058.3</td>
<td>436.90</td>
<td>245.45 &lt;0.001</td>
</tr>
<tr>
<td>+T.S +T.R +S.R</td>
<td>128</td>
<td>224.4</td>
<td>1.75</td>
<td>14</td>
<td>113.3</td>
<td>8.09</td>
<td>4.54 &lt;0.001</td>
</tr>
<tr>
<td>-T.S</td>
<td>130</td>
<td>312.6</td>
<td>2.40</td>
<td>2</td>
<td>88.2</td>
<td>44.10</td>
<td>24.78 &lt;0.001</td>
</tr>
<tr>
<td>-T.R</td>
<td>132</td>
<td>238.5</td>
<td>1.81</td>
<td>4</td>
<td>14.1</td>
<td>3.53</td>
<td>1.98 0.101</td>
</tr>
<tr>
<td>-S.R</td>
<td>136</td>
<td>232.9</td>
<td>1.71</td>
<td>8</td>
<td>8.5</td>
<td>1.06</td>
<td>0.60 0.778</td>
</tr>
<tr>
<td>-T.R -S.R</td>
<td>140</td>
<td>247.8</td>
<td>1.78</td>
<td>12</td>
<td>24.3</td>
<td>2.03</td>
<td>1.14 0.336</td>
</tr>
<tr>
<td>+T.S.R</td>
<td>120</td>
<td>214.0</td>
<td>1.78</td>
<td>8</td>
<td>10.4</td>
<td>1.30</td>
<td>0.73 0.666</td>
</tr>
</tbody>
</table>

T = Pretreatment germination temperature (14°C, 24°C)
S = Duration of pretreatment stratification (0, 10, 20 days)
R = Duration of re-stratification (0, 10, 20, 40, 60 days)
T.S, T.R, S.R = two factor interactions of T, S, R
T.S.R = three factor interaction of T.S.R

\(^1\) change from the two factor interaction model [T+S+R+T.S+T.R+S.R]

---

![Graph](image)

**Figure 5.10** The predicted germination capacities of the [T.S] interaction from the analysis in Table 5.12

Induction of dormancy as a result of a treatment could be expected
to be related to the germination capacity of the sample as a result of pretreatment. The relationship between germination capacity during pretreatment and the germination capacity following restratification could be tested much more rigorously by replacing the pretreatment factors ([T] and [S]) in the analysis with the predicted germination capacity for the pretreatment from the analysis of the pretreatment germination capacity (Table 5.12, Figure 5.6). The importance of this relationship was assessed from the difference in the residual deviance of the two models ([Pretr.Restr] and [P.Restr] in Table 5.13).

### TABLE 5.13

Summary of analysis of deviance for the replacement of the pretreatment effects with the predicted pretreatment germination.

<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>MCD</th>
<th>F Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>149</td>
<td>3396.</td>
<td>4</td>
<td>2974.3</td>
<td>743.58</td>
<td>417.74</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Restr</td>
<td>145</td>
<td>421.7</td>
<td>4</td>
<td>173.0</td>
<td>34.60</td>
<td>19.44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>+Pretr +Restr</td>
<td>140</td>
<td>248.7</td>
<td>5</td>
<td>34.70</td>
<td>1.74</td>
<td>0.97</td>
<td>0.022</td>
</tr>
<tr>
<td>+Pretr .Restr</td>
<td>120</td>
<td>214.0</td>
<td>20</td>
<td>34.70</td>
<td>1.74</td>
<td>0.97</td>
<td>0.022</td>
</tr>
</tbody>
</table>

Replacement of factor Pretr with the variable P

| P+Restr             | 144           | 407.8             | 4           | 159.1           | 39.78 | 22.35  | <0.001 |
| P.Restr             | 140           | 401.9             | 20          | 187.9           | 9.40 | 5.28   | <0.001 |

Pretr = Pretreatment (0,10,20 days at 5°C and 14,24°C germination) = T+S+T.S, Table 5.12

Restr = Restratisation (0,10,20,40,60 days at 5°C)

P = Predicted germination capacity during pretreatment from Table 5.9 model [Temp+Strat+Temp +Strat]

1 change compared to [Pretr +Restr]

2 change compared to [Pretr .Restr]

The germination capacity during pretreatment accounted for significantly less deviance than the pretreatment factors (p<0.001), but it did reduce the residual deviance compared to restratification
alone (p<0.001). The interaction \[P\text{.Restr}\] was not significant (p=0.989) compared to \[P\text{+Restr}\] and the coefficients of the additive model, \[P\text{+Restr}\], show that there is a positive relationship between the germination capacity during pretreatment and the incremental germination capacity.

The inability of pretreatment germination capacity to account for as much of the deviance as the pretreatment factors indicates that these factors had a different effect on the germination capacities during pretreatment and after restratification. A large part of this effect may be attributed to the tendency for temperature of pretreatment to have the opposite effect on the germination capacity during pretreatment compared to the germination capacity after restratification. Increasing the duration of pretreatment stratification led to an increase in the germination capacity of the seed both during pretreatment and after stratification.

(iv) Total germination capacity over the whole experiment

The long period of stratification required to break the dormancy of the seed which did not germinate during pre-treatment confirms that dormancy was induced in the seeds during pretreatment (for example, 60 days restratification did not increase the total germination capacity of the seed to the level attained after 20 days stratification during pretreatment (Figure 5.11)).

The viable seeds which did not germinate during the period of pretreatment germination were a 'selection' from the original population of seeds. The differences in the strength of dormancy of the seeds which did not germinate during pretreatment, judged by
Figure 5.11 The total germination capacity during the pretreatment germination period, restratification and the germination period after restractification 14°C (-----) 24°C (-----).

differences in the incremental germination capacity, may have been due to differences in the strength of the dormancy induced or to differences in the dormancy inherent in the 'selected' portions of the seed sample. One means of investigating this difference in the origin of the dormancy is to consider the responses in terms of the total germination capacity.

If the differences in the strength of dormancy noted in section (iii) were a consequence of inherent differences in the dormancy of the portion of the seed which remained after pretreatment then the responses of total germination capacity to duration of stratification of the various pretreatments should not provide evidence of differences in the strength of dormancy of similar portions of the seed samples. There is, however, evidence of differences in the strength of dormancy. The most obvious case is that of unstratified seed set to germinate at 24°C which has a total germination capacity which exceeds that of
restratification. This difference represents a difference in the strength of dormancy, since a portion of the seed population which germinated after 40 and 60 days restratification in the seed pretreated at 24°C is still dormant in the sample pretreated at 14°C.

Similar, though less dramatic, evidence of differences in the strength of dormancy occurring due to pretreatment comes from the response of total germination capacity to restratification in the seed set to germinate after 10 days pretreatment stratification. In this situation the total germination capacity of seed germinated at 14°C rose above that of seed germinated at 24°C after 60 days restratification.

In summary, the response of the total germination capacity of the seed to restratification shows that the differences in the incremental germination capacity are unlikely to be the result of inherent differences in the dormancy of the seed remaining after pretreatment. Rather they have their origins in differences in the strength of the dormancy induced in the seed.

(v) Times-to-germination after restratification

Samples which had been restratified for 0, 10 and 20 days were excluded from the analysis because of the low numbers of seeds which germinated. The Cox model thus is based on a germination period of 20 days following either 40 or 60 days restratification.

The analysis of times-to-germination after restratification was summarized in Table 5.14 and shows that the three factor interaction is significant ($p<0.001$). The treatment constants of the full model
(Figure 5.12) show that the response of times-to-germination to pretreatment temperature and stratification, after 40 days restratification, bore some similarity to the response of germination capacity to the pretreatment factors shown in Figure 5.12. The effects of pretreatment on times-to-germination were not apparent after 60 days of restratification but the manner in which the times-to-germination changed compared to those of seed stratified for 40 days indicates that the rate of germination may decline after long durations of stratification after treatments which tend to break the dormancy of the seed. A similar effect was noted in seed soaked for 3 days and then stratified for 6 weeks (Section 5.1).

### TABLE 5.14

Summary of analysis of deviance for times-to-germination for seed pretreated and then germinated following restratification.

<table>
<thead>
<tr>
<th>Model terms</th>
<th>Residual d.f. deviance</th>
<th>Residual d.f. deviance</th>
<th>Change d.f. deviance</th>
<th>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>728 3808.0</td>
<td>5.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>710 971.4</td>
<td>1.37</td>
<td>18 2836.5</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>T+S+R</td>
<td>706 931.1</td>
<td>1.32</td>
<td>5 40.3</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>T+S+R+T+R+R</td>
<td>701 918.2</td>
<td>1.31</td>
<td>5 12.9</td>
<td>p=0.024</td>
</tr>
<tr>
<td>T+R+R</td>
<td>699 904.2</td>
<td>1.29</td>
<td>2 14.0</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

Time = Day of observation  
T = Pretreatment germination temperature (14°C, 24°C)  
S = Duration of pretreatment stratification (0, 10, 20 days)  
R = Restrataification (40, 60 days)  
T.S.T.R.S.R = two factor interactions of T,S,R  
T.S.R = three factor interaction of T,S,R

A comparison of the predicted and observed germination curves shows that the differences between the treatments at the extremes of the range is small (between 1 and 2 days at 50% germination). However, the existence of differences in the rate of germination due to
experiences which occurred at least 40 days previously emphasizes the importance of recognizing the potential of events far removed from the immediate conditions in influencing the timing of germination.
Figure 5.12 The treatment constants for the Cox model fitted to seed which germinated after either 40 or 60 days restratification.

Figure 5.13 The predicted and observed germination curves for seed stratified for either 0 or 20 days, germinated at 15°C and then restratified for 40 days.
(vi) Seed mortality

The relative mortality of seeds exposed to the various treatments can be assessed from the number of seeds found to be viable, either by squashing or germination, if the assumption is made that the number of viable seeds were similar in all replicates at the beginning of the experiment. Analysis of variance shows that the number of viable seeds was influenced significantly by both the pretreatment stratification period and the temperature of germination, but not by the period of re-stratification or by the interactions between the treatment effects (Table 5.15).

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>d.f</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Ratio</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strat</td>
<td>2</td>
<td>2258.0</td>
<td>1129.0</td>
<td>9.925</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Temp</td>
<td>1</td>
<td>1120.7</td>
<td>1120.7</td>
<td>9.852</td>
<td>p = 0.002</td>
</tr>
<tr>
<td>Restr</td>
<td>4</td>
<td>1301.7</td>
<td>325.4</td>
<td>2.661</td>
<td>p = 0.025</td>
</tr>
<tr>
<td>Strat . Temp</td>
<td>2</td>
<td>73.2</td>
<td>36.6</td>
<td>0.322</td>
<td>p = 0.730</td>
</tr>
<tr>
<td>Strat . Restr</td>
<td>8</td>
<td>1225.8</td>
<td>153.2</td>
<td>1.347</td>
<td>p = 0.225</td>
</tr>
<tr>
<td>Temp . Restr</td>
<td>4</td>
<td>373.0</td>
<td>93.2</td>
<td>0.820</td>
<td>p = 0.517</td>
</tr>
<tr>
<td>Residual</td>
<td>128</td>
<td>14560.0</td>
<td>113.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Stratification reduced the mortality of seeds during pretreatment since the number of viable seeds increased with the duration of stratification (Table 5.16). This confirms the results of the previous experiment which showed that there was a reduction in the
number of viable seeds as the duration of treatment at 15°C was increased (Table 5.8). It is suggested that the exposure of unstratified seed to high temperatures leads to the death of a proportion of the seeds, but that this proportion may be reduced as a result of rapid germination if the seed has been stratified prior to exposure. Temperature conditions during germination also caused a differential in mortality between treatments since seed germinated at 24°C exhibiting higher mortality than seed germinated at 14°C.

**TABLE 5.16**

The mean number of viable seeds for the pretreatment temperature and stratification and restratification treatments.

<table>
<thead>
<tr>
<th>Stratification</th>
<th>0 days</th>
<th>10 days</th>
<th>20 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>14°C</td>
<td>24°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>83.5</td>
<td>78.0</td>
<td></td>
</tr>
<tr>
<td>Restratification</td>
<td>0 days</td>
<td>10 days</td>
<td>20 days</td>
</tr>
<tr>
<td></td>
<td>77.7</td>
<td>80.1</td>
<td>85.9</td>
</tr>
</tbody>
</table>

For comparison between means:
- Stratification : LSD_{0.01} = 5.58
- Temperature : LSD_{0.01} = 4.56
- Restratification : LSD_{0.01} = 7.20

The absence of a significant effect of restratification (Table 5.15) shows that the number of viable seeds was not significantly affected by restratification over periods of up to 60 days following pretreatment.
Induction of dormancy:

Discussion:

The changes in the response of the seed to the temperature environment have been discussed previously in relation to seed which has not been stratified (Section 5.1.2). The study reported in this section has extended these observations by looking at the dormancy induced in seed which does not germinate in conditions which may sustain germination in a portion of the seed sample. The seed germinated at 14°C with no pretreatment stratification may be compared in this and the previous experiment (Figure 5.14) and the dormancy induced was found to be similar. The dormancy induced in moist seed exposed to 24°C for 16 days was weaker than the dormancy induced at 14°C/15°C. It is evident from this result that a treatment which produces a low germination capacity does not necessarily induce a stronger dormancy compared to a treatment which leads to a high germination capacity.

The impact of the weaker dormancy induced in seed at higher temperatures is not likely to be great in seed shed in the field since the higher temperatures of summer will be followed by lower temperatures which will induce dormancy more fully. The low germination capacity of seed germinated at higher temperatures (24°C) will make autumn germination of seed shed in summer very unlikely. On the other hand, a proportion of the seed shed later in autumn, when temperatures are lower, may germinate before dormancy is induced. The changes in dormancy in the field and their impact on the emergence of seedlings will be further discussed in Chapter 7.

The strength of dormancy induced in seed by moist treatment at high temperatures is highly dependent on the dormancy of the seed prior
to treatment and the temperature of germination. The influence of the pretreatment stratification and temperature of germination are very different. Longer durations of pretreatment stratification result in a weaker dormancy and so the germination capacities before and after re-stratification are both positively related to the pretreatment stratification period.

The response to temperature differs, depending on the prior stratification treatment. Generally pretreatments which resulted in higher germination capacities within a stratification treatment induced a stronger dormancy. The possibility that the stronger dormancy was a consequence of inherent differences in the dormancy of the seed remaining after pretreatment was discounted (Figure 5.11).

![Graph showing germination capacity over duration of re-stratification](image)

**Figure 5.14** A comparison of the germination capacity of seed treated at 15°C for 15 days and stratified before germination at 15°C (Section 5.1.2) and the unstratified pretreatments (14°C, 24°C for 16 days) in this experiment.

Seeds died during the pretreatment germination. The observations of mortality during pretreatment stratification and re-stratification
Induction of dormancy:

suggest that there is a portion of the seed population which may only survive a single experience of conditions suitable for germination. This portion of the seed sample could only become established seedlings if the seed was shed in late autumn or spring, when the chances of dormancy being induced are low.
5.3 Induction of dormancy by desiccation of non-dormant seed

Field observations suggest that in spring the soil may dry out prior to the emergence of seedlings; a logical corollary is that the seed may also dry out before germination takes place. This section describes an experiment which tests the effect on germination of desiccation of the seed at various temperatures following stratification.

Grose (1963) studied the effect of dry storage on the dormancy and viability of *E. delegatensis* seed. He showed that the degree of dormancy which was induced in dried, stratified seed increased with increases in temperature over the range $5^\circ C$ to $37^\circ C$ and decreased with reductions in the water content of the seed. Additionally, the rate at which dormancy was induced in the seed of *E. delegatensis* was found to increase as the temperature of storage increased from $5^\circ C$ (19% water content) to $37^\circ C$ (4% water content). The viability of the seeds declined dramatically over the period of storage (30 weeks) when maintained at $5^\circ C$ but showed a more modest decline in those treatments at high temperatures in which dormancy was induced more rapidly.

Methods:

Replicates of cleaned seed (0.2 g, 33.7 viable seeds) collected near Dicky Cooper Ck. in December 1980 were moistened and stratified for 4 weeks at $5^\circ C$. Following stratification, each replicate was weighed and dried by placing the petri dishes on a bed of Silica gel in airtight plastic boxes at either $5^\circ C$, $15^\circ C$, or $40^\circ C$.

After 1 week of the drying treatment the seed was weighed,
moistened and placed in an incubator at 15°C to germinate. An additional group of five replicates continued the drying treatment at 5°C for 12 weeks prior to rewetting.

Results:

(i) Germination capacity

The germination of the seed was reduced by drying for 1 week at all temperatures (Table 5.17 and Table 5.18). The analysis indicates that drying at 40°C severely reduced the proportion of seed germinating when compared to drying at 5°C or 15°C or to no drying (Table 5.18).

**TABLE 5.17**

Summary of analysis of deviance for the germination capacity of seed which was dried under various regimes following stratification.

<table>
<thead>
<tr>
<th>Model terms</th>
<th>Residual d.f. deviance</th>
<th>RMD</th>
<th>Change d.f. deviance</th>
<th>MCD</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>24</td>
<td>288.70</td>
<td>12.03</td>
<td></td>
<td>236.28</td>
<td>59.07</td>
</tr>
<tr>
<td>Treatment</td>
<td>20</td>
<td>52.42</td>
<td>2.62</td>
<td>4</td>
<td>236.28</td>
<td>59.07</td>
</tr>
</tbody>
</table>

An increase in germination capacity was observed in samples exposed to dry conditions for prolonged periods. This result is perplexing since an examination of the weight taken before the seed was set to germinate confirmed that the seed stored for 12 weeks under dry conditions was indeed dry.

The reduction of weight by the end of drying was the same in all treatments and the water content of the seed was similar to that of
Induction of dormancy: 

freshly fallen seed. The reduction in dormancy occurred, therefore, at water contents equivalent to those of dry seed after shedding from the capsule (8-10%). The decline in the germination capacity of *E. pauciflora* seed stored under dry conditions at 5°C was much greater in the experiments reported in this section than the decline reported for *E. delegatensis* seed by Grose (1963). The water content of the seed was much lower (10%) in the present experiments. One explanation for this difference could be that the dormancy induced by drying was balanced by the breaking of dormancy at higher water contents. But, as the water content declined the rate of breaking of dormancy declined leaving a net induction of dormancy. However, this explanation would not account for the high germination capacity of seed stored at 5°C for 12 weeks unless the rate of breaking dormancy under dry conditions was high enough to permit some breaking of dormancy. Further work is necessary if the response of seed to drying is to be fully elucidated, but the experimental manipulation of water content presents many problems.

**TABLE 5.18**

The coefficients and predicted values from the treatment model in of Table 5.17.

<table>
<thead>
<tr>
<th>DRYING TREATMENTS</th>
<th>Coefficients Estimate</th>
<th>s.e.</th>
<th>Predicted germination (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No drying</td>
<td>0.446</td>
<td>0.150</td>
<td>61.0</td>
</tr>
<tr>
<td>5°C for 1 week</td>
<td>-1.386</td>
<td>0.201</td>
<td>20.0</td>
</tr>
<tr>
<td>5°C for 12 weeks</td>
<td>0.983</td>
<td>0.167</td>
<td>72.9</td>
</tr>
<tr>
<td>15°C for 1 week</td>
<td>-1.293</td>
<td>0.193</td>
<td>21.5</td>
</tr>
<tr>
<td>40°C for 1 week</td>
<td>-2.226</td>
<td>0.271</td>
<td>9.7</td>
</tr>
</tbody>
</table>

The actual response of the induction of dormancy to the water
content of the seed is not entirely clear since the final water content and the rate of drying were confounded in Grose’s experiments. *Eucalyptus delegatensis* seed was stratified, dried at a temperature of 17°C for 56 hours, then moistened and set to germinate at 17°C. The germination capacity of seed at 35% water content was almost as high as for seed which had not been dried. The germination capacity declined with decreasing water content until a water content of around 15% was reached. As the water content of the seed was further reduced the degree of dormancy was also reduced. These results indicate that rapid drying to low water contents does not influence the dormancy of the seed. However, a reduction in the rate of drying, or the maintenance of higher moisture contents, results in an increasing rate of induction of dormancy.

Unfortunately, we have at present little knowledge of the changes which occur in the water content of the seed in the field. Some attempts were made to collect this information in the present study (Section 7.3) but contamination of the seed with soil prevented accurate assessments of seed water content. Further work is necessary to determine the response of seed to drying at various temperatures. At present, I can only conclude that the rate of induction of dormancy increases with increases in temperature and water content and that the interaction between the two environmental factors is far from defined.
5.4 Conclusions

1. The strength of dormancy induced in moist unstratified seed depends on the temperature and duration of treatment.

2. The strength of the induced dormancy far exceeds the innate dormancy of the seed.

3. Factors which increase the strength of dormancy also increase the times-to-germination.

4. The strength of the dormancy induced in seed which did not germinate during a time when conditions were adequate for germination is dependent on the temperature of exposure and the dormancy of the seed prior to the germination.

5. For a given temperature of germination, the dormancy induced will be weaker in seed which has been stratified for longer.

6. Drying stratified seed at high temperatures results in the induction of dormancy in the seed.

The studies in this chapter show that a seed which falls to the ground in summer or early autumn is likely to become strongly dormant although the specific strength of the dormancy will be dependent on the temperature and moisture conditions which it is exposed to. If the seed falls to the ground in late autumn it may become moist at temperatures which are low enough to break dormancy, however, the temperature conditions it will experience subsequently will be low and germination is unlikely. Such conditions will only permit the
Induction of dormancy: 200.

germination of seed stratified for long durations.

The expected behaviour of the seed in spring, having spent a winter on the soil will be very different. This seed will have experienced a long period of low temperatures which will mean that the seed will germinate at a wide range of temperatures and that if dormancy is induced it will be relatively weak. If this seed becomes dry dormancy will be induced but the strength or degree of this dormancy has not been investigated.
Induction of dormancy:

5.6 Appendix

This appendix describes the fitting of an empirical model in place of the factors [Treat] and [Strat] in the analysis of germination capacity in Section 5.1.2.

The effect of stratification may be replaced by a variable, ST, the duration of stratification in days (1).

\[
\text{logit}(G_i) = \mu + \text{Treat}_i \times \beta_1 \times \text{ST}
\]

\(G_i\) is the probability of germination in treatment at 15°C treatment \(i\). \(\beta_1\) is a constant and \(\text{ST}\) is the duration of stratification in days.

The difference in the residual deviance of this model and [Treat+Strat] is not significant (p=0.168) and the effect of stratification may, therefore, be replaced by a constant multiplied by the duration of stratification. The value of the constant (\(\beta_1=0.113, \text{s.e.}=0.005\)) is slightly lower than that noted in Section 4.2.2 which shows that the log of the odds ratio of germination increased at a slower rate during the breaking of dormancy in this experiment.

The effect of treatment at 15°C can be investigated in a similar way. Table 5.19 shows that a linear function of S, the duration of treatment at 15°C in days, fitted in place of the factor [Treat] results in a highly significant difference in residual deviance compared to [Treat+Strat]; however, when a quadratic function [S+SQ] is fitted in place of the factor [Treat] in the model (2) the residual deviance is not significantly different (p=0.019) from [Treat+Strat].
TABLE 5.19

Summary of analysis of deviance for the replacement of [Treat] and [Strat] by the variables S, SQ, ST in the analysis of germination capacity of seed stratified for 10, 20, 40 days after treatment at 15°C for 0.5, 10, 15 days at 15°C.

<table>
<thead>
<tr>
<th>Model terms</th>
<th>Residual d.f. deviance</th>
<th>RMD</th>
<th>d.f. deviance</th>
<th>MCD</th>
<th>F Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treat +Strat</td>
<td>54</td>
<td>133.8</td>
<td>2.48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replacement of factor Strat by variable ST.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treat +ST</td>
<td>55</td>
<td>140.3</td>
<td>2.55</td>
<td>1</td>
<td>6.5</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replacement of factor Treat by variables S, SQ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S +Strat</td>
<td>56</td>
<td>349.1</td>
<td>6.23</td>
<td>2</td>
<td>215.30  107.63</td>
<td>32.041 0.001</td>
</tr>
<tr>
<td>S+SQ +Strat</td>
<td>55</td>
<td>153.2</td>
<td>2.79</td>
<td>1</td>
<td>19.4</td>
<td>19.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replacement of both Treat and Strat by variables S, SQ, ST</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S +SQ +ST</td>
<td>56</td>
<td>158.5</td>
<td>2.63</td>
<td>2</td>
<td>24.7</td>
<td>12.35</td>
</tr>
</tbody>
</table>

Factors:
Treat = Duration of treatment at 15°C
Strat = Duration of stratification

Variables:
ST = Duration of stratification (10, 20, 40 days)
S = Duration of treatment at 15°C (0.5, 10, 15 days)
SQ = $S^2$

1 Compared to [Treat + Strat]

logit($G_1$) = $\mu + b_1S + b_2SQ + Strat$ .... 2

The variables S and SQ refer to the duration of treatment at 15°C in days and the duration of treatment at 15°C in days squared respectively. The final step in the derivation of an empirical model for the germination capacity of E. pauciflora seed soaked at 15°C is the investigation of the model (3) in which all the factors have been
replaced by variables.

\[ \text{logit}(G_1) = \mu + \beta_1 S + \beta_2 SQ + \beta_3 ST \]

...3

The model describes the variation noted equally as well as [Treat+Strat] (p=0.032) and can be used for the prediction of the germination capacity of seed exposed to treatment at 15°C and then stratification. The values of the coefficients of the model, and their standard errors are \( \mu = -0.991 \text{ s.e.} = 0.107 \), \( \beta_1 = -0.77 \text{ s.e.} = 0.033 \), \( \beta_2 = 0.029 \text{ s.e.} = 0.002 \), \( \beta_3 = 0.111 \text{ s.e.} = 0.005 \). The germination capacities predicted by this model are compared to the observed values.
5.5 Summary of methods and results

A. Section 5.1.1
B. Section 5.1.2
C. Section 5.2  Germinated at 15°C
D. Section 5.2  Germinated at 24°C
E. Section 5.3
USE OF THESSES

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

by

Douglas Graham Abrecht

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 6.1

The temperature regimes for germination on the temperature gradient plate.

<table>
<thead>
<tr>
<th>TEMPERATURE</th>
<th>TEMPERATURE FOR PERIOD</th>
<th>GERMINATION PERIOD(^2)(DAYS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>REGIME</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 12.8</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).

(i) 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
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  d. 0 days at 5°C, 24°C  
b. 10 days at 5°C, 14°C  e. 10 days at 5°C, 24°C  
c. 20 days at 5°C, 14°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
Temperature of germination:

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>Residual d.f deviance</th>
<th>RMD</th>
<th>Change d.f deviance</th>
<th>CMD</th>
<th>F Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>71</td>
<td>3799.00</td>
<td>53.51</td>
<td></td>
<td></td>
<td></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)
(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.</th>
<th>Deviance RMD</th>
<th>Change d.f.</th>
<th>Deviance</th>
<th>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>265</td>
<td>2741</td>
<td>10.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>252</td>
<td>851.4</td>
<td>3.38</td>
<td>7</td>
<td>1880.6</td>
</tr>
<tr>
<td>+Temp +Strat</td>
<td>246</td>
<td>388.6</td>
<td>1.58</td>
<td>6</td>
<td>462.8</td>
</tr>
<tr>
<td>+Temp .Strat</td>
<td>241</td>
<td>372.2</td>
<td>1.54</td>
<td>5</td>
<td>16.4</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
Temperature of germination: 213.

![Cumulative germination graph]

Figure 6.3 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.

on the gradient plate was assessed by analysis of 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 screening 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
Figure 6.4 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.</th>
<th>Residual deviance</th>
<th>Change d.f.</th>
<th>Change deviance</th>
<th>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>71</td>
<td>2011</td>
<td></td>
<td></td>
<td>p&lt;0.005</td>
</tr>
<tr>
<td>Temp +Strat</td>
<td>58</td>
<td>309.9</td>
<td>5.34</td>
<td>13</td>
<td>1701.1</td>
</tr>
<tr>
<td>+Temp .Strat</td>
<td>36</td>
<td>48.08</td>
<td>1.34</td>
<td>22</td>
<td>261.2</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
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.
in the seed which had not been stratified whilst it induced it in seed which was stratified.

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>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Sum of Squares</th>
<th>Mean Variance</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp</td>
<td>11</td>
<td>1926.7</td>
<td>175.2</td>
<td>1.153</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>
<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 = 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.
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 was 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 vs Temperature](image)

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).

![Germination Capacity Chart](chart.png)

**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
Temperature of germination:

'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>-Source</td>
<td>42</td>
<td>454.1</td>
<td>10.81</td>
<td>1</td>
<td>355.40</td>
<td>355.40</td>
</tr>
<tr>
<td>-Temp</td>
<td>54</td>
<td>788.6</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 Ck. seed after 16 and 40 days on the gradient plate.

<table>
<thead>
<tr>
<th>Seed Source</th>
<th>Estimated Peak</th>
<th>Shoulder</th>
<th>'Breath'</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temp °C % germ.</td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>Waste Point</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 days</td>
<td>16.6 81.5</td>
<td>10.8</td>
<td>20.7</td>
</tr>
<tr>
<td>40 days</td>
<td>17.2 84.7</td>
<td>8.2</td>
<td>20.8</td>
</tr>
<tr>
<td>Baker's Creek</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 days</td>
<td>16.0 58.0</td>
<td>12.5</td>
<td>17.8</td>
</tr>
<tr>
<td>40 days</td>
<td>14.9 57.0</td>
<td>14.2</td>
<td>17.9</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: 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.
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.</th>
<th>Residual deviance</th>
<th>RMD</th>
<th>Change d.f.</th>
<th>Change 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>
<td></td>
<td></td>
</tr>
<tr>
<td>Temp +Source</td>
<td>14</td>
<td>73.92</td>
<td>5.28</td>
<td>5</td>
<td>422.08</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>+Temp .Source</td>
<td>10</td>
<td>11.97</td>
<td>1.20</td>
<td>4</td>
<td>61.95</td>
<td>p&lt;0.001</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.

![Graph showing germination capacity](image)

**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 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:

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

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
USE OF THESES

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

by

Douglas Graham Abrecht

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

February 1985
CHAPTER 7

DORMANCY, GERMINATION AND EMERGENCE

IN THE FIELD
CHAPTER 7

Experiments in the previous chapters have considered the influence of some environmental factors on the dormancy and the timing of germination of *Eucalyptus pauciflora* seed under controlled conditions. The studies in this chapter examine changes in dormancy and germination of the seed and the emergence of seedlings in the field in order to test the importance of the responses noted in the laboratory under field conditions.

The responses noted in Chapters 4 and 5 suggest that the strength of dormancy of seed which becomes moist at high temperatures will be increased and as the temperature declines to temperatures below 9°C dormancy will be broken. The first study in this chapter tests whether there are changes in the strength of dormancy or germination of seed planted in late summer and relates the changes to the physical environment.

Successful regeneration depends on seed germination and seedling emergence at a time when conditions are suitable for survival and growth of the seedling. Experiments in Chapters 3, 4 and 6 have shown that differences in the germination behaviour of seed from different altitudes are not likely to result from variation in the temperature response of breaking dormancy or germination but are more likely to be associated with the strength of dormancy. The differences in the strength of innate dormancy in seed collected at higher altitudes suggests that this seed would be less likely to germinate in autumn than seed from lower altitudes (Section 3.2, Section 4.2.1). The second section of this chapter examines the emergence of seedlings from
seed transplanted between altitudes with a view towards assessing the ability of the transplanted seed to produce emergent seedlings at altitudes other than those of its origin.

As soil temperatures decline during autumn, the conditions will be less likely to induce dormancy and will become increasingly favourable for germination of the seed and, in late autumn for the breaking of dormancy. The time of planting of seed could therefore be expected to have a critical influence on survival of the seed and the number of seedlings which emerge in spring. The final study in this chapter investigates the emergence of seedlings from seed planted at a range of times during autumn and in late winter.
7.1 Changes in dormancy and germination in the field

Methods:

Bags made of fine synthetic mesh (120 mm X 80 mm) were divided into 3 sections and 0.3 g (51.9 viable seeds) of cleaned seed, collected near Dicky Cooper Ck., was placed in each section.

A plot (1.5 m X 2.0 m) located on the southern edge of a clearing in a stand of *E. pauciflora* near Rennix Gap (1610 m) was cleared of vegetation and leveled in October 1982. On February 8 1983, forty-four bags of seed were buried at approximately 10 mm depth. The plot was fenced to minimize disturbance by animals.

Five bags of seed were randomly selected for retrieval at approximately fortnightly intervals during the period February 15 to May 5 and again on May 28 1983. Field germination during spring was assessed by retrieving one bag at weekly intervals, beginning on August 28. Bags were retrieved with the surrounding soil intact (to a depth of 15 mm) and were sealed in a plastic bag to minimize changes in the moisture content of the seed.

On the day following retrieval, the soil surrounding the mesh bags was brushed off and used for determination of gravimetric soil moisture content. The mesh bags containing the seed were rinsed to remove the remnants of soil. Seed was removed from each section of the bag and seeds which had germinated in the field were counted and discarded. The seed which remained was placed on filter paper in a 60 mm petri dish to which 2.0 ml of Benomyl® fungicide in water (0.2 g/l) had been added. The seed from one section of each bag was stratified (5°C) for either
Dormancy, germination and emergence in the field: 239.

0, 4 or 8 weeks and then set to germinate at 15°C.

A CR21 data logger (Campbell Scientific Inc., Logan, UTAH), was used for continuous monitoring of the climatic environment in the field. The previous experiments had shown the importance of temperature and moisture availability in determining the changes in dormancy of seed and it was decided to monitor these aspects of the environment closely. Screen temperature (1.5 m), and soil temperatures at 0.01 m, 0.1 m, 0.2 m depth were sampled every 30 minutes using matched, calibrated thermistors. Rainfall was measured with a tipping bucket rainguage and soil moisture was monitored by daily readings of gypsum soil moisture blocks (Beckman Instruments Inc., Cedar Grove, NEW JERSEY) installed at 0.1 m and 0.2 m. Rainfall, gravimetric soil moisture content (core from the surface to 0.05 m), and soil temperatures at 0.5 m, 1.0 m were monitored weekly in addition to the continuous records from the data logger.

Results:

(i) Weather measurements

Rainfall, soil moisture and soil temperature (0.01 m) data are summarized in Figure 7.1. The soil moisture blocks were found to be unreliable and therefore the weekly gravimetric soil moisture measurements have been used as an indication of soil moisture status.
Figure 7.1 The changes in dormancy in relation to the climate during spring and autumn 1983 at Rennix Gap (1610 m).

a. % germinated seeds at retrieval
b. % germinated seeds at 15°C
c. % germinated seeds at 15°C after 4 weeks at 5°C
d. % germinated seeds at 15°C after 8 weeks at 5°C

(soil temperature is the mean temperature of 48, 30-minute samplings for each day)

(ii) Changes in dormancy during autumn

The analysis of the germination capacities of the seed retrieved and stratified prior to germination at 15°C confirms that there are significant differences in the response of seed, retrieved at different times to stratification (Table 7.1).
TABLE 7.1

Summary of analysis of deviance for germination capacities of seed retrieved from Rennix Gap in autumn and germinated in the laboratory.

<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 CMD</th>
<th>F Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>104</td>
<td>3492</td>
<td>33.58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retr +Strat</td>
<td>96</td>
<td>605.9</td>
<td>6.31</td>
<td>8</td>
<td>2886.1</td>
<td>360.76</td>
</tr>
<tr>
<td>Retr -Strat</td>
<td>84</td>
<td>381.8</td>
<td>4.55</td>
<td>12</td>
<td>224.1</td>
<td>18.68</td>
</tr>
</tbody>
</table>

Retr = Date of retrieval from Rennix Gap
Strat = Stratification treatment (0, 4, 8 weeks)

The germination capacity of seed samples after various periods of stratification permits an assessment of the strength of dormancy since seed with a lower germination capacity after a certain duration of stratification has a stronger dormancy. The trends in the germination capacity of the seed show that the seed became strongly dormant within a week of planting and that there was a progressive weakening of dormancy in late autumn as the soil temperatures fell and the soil moisture rose to high levels (Figure 7.1). Weakening of dormancy in late March and April was first apparent in the seed retrieved on April 13 which showed an increase in germination capacity after 8 weeks stratification but not after 4 weeks stratification. By April 28 the germination capacity of the seed stratified for 4 weeks was beginning to rise whilst the germination capacity of the seed stratified for 8 weeks remained at a high level. This trend continued until the final retrieval during autumn (May 28).

The refrigerator used for stratification had a mean temperature of 2°C in the first week of stratification of the seed retrieved on February 15, compared to 5°C for all other weeks in the experiment.
Dormancy, germination and emergence in the field: 242.

This occurrence would be expected to have reduced the rate at which dormancy was broken and may have resulted in the very low germination capacity of seed stratified for 8 weeks after retrieval on that day compared to seed retrieved in subsequent weeks. The contrasting lack of difference in the germination capacity of seed retrieved on February 15 compared to subsequent weeks after 4 weeks stratification is probably accounted for by the inability to break the dormancy of the seed.

(iii) Changes in dormancy and germination in spring

The weakening of dormancy due to low temperatures continued throughout winter. By August 28 the seed was non-dormant at 15°C, and in the ensuing weeks the seed germinated in the field. The daily mean temperatures during this period did not exceed 5°C (Figure 7.1).

(iv) Seed mortality

The number of viable seeds in the samples retrieved from the field were analysed in order to investigate mortality of seed due to storage in the field over the autumn period. The number of viable seeds was calculated as the sum of those seeds which germinated before and after retrieval from the field and the seeds judged to viable by squashing at the end of the laboratory germination period. Analysis of variance showed that the time of retrieval may have had a significant effect on the number of viable seeds (p=0.048), but there was no significant trend in the mean number of viable seeds with date of retrieval from Rennix Gap (linear regression p=0.155) during autumn.
Dormancy, germination and emergence in the field:

TABLE 7.2

The mean number of viable seeds for each of the days of retrieval

<table>
<thead>
<tr>
<th>Date (1983)</th>
<th>FEB 15</th>
<th>MAR 1</th>
<th>MAR 16</th>
<th>MAR 30</th>
<th>APR 13</th>
<th>APR 28</th>
<th>MAY 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day from Jan 1</td>
<td>46</td>
<td>60</td>
<td>75</td>
<td>89</td>
<td>103</td>
<td>118</td>
<td>148</td>
</tr>
<tr>
<td>Viable seeds</td>
<td>51.9</td>
<td>56.9</td>
<td>58.7</td>
<td>50.1</td>
<td>53.8</td>
<td>48.7</td>
<td>52.5</td>
</tr>
</tbody>
</table>

LSD₀.₀₅ = 7.58

Seed retrieved in spring also showed significant differences in the number of viable seeds (ANOVA, p=0.004); however, there was no trend in the number of viable seeds with time of retrieval between August 28 and October 4 (linear regression, p=0.58). The mean number of viable seeds in the autumn and spring retrievals were not significantly different indicating that there was little mortality of seeds due to storage in the soil during winter.

Discussion:

Changes in the dormancy of seed stored in the soil in the field have an important bearing on the chances for successful germination and seedling establishment. Seed planted in late summer became strongly dormant as a result of the exposure of moist seed to high soil temperatures; this behaviour is consistent with the induction of dormancy noted in Chapter 5. Rainfall caused an increase in soil moisture during early April when temperatures were too hot for dormancy to be broken. At this time a low proportion of the seeds germinated but no further strengthening of dormancy was observed. As the soil temperatures declined during April, with soil moisture at a high level, the dormancy of the seed became progressively weaker.
Dormancy was broken at temperatures below 9°C in the laboratory (Chapter 4). Figure 7.2 shows that the increase in the germination capacity after 4 weeks stratification of seed retrieved from the field was associated with the duration of exposure (number of 30-minute periods) to temperatures below 9°C in the field.

![Graph showing germination capacity against number of 30-minute periods less than 9°C](image)

**Figure 7.2** The germination capacity of seed stratified for 4 weeks plotted against the number of 30-minute periods below 9°C.

Field germination of seed would be very unlikely in early winter since seed retrieved from the field on May 28 required a further 4 weeks stratification at 5°C in the laboratory for germination to occur at 15°C. Stratification of seed in the field is likely to take considerably longer because the soil temperatures will be lower than 5°C, and will result in a much slower breaking of dormancy (Section 4.3.1). The duration of stratification required would be further increased because germination at the low soil temperatures occurring during autumn and winter would necessitate a much longer period of stratification than is required at 15°C (Section 6.1).
The absence of significant mortality of seed in the field during the autumn and winter period is not consistent with the results of laboratory experiments which showed that mortality was associated with the induction of dormancy in moist conditions at 15°C. Grose (1963) also observed mortality of *E. delegatensis* seed during the autumn and winter period. The large variation in the number of viable seeds per replicate in the present experiment may have masked a low level of mortality during autumn and winter.
7.2 Seedling emergence from seed planted at four altitudes.

Seasonal changes in dormancy and germination of *E. pauciflora* seed tend to delay the germination of most of the seed in the soil until spring. Seed from higher altitudes has a stronger innate dormancy than seed from lower altitudes. The soil temperatures in autumn become more favourable for stratification at high altitudes and it is possible that seed with weaker dormancy would have an increased risk of germination during winter at higher altitudes. On the other hand, at low altitudes, the strong dormancy of the seed from higher altitudes may delay the germination of seed beyond the period of favourable conditions during spring. This section examines the extent and timing of seedling emergence from seed planted at four altitudes.

Methods:

Seed from four altitudes, Waste Point (960 m), Sawpit Ck. (1240 m), Dicky Cooper Ck. (1740 m) and Baker’s Ck. (1910 m) was divided into units (1.0 g Waste Point, the rest 0.5 g). The approximate number of viable seeds in a unit of each seed source is shown in Table 7.3.

Four sites in clearings with northerly aspects, and a slope not greater than 5%, were selected near Waste Point (960 m), Sawpit Ck. Ranger Station (1215 m), Rennix Gap (1610 m) and Pipers Gap (1740 m). At each site, four plots (0.7 m X 0.7 m) were cleared of vegetation and the soil was dug over to a depth of 0.2 m, and rocks and roots were removed to provide an homogenous seed bed. Soil collected at Dicky Cooper Ck., which had been dried and sieved, was spread on the plots to a depth of 10 mm, to provide a uniform substrate for seed germination.
Dormancy, germination and emergence in the field:

### TABLE 7.3

The numbers of viable seeds in units of the seed collected near Waste Point, Sawpit Ck., Dicky Cooper Ck. and Baker's Ck.:

<table>
<thead>
<tr>
<th>SEED SOURCE SITE</th>
<th>ALTITUDE</th>
<th>SAMPLE</th>
<th>MEAN NUMBER OF HEIGHT OF VIABLE SEEDS¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waste Point</td>
<td>960 m</td>
<td>1.0 g</td>
<td>24.9 ± 6.0</td>
</tr>
<tr>
<td>Sawpit Ck.</td>
<td>1240 m</td>
<td>0.5 g</td>
<td>27.5 ± 8.8</td>
</tr>
<tr>
<td>Dicky Cooper Ck.</td>
<td>1740 m</td>
<td>0.5 g</td>
<td>32.0 ± 5.6</td>
</tr>
<tr>
<td>Baker's Ck.</td>
<td>1910 m</td>
<td>0.5 g</td>
<td>43.0 ± 5.4</td>
</tr>
</tbody>
</table>

¹ Means of 8 replicates ± standard deviation

The sites of planting were not identical to the sources of the seed because of difficulties in collecting the amount of seed required from trees at sites which could be easily accessed during spring. An attempt was made to collect seed from populations of *P. pauciflora* from a wide altitudinal range (960 m to 1910 m).

On February 8 and April 13 1983, two of the plots at each altitude were planted with four replicates of the seed from each of the seed sources, using a latin square design. This design was used to permit an analysis of the effect of slope (rows) on the emergence of seedlings since some soil and water movement across the plot was expected. Each replicate of seed was confined to an area within the plot by a section of PVC water pipe (75 mm diameter) pushed into the soil to a depth of 90 mm to form a ring; the rings prevented the mixing of seed from different sources due to disturbance by needle-ice and runoff water.

Each replicate of seed was mixed with about 50 ml of sieved soil collected near Dicky Cooper Ck. which was then placed in a ring. The seed was planted in this way to ensure a range of planting depths for
the seed ranging from 0 mm to 10 mm in an attempt to remove any
differential effect of depth of planting on the germination response of
the seed.

After planting, each plot was covered with a fine white gauze to
minimize any effects due to rainfall splash; the whole plot was then
covered with a chicken wire cage (10 mm mesh), to prevent disturbance
by animals. The gauze was removed from plots at low altitudes in
early August, and after snow melt at higher altitudes.

Measurements of soil temperature at 0, 0.1, 0.2, 0.5, 1.0 m depth,
grass minimum temperature and rainfall were made at each site on a
weekly basis. The temperature environments at the various sites were
compared using temperature recorded at 0.5 m, because this was the
shallowest depth at which the fluctuations in temperature observed over
the 6 hours taken to visit all sites were negligible.

_Eucalyptus pauciflora_ seedlings emerge from the soil with the
cotyledons folded and often partially enclosed in the seed coat
(personal observation). The seed coat falls off as the cotyledons
expand. A seedling was classified as having emerged when the
cotyledons were fully expanded. The numbers of emergent seedlings in
each ring and the number of dead seedlings were recorded at
approximately weekly intervals, and the tops of dead seedlings were
removed.

The study reported in this section was first attempted in 1982.
October and November were very dry in that year, and drought resulted
in heavy mortality of seedlings at all altitudes of planting. The
results presented in this section were obtained in the spring of 1983.
in which soil moisture remained high throughout the period of emergence.

Results:

(1) The emergence of seedlings in autumn.

Seedlings began to emerge in early April at Waste Point and the timing of the first emergence was progressively delayed at higher altitudes. The emergence of seedlings in autumn is reflected in the number of emergent seedlings observed on May 28, 1983 (Table 7.4).

<table>
<thead>
<tr>
<th>ALTITUDE</th>
<th>960 m</th>
<th>1215 m</th>
<th>1610 m</th>
<th>1740 m</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCCASION</td>
<td>FEB</td>
<td>APR</td>
<td>FEB</td>
<td>APR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>FEB</td>
<td>APR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEED SOURCE</td>
<td>960 m</td>
<td>3 4</td>
<td>7 1</td>
<td>2 0</td>
</tr>
<tr>
<td>1240 m</td>
<td>6 10</td>
<td>2 0</td>
<td>4 0</td>
<td>1 0</td>
</tr>
<tr>
<td>1740 m</td>
<td>3 10</td>
<td>3 5</td>
<td>1 0</td>
<td>4 0</td>
</tr>
<tr>
<td>1910 m</td>
<td>3 3</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
</tbody>
</table>

1 Sum of seedlings emerging in 8 replicates at each planting time at each altitude. The approximate number of seeds per replicate is given in Table 7.3

The number of seedlings which emerged in autumn was small in relation to the amount of viable seed planted and declined with increasing altitude of planting. The number of seedlings which emerged from seed planted on April 13 was generally less than from the later...
planting, although this trend was reversed at Waste Point (960 m) where more seedlings emerged from seed planted in April than February.

(ii) The emergence of seedlings in spring

There are two aspects of the emergence of seedlings to be considered. The first is the general relationship between emergence of the seedlings and climate, and the second is variation in the emergence of the seedlings due to seed source at the different altitudes of planting.

The emergence curves generally show a gradual rise to a plateau (corresponding to the maximum number of emergent seedlings) then a decline as seedlings begin to die (Figure 7.3). The number of seedlings dying was negligible during the time when the number of seedlings emerging was increasing. There were, however, two notable departures from this trend. Severe needle-ice activity at Waste Point (960 m) during the week ending September 7 (day 250) resulted in the deaths of most of the seedlings which had emerged by that time. The marked reduction in the numbers of seedlings at Sawpit Ck. (1215 m) after September 27 (day 270) was due to flooding of the plots which caused death of most of the seedlings in the plots planted on April 13 and substantial mortality in the plots planted on February 8. The differences in the mortality of seedlings emerging from seed planted at different times appeared to be due to the position of the plots and not to differences in the characteristics of seedlings. The data from Sawpit Ck. site were excluded from further analyses.
Figure 7.3
(a) The total number of seedlings emerging during spring 1983 from seed collected at 4 altitudes [• Baker's Ck. (1910 m); ▲ Dicky Cooper Ck. (1740 m); + Sawpit Ck. (1240 m); □ Waste Point (960 m)] and then planted at 4 altitudes [PG (1740 m); RX (1610 m); SC (1215 m); WP (960 m); see Figure 1.1 for site locations] during autumn 1983.
(b) The soil temperature at 50 cm over time at the 4 sites of planting during spring 1983
(c) The soil moisture (0-5 cm) over time at the 4 sites of planting during spring 1983
Spring emergence of seedlings predominated at all altitudes of planting, with differences in the timing of emergence shown in Figure 7.3. Seedlings from seed collected at 960 m emerged rapidly at 960 m and 1215 m compared to those from seed collected at higher altitudes. The difference in emergence of seed from 960 m compared with the other sources amounted to 20 days at 1215 m. The period from the emergence of the first seedling until the maximum number of seedlings had emerged decreased with altitude of planting. Conditions were suitable for germination over a long period at Waste Point; the higher soil temperatures may have permitted a fuller expression of the strength of dormancy of the seed both within and between the seed sources. The emergence of seedlings at higher altitudes was probably delayed by low temperatures under snow. When the soil temperatures began to rise following the snow melt, a greater proportion of the seed germinated and the seedlings emerged and established rapidly.

Seedling mortality increased in late December at the same time as soil moisture levels began to decline rapidly (Figure 7.3) and it is assumed that the mortality was related to drought. The onset of mortality was delayed at lower altitudes even though the decline in soil moisture showed a similar pattern at all altitudes. This is probably because the seedlings at lower altitudes were larger (personal observation) and presumably had deeper root systems, and therefore better access to water than those at higher altitudes. This interpretation may be further complicated by differences in root to shoot ratios of seedlings growing at different altitudes which were not investigated.

As the number of viable seeds planted in each ring was not known, the comparison of the seed sources could not be based on the proportion
of the seed emerging. Neither could the comparison of the seed sources be based on absolute differences in the number of emergent seedlings because differences in the processes which lead to emergence would be confounded with differences in the initial numbers of viable seeds (Table 7.3). However, comparisons of the behaviour of seed from each source planted at a range of altitudes will indicate whether there was any difference in emergence due to altitude of planting. The plateau region of the emergence curves of Figure 7.3 was used to investigate the differences due to seed source; the sampling was not intense enough to investigate the rising part of the curves, and the curves of the different seed sources show little variation after they reach the plateau. The variation in the number of emergent seedlings on November 16 will now be considered in detail.

The analysis summarized in Table 7.5 shows that the number of seedlings emerging from seed from different seed sources was significantly different (Source, p<0.001). Comparison of the mean number of viable seeds for each source (Table 7.6) with the estimated number of seeds planted (Table 7.3) shows that a high proportion of the seed emerged as seedlings (960 m, 71%; 1240 m, 91%; 1740 m, 93%; 1910 m, 83%).

The altitude of the seed source had a significant effect on the number of emergent seedlings. This could be expected from the differences in the numbers of viable seeds which were planted (Table 7.3). The results which are of particular importance are the significant interactions between planting time and seed source (Time*Source, p=0.031) and the altitude of planting and seed source (Altitude*Source, p=0.002): they show that the number of emergent seedlings differed within a seed source depending on the altitude and
TABLE 7.5

Summary of analysis of variance for the number of seedlings on November 16, 1983 which emerged from seed, from four altitudes, planted on February 9 and April 13 at four altitudes.

<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>Altitude. Plot Stratum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Altitude</td>
<td>2</td>
<td>176.3</td>
<td>88.1</td>
<td>0.18</td>
<td>0.843</td>
</tr>
<tr>
<td>Time</td>
<td>1</td>
<td>656.4</td>
<td>656.4</td>
<td>1.31</td>
<td>0.296</td>
</tr>
<tr>
<td>Altitude * Time</td>
<td>2</td>
<td>1408.8</td>
<td>704.4</td>
<td>1.41</td>
<td>0.316</td>
</tr>
<tr>
<td>Residual</td>
<td>6</td>
<td>3000.5</td>
<td>500.1</td>
<td>3.67</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>5241.9</td>
<td>476.5</td>
<td>3.49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Altitude. Plot. Row Stratum</td>
<td>36</td>
<td>5478.8</td>
<td>152.2</td>
<td>1.12</td>
<td>0.327</td>
</tr>
<tr>
<td>Altitude. Plot. Column Stratum</td>
<td>36</td>
<td>4410.8</td>
<td>122.5</td>
<td>0.90</td>
<td>0.634</td>
</tr>
<tr>
<td>Altitude. Plot. Row. Column Stratum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source</td>
<td>3</td>
<td>8397.1</td>
<td>2799.0</td>
<td>20.52</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Altitude * Source</td>
<td>6</td>
<td>3196.5</td>
<td>532.7</td>
<td>3.91</td>
<td>0.002</td>
</tr>
<tr>
<td>Time * Source</td>
<td>3</td>
<td>60.3</td>
<td>20.1</td>
<td>3.08</td>
<td>0.031</td>
</tr>
<tr>
<td>Altitude * Time * Source</td>
<td>6</td>
<td>764.5</td>
<td>127.4</td>
<td>0.934</td>
<td>0.524</td>
</tr>
<tr>
<td>Residual</td>
<td>90</td>
<td>12279.0</td>
<td>136.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>108</td>
<td>25897.4</td>
<td>239.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grand Total</td>
<td>191</td>
<td>41029.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Split plot with a Latin Square design in the sub-plot.

The number of emergent seedlings associated with the significant interaction between altitude of planting and seed source show that seed from 960 m produced less emergent seedlings as the altitude of planting was increased whereas seed from 1740 m and 1910 m both showed increases in the numbers of emergent seedlings with altitude. The seed collected...
near Sawpit Ck. (1240 m) had a similar number of emergent seedlings at all altitudes (Table 7.6).

Although the time of planting did not have a significant effect overall, the interaction between time of planting and seed source was significant (Time*Source, p=0.031 Table 7.5). When the emergence of seedlings at each of the altitudes of planting was considered separately, the interaction between planting time and seed source was found to be significant only for seed planted at Piper's Gap (p=0.002). The means for this site show that the tendency for the later planting to produce fewer seedlings from seed collected at high altitudes was not apparent in the emergence of seed from low altitudes (Table 7.7). The main source of this interaction is the large effect of planting time on emergence of seed from Dicky Cooper Ck. (1740 m) and Baker's Ck. (1910 m) compared to the small differences in seed from lower altitudes.
TABLE 7.6

The mean number of emergent seedlings at 960 m, 1610 m and 1740 m from seed collected at 1000 m, 1230 m, 1610 m, 1740 m.

<table>
<thead>
<tr>
<th>PLANTING LOCATION</th>
<th>ALTITUDE OF SEED SOURCE</th>
<th>MEAN FOR LOCATION¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>960 m</td>
<td>1240 m</td>
</tr>
<tr>
<td>Waste Point 960 m</td>
<td>24.6</td>
<td>24.5</td>
</tr>
<tr>
<td>Rennix Gap 1610 m</td>
<td>15.6</td>
<td>27.1</td>
</tr>
<tr>
<td>Piper's Gap 1740 m</td>
<td>12.5</td>
<td>23.7</td>
</tr>
</tbody>
</table>

Mean for source²: 17.6 25.1 29.7 35.7

For comparison between:
- Seed sources within an altitude of planting (rows) \( \text{LSD}_{0.05} = 10.6 \)
- Altitudes of planting within a seed source (columns) \( \text{LSD}_{0.05} = 8.2 \)
- Means for altitude of planting (¹) \( \text{LSD}_{0.05} = 7.9 \)
- Means for seed source (²) \( \text{LSD}_{0.05} = 4.7 \)

Sawpit Ck. (1215 m) data omitted due to flood damage.

TABLE 7.7

The mean number of emergent seedlings from seed collected at four altitudes and planted either on February 8 or April 13 1983 at Piper's Gap (1740 m).

<table>
<thead>
<tr>
<th>TIME OF PLANTING</th>
<th>ALTITUDE OF SEED SOURCE</th>
<th>MEAN FOR PLANTING</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>960 m</td>
<td>1240 m</td>
</tr>
<tr>
<td>February 8, 1983</td>
<td>10.3</td>
<td>26.0</td>
</tr>
<tr>
<td>April 13, 1983</td>
<td>14.8</td>
<td>21.4</td>
</tr>
</tbody>
</table>

For comparison between:
- Seed sources within a time of planting \( \text{LSD}_{0.05} = 9.2 \)
- Time of planting within a seed source \( \text{LSD}_{0.05} = 8.9 \)
Discussion:

The differences observed in seedling emergence may be attributed to an increase in the strength of dormancy of the seed with altitude of seed source (Section 4.2.1) since seed from different altitudes showed no differences in the temperature response of either stratification (Section 4.3) or germination (Section 6.2). Differences in the number of seedlings which emerge could be expected to reflect differences in the proportion of seed from each source which has a strength of dormancy which is appropriate for the environment of planting.

The reduction in the number of seedlings which emerged from seed from Waste Point planted at altitudes above 1215 m is consistent with the earlier germination and death of seedlings, as a result of the weaker dormancy of that seed compared to seed from higher altitudes. The reduction in the number of seedlings emerging from seed from Dicky Cooper Ck. and Baker's Ck. planted at 960 m could be explained by a reduction in the number of seeds germinating as a result of an incomplete breaking of dormancy at 960 m compared to seed planted at 1610 m and 1740 m. A high proportion (91%) of the seed from Sawpit Ck. emerged as seedlings at all altitudes of planting.

Differences in the strength of dormancy may explain differences observed in the response of seed collected at various altitudes to the time of planting at the highest altitude (1740 m) (Table 7.2). The reduction in the number of seedlings emerging from seed from Dicky Cooper Ck., and Baker's Ck. planted on April 13 compared to February 9 is unlikely to be due to the inability of the conditions at Piper's Gap to break the dormancy of the seed from higher altitudes; the experiment reported in the next section shows that a high proportion of seed from
Dormancy, germination and emergence in the field

Dicky Cooper Ck. planted on August 28 at 1610 m was able to break dormancy and emerge as seedlings. It is possible that the lower number of emerged seedlings in the later planting was due to premature germination of the seed during winter.

Conditions which were suitable for the breaking of dormancy must have occurred at Brennix Gap (1610 m) prior to April 13 because the strength of dormancy of the seed had begun to decline by that date (Figure 7.1). It could be expected that the onset of these conditions would have been earlier at Piper's Gap because of generally lower temperatures at the higher altitude. Seed planted on April 13 at Piper's Gap would therefore have been exposed to conditions which were suitable for the breaking of dormancy from the time it was planted. In contrast, the dormancy induced in the seed planted on February 8 would be far stronger on April 13 than the innate dormancy of the seed which was planted on that day. The germination of seed planted in late summer would thus be expected to occur at a later time than seed planted later in autumn.

There are two possible explanations for the absence of significant differences in the number of seedlings emerging from seed planted in February and April at lower altitudes: the warmer conditions at lower altitudes after April 13 may have permitted the induction of dormancy in the seed, thereby delaying germination; or the shorter duration of winter at lower altitudes may have reduced the critical nature of the delay in germination (caused by the induction of dormancy) for the survival of the seedling. The breaking of dormancy observed in seed planted at 1610 m after April 13 (Section 7.1) renders the first possibility unlikely. However, in the absence of further information on the changes in dormancy at lower altitudes during autumn, it was not
possible to determine the actual cause.

Interpretation of the results of the present experiment in terms of the adaptation of *E. pauciflora* to variation in environmental conditions with altitude requires an assessment of the characteristics of the season in which the study was carried out compared to the mean climatic conditions. The temperature conditions during the spring of 1983 were similar to the average (Figure 7.4) and the monthly precipitation was very high during September, October and November of that year. The consistently high soil moisture levels during the spring of 1983 provided an ideal opportunity to examine the effects of altitude, independent of soil moisture constraints. The dry conditions during October and November of 1982, which resulted in the mortality of almost all of the seedlings which emerged, shows the critical importance of soil moisture conditions in the spring following dispersal for the successful establishment of newly-germinated seedlings.
Figure 7.4 The climatic conditions during 1982 and 1983 at Sawpit Ck. (1215 m) in relation to the mean conditions over the period 1968-1983. Solid lines are 1 standard deviation above and below the mean.
7.3 The influence of time of planting on emergence of seedlings.

The previous experiment showed that the emergence of seed collected at a range of altitudes depended on the altitudinal difference in the seed source and the site of planting. Changes in the dormancy of seed planted in early autumn suggests that seed planted at later times might be expected to have a different dormancy compared with seed planted in early February (Section 7.1). Although replicate studies of the changes in dormancy of seed for different times of planting were not possible, some indication of the differences was obtained by examining the emergence of the seedlings from seed planted at different times. This study describes a study in which the emergence of seedlings from seed planted at a range of times in autumn and spring was monitored.

Methods:

A plot (0.8 m x 0.8 m) at the southern edge of a clearing in an *E. pauciflora* stand near Rennix Gap (1610 m) was prepared in a similar manner to the plots described in Section 7.2. Replicates of seed (0.5 g, 32 viable seeds), collected near Dicky Cooper Ck. (1740 m) were planted in five randomly selected rings each fortnight from February 15 to May 5 and then on May 28. All of the seed for the autumn plantings was weighed on the same occasion and was stored in dry conditions in a refrigerator until it was required for planting. A further planting on August 28 was undertaken in order to examine the breaking of dormancy in spring. The seed for the spring planting was from the same source but was collected a year later than the seed used for the autumn planting, and a larger amount of seed was planted in spring (0.7 g, 58 viable seeds). Seed was planted at a depth of between 7 mm and 10 mm.
The plot was inspected at approximately weekly intervals for emergent seedlings during autumn and spring. The number of seedlings with fully expanded cotyledons and the number of dead seedlings were recorded, and the dead seedlings were removed.

Results:

(i) Climatic conditions

The climatic conditions during the period of planting and seedling emergence are shown in Figure 7.5. This figure also summarizes the relevant findings of Section 7.1 and shows that there was a progressive breaking of dormancy during the late autumn but that the proportion of seeds which germinated during autumn was low. Dormancy was broken during the winter, and a large proportion of the seed germinated in September; the emergence of the seedlings was, however, delayed by more than a month. The emergence of seedlings will now be considered in more detail for seed planted at different times during autumn.

(ii) Emergence of seedlings in autumn and winter

Some of the seed planted in autumn emerged before winter; however, the seedlings which did emerge were associated with particular planting times (Table 7.8) and all of these seedlings had died by August 3. The lower number of emergent seedlings associated with the earlier plantings reflects the induction of dormancy noted for seed planted on February 8 (which was discussed in Section 7.1). The lower number of emergent seedlings from seed planted later than March 30 could have resulted from conditions which were not suitable for immediate
germination or from the shorter time available in which emergence may occur as winter approaches.

Figure 7.5 The dormancy and germination of seed and emergence of seedlings in relation to the climate during autumn and spring.
- a. % germinated seeds at retrieval
- b. % germinated seeds at 15°C after 4 weeks at 5°C
- c. number of emergent seedlings

(soil temperatures are based on the mean of 48, 30-minute samples of temperature per day)

The site was visited at monthly intervals during winter and no emergence of seedlings was observed, probably as a result of the low temperatures and action of needle ice which frequently stirred the surface of the soil to a depth of 2 mm to 5 mm.
Dormancy, germination and emergence in the field.

### TABLE 7.8

The total number of seedlings which had emerged by various dates during autumn.

<table>
<thead>
<tr>
<th>SEED PLANTING</th>
<th>DATE OF OBSERVATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13 APR</td>
</tr>
<tr>
<td>8 FEB</td>
<td>1 0</td>
</tr>
<tr>
<td>15 FEB</td>
<td>2 0</td>
</tr>
<tr>
<td>1 MAR</td>
<td>3 1</td>
</tr>
<tr>
<td>16 MAR</td>
<td>4 5</td>
</tr>
<tr>
<td>30 MAR</td>
<td>5 0</td>
</tr>
<tr>
<td>13 APR</td>
<td>6 0</td>
</tr>
</tbody>
</table>

1. Total number of seedlings in 5 replicates
2. From Figure 7.3
( ) indicate the number of additional seedlings which had emerged but did not have expanded cotyledons.

(iii) Emergence of seedlings in spring

The counts of emergent seedlings at successive times represent repeated measures which are not independent of each other and therefore the counts at each time were analysed separately. The spring planting of seed (August 28, [9]) was not included in the analysis because the number of seeds which were planted was different and therefore this treatment would be expected to show a significant difference in the numbers of seedlings which emerged. The soil in 4 rings was disturbed prior to emergence of the seedlings; two of these rings included seed planted on May 28 [8] and these plots were included as missing values in the analysis.

Analysis of variance showed that the numbers of seedlings emerging
in spring from seed planted at different times in autumn was not significant (Table 7.9). The lack of significance of the effect of planting time on the number of emerged seedlings for all of the days of observation except October 18 may be due to the large amount of variation among the replicates (shown by the large value of the Least Significant Differences LSD). The major differences in the types of response resulted from variation in the maximum number of emergent seedlings and the rate at which the seedlings emerged (Figure 7.6). The mean number of seedlings for each planting date and day of observation are shown in Table 7.10 to permit the comparison of each of the planting dates. They show that, generally, the number of emergent seedlings from seed planted on occasions 3 (March 1), 4 (March 16), 5 (March 30) and 8 (May 28) was lower than seed planted at the other times.

**TABLE 7.9**

The summary of analyses of variance of the number of emergent seedlings from seed planted at various times for each observation occasion in spring.

<table>
<thead>
<tr>
<th>Day of Observation</th>
<th>Source of Variation</th>
<th>Variance ratio</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>284</td>
<td>Planting Date</td>
<td>2.10</td>
<td>0.075</td>
</tr>
<tr>
<td>291</td>
<td>&quot;</td>
<td>2.62</td>
<td>0.031</td>
</tr>
<tr>
<td>298</td>
<td>&quot;</td>
<td>2.17</td>
<td>0.067</td>
</tr>
<tr>
<td>305</td>
<td>&quot;</td>
<td>2.27</td>
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</tr>
<tr>
<td>320</td>
<td>&quot;</td>
<td>2.25</td>
<td>0.058</td>
</tr>
</tbody>
</table>

1 Day from January 1, 1983
Dormancy, germination and emergence in the field:

TABLE 7.10

The mean number of seedlings from each planting date which had emerged on each day of observation.

<table>
<thead>
<tr>
<th>DATE OF OBSERVATION 8 FEB</th>
<th>DATE OF PLANTING</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 FEB</td>
<td>16 MAR</td>
<td>30 MAR</td>
<td>13 APR</td>
<td>27 APR</td>
<td>28 MAY</td>
</tr>
<tr>
<td>11 OCT</td>
<td>16.4</td>
<td>10.2</td>
<td>5.3</td>
<td>13.6</td>
<td>9.0</td>
<td>15.6</td>
</tr>
<tr>
<td>18 OCT</td>
<td>30.6</td>
<td>19.8</td>
<td>13.8</td>
<td>18.6</td>
<td>14.6</td>
<td>26.4</td>
</tr>
<tr>
<td>25 OCT</td>
<td>31.6</td>
<td>24.6</td>
<td>18.8</td>
<td>20.0</td>
<td>16.2</td>
<td>27.4</td>
</tr>
<tr>
<td>1 NOV</td>
<td>32.4</td>
<td>25.8</td>
<td>18.5</td>
<td>19.6</td>
<td>16.2</td>
<td>28.2</td>
</tr>
<tr>
<td>16 NOV</td>
<td>31.8</td>
<td>25.4</td>
<td>18.0</td>
<td>19.4</td>
<td>14.0</td>
<td>29.6</td>
</tr>
</tbody>
</table>

^1 value for significant difference between means within a day of observation at p=0.05.

Figure 7.6 The mean number of seedlings which emerged from 5 replicates of seed planted at a range of times.

1: February 8  
4: March 16  
5: March 30  
7: April 27  
9: August 28
Discussion:

Although the variation between replicates means that the effect is not statistically significant, the trend in seedling emergence shows that the time of planting can affect the number of seedlings which emerge. This trend is consistent with the response predicted from earlier studies in this thesis.

Seed planted on February 8, at the same time and at the same site as the seed used in the experiments investigating changes in dormancy during autumn (Section 7.1), produced a large number of seedlings in spring. Section 7.1 showed that the temperature and moisture conditions were conducive to the induction of dormancy, following planting on February 8, and that only a small proportion of the seeds germinated (ca. 2%) and few seedlings emerged during autumn (Table 7.8). Seed planted on February 15 produced a similar number of seedlings to that planted on February 8.

The dry conditions following planting on March 1 probably meant that the seed planted on that occasion would have responded similarly to the seed planted on March 16 because changes in dormancy occurring at low seed moisture content occur very slowly (Section 4.1.1) and the seed would not have become moist until March 16 when it rained. The lower number of seedlings emerging in spring from seed planted on March 1, March 16, March 30 were all associated with higher numbers of seedlings emerging in autumn as a result of favourable moisture and temperature conditions for germination following planting. However, the number of seedlings which emerged in autumn from the latter plantings cannot account for the differences in the numbers of emerged seedlings in spring. Therefore, it was assumed that more seedlings
germinated than emerged and that all of these seedlings died before spring.

Soil temperature and moisture conditions were apparently suitable for the breaking of dormancy from about March 30 onwards since the germination capacity of seed planted on February 9 began to weaken from that time (see Figure 7.1, curve d.). The seed planted on April 13 and April 27 was therefore exposed to conditions which consistently broke dormancy and produced higher numbers of seedlings in spring than the seed planted between March 1 and March 30.

The low number of seedlings originating from seed planted on May 28 is not consistent with the other observations in this chapter. The low numbers are unlikely to be due to the premature germination of the seed because seed planted on April 13 and April 27 would be expected to be more severely affected through exposure to even longer periods of conditions suitable for breaking dormancy. The low numbers are also unlikely to be due to incomplete breaking of the dormancy of the seed because a high proportion of the seed planted on August 28, at the beginning of spring, produced emergent seedlings. In addition, the studies in Section 7.1 showed that seed planted on February 9 and retrieved on May 28 had about the same strength of dormancy as fresh seed from the same source (the same seed used for the studies in this section), yet a high proportion of the former seed germinated. The low numbers are also unlikely to be due to a failure of seed planted on February 9 to emerge since seed planted on February 9 in the present experiment produced a large number of emergent seedlings. The reason for the low number of seedlings in seed planted on May 28 remains unresolved.
Planting seed in spring (August 28) resulted in a different germination behaviour compared to seed planted in autumn. Although the absolute numbers of seed cannot be compared, the pattern of emergence shows that the seed planted in spring broke dormancy and then emerged at a later time than the seed which had over-wintered in the soil.
7.4 Conclusions

1. The changes in dormancy in autumn are consistent with the results of the laboratory experiments since moist conditions and temperatures above 9°C resulted in the induction of dormancy in seed planted in late summer.

2. The induction of dormancy is important in limiting the germination of seed during autumn and in preventing germination during winter.

3. Seed germination in the field occurs when the soil temperatures are low at the end of winter (temperature at 10 mm did not exceed 9°C).

4. Seedling emergence occurred about 30 days after seed germination.

5. The proportion of seed which emerges as seedlings depends on the altitude of seed source and the altitude of planting. The reduction in the proportion of emergent seedlings was most marked when seed from high altitudes was planted at low altitudes and vice versa.

6. Drought may result in 100% mortality of seedlings at all altitudes.
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THE REGENERATION OF

Eucalyptus pauciflora Sieb. ex Spreng.

FROM SEED

by

Douglas Graham Abrecht

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

February 1985
CHAPTER 8

GENERAL DISCUSSION
CHAPTER 8

Studies in the previous chapters have examined the variation in the dormancy and germination behaviour of seeds and the emergence of seedlings of *Eucalyptus pauciflora* from a range of altitudes. The differences in the response of seed from different altitudes, which is primarily mediated by seed dormancy, provides a basis for understanding the regeneration of the species.

The mechanism of dormancy has not been considered in the studies reported in this thesis because the main concern was the influence of dormancy on the timing of germination and emergence. A precise understanding of the mechanism of dormancy would not achieve this objective. A study which investigates mechanism requires an understanding of the behaviour of the whole process to ensure that the mechanism which is eventually proposed is not an artefact of the laboratory but is relevant to the behaviour of the seed in the field. The studies in this thesis have contributed to the understanding of the response of the dormancy to environmental factors, in particular temperature.

The first section of this chapter evaluates the statistical techniques which were used to test differences in the germination capacity and times-to-germination. The second section of this chapter discusses the results from previous chapters in terms of the overall process of the regeneration of *Eucalyptus pauciflora*. The final two sections discuss the results in terms of the mechanism of dormancy and the regeneration of *E. pauciflora*. 
8.1 Evaluation of statistical techniques

The analysis and interpretation of the data from germination tests was discussed in Chapter 2; particular attention was paid to the use of Generalized Linear Models in the analysis of germination capacity, and to the use of Cox models in the analysis of germination curves. This section reviews the usefulness of these techniques together with their limitations.

(1) Germination Capacity

The use of Generalized Linear Models to analyse germination capacity has permitted not only the elucidation of the effects of experimental treatments but also the investigation of the mathematical form of the responses to temperature in a similar manner to regression analysis. The main advantage of using Generalized Linear Models (with a Logit link function and Binomial errors), instead of angular transformation and analysis of variance, is their statistical validity (Section 2.4.1).

(ii) Times-to-germination

Previous approaches to the analysis of the times-to-germination have relied on invalid assumptions, mainly related to the treatment of Binomial data as if it were Normal (by using techniques which rely on least squares criteria for fitting various regressions to germination curves) or, in the case of Hunter et al. (1983), by assuming that the times-to-germination of a seed sample have a Normal or log-Normal distribution (Section 2.2).
Constraints imposed by field programs meant that very few of the experiments in the present studies had records of germination which were collected on a strict daily basis. This detracted from the analyses using the Cox model in many cases, particularly when the germination treatments began on different days in various parts of the experiment. The use of the Cox model would be more powerful where records could be kept on a strict daily basis.

The Cox model has much wider application in the analysis of the effects of experimental treatments on data such as the mortality or changes in life stage of individuals in a population over time. Weiss (1982) used a similar model to examine the mortality of Chrysanthemoides monilifera seedlings under a range of treatments in the field.

Whilst the Cox model permits the analysis of the times-to-germination it does not generate a measure which can be used to compare the rate of germination between experiments. This deficiency stems from the absence of an assumption about the theoretical form of the distribution of the times-to-germination which is part of the strength of the analysis. The rate of germination could be compared in different experiments either by combining the experiments in the same Cox model or deriving some other measure of the rate of germination, such as the time to 50% germination, from the germination curves predicted by the Cox models fitted to the experiments separately.
8.2 Synthesis

The studies in this thesis have concentrated on the influence of environmental factors on the regeneration of *E. pauciflora* from seed. This section discusses the influence of the timing of germination and emergence in increasing the chances of survival of seedlings at a range of altitudes.

The size and dispersal of *Eucalyptus pauciflora* seed

Examination of the physical characteristics of seed collected from a range of altitudes (960 m to 1910 m) showed that the size of viable seed decreases as the altitude of seed source increases. The reduction in the size of the seed could be associated with a restricted period of seed filling due to slightly later flowering and the reduction in the length of the growing season as altitude increases. Seed size is also likely to be under some degree of genetic control; this was not investigated in the present study.

*Eucalyptus pauciflora* does not have a long-lived soil seed bank but holds a store of seed in capsules in the canopy. The dispersal of the seed from the canopy may occur at any time of the year but studies on other eucalypt species in southern Australia show that it is likely to be concentrated in summer and autumn (Christensen 1971, Cremer 1965b). The variability in the timing of dispersal means that the conditions to which seed may be exposed after dispersal may vary widely, and the response of the seed to these conditions determines the timing of germination.
The dormancy of *E. pauciflora* seed

A variable proportion of *Eucalyptus pauciflora* seed is dormant at dispersal. The degree of innate dormancy was found to be similar for populations from a range of altitudes from 960 m to 1730 m but was greater in seed from populations near treeline (1910 m, 2000 m). The strength of dormancy increases with altitude but there is no difference in the form of the temperature response for breaking dormancy for seed from different altitudes. The increase in the strength of dormancy with altitude leads to an increase in the duration of cold conditions necessary to break dormancy; this effectively delays the onset of germination of the seed from higher altitudes compared to that from lower altitudes when they are exposed to similar conditions.

There was a large amount of variation in the strength of dormancy of seeds collected from trees growing in close proximity at the same altitude. The extent of this variation in the strength of dormancy has been demonstrated in Section 4.2.2 which showed that, as the duration of stratification was increased, the dormancy of an increasing proportion of the seeds was broken. Variation in strength of seed dormancy provides a basis for variation in the timing of germination which may be important for spreading and reducing the risk of mortality of the total seed population due to infrequent events, such as severe frosts.

The effectiveness of variation in the strength of dormancy of a seed sample in promoting the survival of at least some of the seedlings is indicated by the emergence of some seedlings from all seed sources in reciprocal transplant experiments at altitudes ranging from 960 m to 1740 m (Section 7.2).
Changes in dormancy of the seed during dispersal

Temperature conditions determine whether dormancy is broken or enhanced, and the moisture conditions appear to influence the rate at which changes in dormancy occur in response to the temperature conditions. The dormancy of the seed reaching the ground is largely determined prior to the shedding of the seed, however the strength of dormancy of the seed which is dispersed may be increased by conditions during the shedding of the seed from the capsule. Exposure to temperatures of 5°C and 40°C during seed shedding leads to slight increases in the strength of dormancy. However, the small degree of influence exerted by temperatures within the normal range suggests that the season of dispersal is unlikely to have a large influence on the dormancy of the dispersed seed. Changes in the dormancy of the seed whilst it remains in the capsule also seems unlikely since breaking of dormancy would be expected to occur over winter when temperatures are low. Seed shed from capsules collected in both June and September was found to have a low germination capacity immediately after shedding.

Preliminary evidence indicates that the strength of dormancy of seed dispersed from the canopy following a fire may be greater than that dispersed under normal conditions. However, this assertion is based on an incomplete knowledge of the temperatures to which capsules are exposed during a fire. Further studies are required to check whether the dormancy of seed dispersing following a fire is greater than the dormancy of seed shed under normal conditions.

In summary, the dormancy of the seed reaching the ground is unlikely to be influenced by the conditions around the time of seed shedding, although there is a chance that some of the seed dispersed
after a fire may be more strongly dormant.

The induction of dormancy

The conditions to which the seed is exposed prior to germination will vary widely depending on the timing of seed dispersal and the weather. It is likely that a large proportion of the seed will be dispersed before late February and will therefore be exposed to temperatures and moisture conditions which are conducive to the induction of dormancy.

The present studies have shown that dormancy is induced in moist seed exposed to temperatures above 9°C and broken at temperatures below 6°C (Section 4.2.2.1). Exposure to temperatures in the range 6°C to 9°C may lead to the breaking of dormancy of some of the seed in a population; however, there is evidence from Grose's (1963) studies of \textit{E. delegatensis} that dormancy is induced after prolonged exposure to these temperatures. The precise response of the induction of dormancy to temperature has not been determined, but there is evidence that the rate at which dormancy is induced may vary with temperature (Section 4.2.2.1, Section 5.2).

The importance of the induction of dormancy as a mechanism for minimizing germination in autumn and winter depends on the conditions encountered by the seed after it reaches the ground. The high temperatures and moist conditions common in late summer prevented germination and led to the induction of dormancy in seed planted at that time; the dormancy of this seed was sufficiently strong at the beginning of winter to ensure that germination (Section 7.1) and emergence (Section 7.3) is delayed until spring.
As the time of planting becomes later in autumn the decrease in soil temperatures leads to an increase in the chances that a proportion of the seed will germinate before dormancy is induced. Conditions which are suitable for the germination of a portion of the seed sample also result in an increase in the strength of dormancy of the seed which does not germinate, to the extent that further germination prior to winter is prevented (Section 5.2). As the time of planting is delayed further, the probability of the seed being exposed to conditions which induce dormancy is reduced, and there comes a point where the seed will be exposed to conditions which are wholly suitable for the breaking of dormancy. In this case the capacity of the environment to break dormancy may exceed the strength of dormancy of the seed (especially when it has not been incremented by induction) and the number of seedlings which emerge may be reduced by premature germination of seed during winter (Section 7.3).

The potential for the induction of dormancy in spring would seem to be limited to those seeds which have not received sufficient exposure to cold conditions to ensure germination at temperatures below 9°C. As the dormancy of a seed sample is broken by stratification the proportion of seed which germinates is increased and the strength of dormancy of the seeds which do not germinate is reduced (Section 5.2). It is conceivable that in years when an early snow melt is followed by dry, warm conditions, the capacity of the environment to break dormancy could be reduced to the extent that a proportion of the seed will not germinate at temperatures above 9°C; dormancy will thus be induced in that seed.

In summary, dormancy is induced in moist seed at temperatures
above 9°C and it would seem that the induction of dormancy effectively prevents the germination of a large proportion of the seed dispersed in summer and autumn. Seed dispersed later in autumn, which is not exposed to conditions which induce dormancy, may not have the strength of dormancy necessary to delay germination to a more favourable time in spring.

The breaking of dormancy

Laboratory studies have shown that, at constant temperatures, the breaking of seed dormancy occurs between 0°C and about 9°C, and that the rate at which dormancy is broken varies depending on the temperature within this range (Section 4.2.2). The effect of diurnal fluctuations in temperature on the breaking of dormancy was not investigated in the present study. It could be expected that temperature fluctuations within the range 0°C to 9°C might lead to a consistent breaking of dormancy; however, when the higher end of the temperature range exceeds 9°C, the nett effect on the dormancy of the seed could be expected to depend on any synergistic or antagonistic interactions between the induction and breaking of dormancy.

Soil temperatures decline to levels which are suitable for the breaking of dormancy during autumn but the effectiveness of the autumn and winter conditions for breaking dormancy in the field may vary depending on the temperature regime to which the seed is exposed. The variation in temperature with altitude will be particularly important in this respect. At low altitudes, which do not receive permanent snow cover, the breaking of dormancy will occur in a temperature regime which is fluctuating diurnally. At high altitudes, the period of snow cover will encompass a period of low, but relatively constant soil
temperatures at which the breaking of dormancy will proceed very slowly. The decline in temperature with altitude will mean that, in the first instance, the time when the temperature conditions change from nett induction of dormancy to nett breaking of dormancy will become earlier with increasing altitude and, in the second instance, that there will be a longer period when the rate of breaking dormancy is low at higher altitudes. The duration of snow cover increases with altitude (Slatyer et al. 1984) and the soil temperature conditions under snow (-0.5 to 0.5°C) will permit only very slow breaking of dormancy.

In summary, the induction of dormancy minimizes the germination of seed in autumn and preserves the seed for germination in spring. Survival of the seedling is dependent on the strength of dormancy being matched to the capacity of the environment to break dormancy at a time which is suitable for the germination and establishment of seedlings.

The germination of seed

Moist seed may respond to the temperature environment in three ways: germination, changing the strength of dormancy, or death. The response of the seed to temperature (in particular the proportion of seed germinating) changes as the dormancy of the seed sample is broken. As the duration of the stratification treatment is increased, the range of temperatures which are suitable for germination also increases and a larger proportion of the seed germinates (Section 6.1).

The advantages of seed dormancy as a mechanism for limiting germination to the more favourable spring season have already been discussed. The more precise control of the timing of germination
during spring may be equally critical for the subsequent survival of the seedlings. As the winter progresses and dormancy is broken, the range of temperatures which are suitable for germination increases and the seed is more likely to germinate at low temperatures.

Field germination of seed occurred over a period of 30 days, when soil temperatures were below 9°C. It could be expected that if soil temperatures had been depressed later into the spring, for example by persistent snow cover, then the seed would be likely to germinate at a later time and even lower temperatures. On the other hand, if soil temperatures had risen earlier, then the seed might have germinated earlier.

The strength of dormancy appears to be the critical attribute leading to variation in the germination response of seed from different altitudes to temperature and is sufficient to explain the differences observed in the emergence of seedlings in the field.

Emergence of seedlings

The field studies of Section 7.2 showed that seed from all sources was capable of producing some seedlings at all altitudes despite the wide differences in environmental conditions; this suggests that the variation within a population of seed was sufficient to include seeds which could produce seedlings in widely different conditions. Seed from higher altitudes was found to produce a lower proportion of seedlings when planted at low altitudes than seed collected and planted at low altitudes and vice versa (Section 7.2). When planted at high altitudes the weaker dormancy of seed from low altitudes means that this seed is likely to germinate earlier, thus exposing the seedling to
more severe conditions either under the snow or soon after the snow melts. When planted at low altitudes, the stronger dormancy of seed from higher altitudes may delay germination of a proportion of the seed population beyond the time of favourable conditions in spring.

In seed from a particular source, the decrease in the strength of dormancy with increasing seed size (Section 3.2) may permit a test of the importance of the strength of dormancy in relation to the capacity of the environment to break dormancy. Grose (1963) has shown that the area of the cotyledons in *E. delegatensis* seed is related to the seed size. The results of the studies of Section 3.2 would suggest that it was likely to be the smaller seed from low altitudes which emerged at high altitudes and the larger, less dormant seed from high altitudes which emerged at low altitudes. A comparison of areas of cotyledons of seedlings emerging at different altitudes of planting may provide a simple field test of the basis for the differences in the proportion of the seed emerging.

The studies in Sections 7.1 and 7.2 show that there was a delay of 30 days between the germination of seed and emergence of the seedlings. It is presumed that the growth of the seedling between germination and emergence is also influenced by temperature, with low temperatures effectively delaying the emergence of the seedling and high temperatures hastening emergence. An advantage of the delay in emergence caused by low temperatures may lie in the avoidance of damage to the expanded cotyledons by needle ice or severe frosts. Similarly, the more rapid emergence at higher temperatures would be an advantage because the soil moisture will decline more rapidly at those temperatures.
Footnote to Section 8.2.

The influence of environmental factors before, during and after the dispersal of seed from the capsules has been summarized. The fate of seed depends on these processes together with the timing of the shedding of seed from the capsule. The following decision tree is included to clarify the discussion.

Seed shed from the capsule in the canopy?

YES  NO
Viable free seed *1  Seed shed from capsule after dispersal? *2

YES  No
Viable free seed *3  Dead seed *4

*1 Seed is shed following desiccation of the capsule. Seed could be shed in this manner at any time of year and its fate will depend on the sequence of conditions that it encounters following dispersal.

*2 The liberation of seed from the capsule after dispersal depends on the desiccation of the capsule to release the seed and the action of gravity or some other agent to cause shedding of the seed (Cremer 1965a). Weather conditions and the microenvironment of the capsule will be the primary determinants of the rate of desiccation of the capsule. For example, the chances of desiccation are increased if the capsules are attached to branches or twigs which can hold the capsule away from the soil surface. The shedding of the seed will also be favoured if the capsule is suspended so the seed can fall out.
The dormancy of seed released from capsules after dispersal could be expected to be strengthened but not reduced as a result of the conditions after dispersal. Grose (1960) noted that the dormancy of seed extracted from *E. delegatensis* capsules which had been held under cold, moist conditions was similar to seed from untreated capsules. The desiccation of the capsule prior to seed release may also result in the induction of dormancy in the seed (Section 5.3).

As the capsule decomposes the chance of the seed being released becomes more remote due to hardening of the valves (Grose 1960). If the seed is not released from the capsule it will eventually become non-viable.

The discussion above has proposed two pathways by which viable seed may be dispersed. Differences in the timing of germination of seed dispersed in these two ways (*1, *3) are most likely to be observed when dispersal occurs during winter since seed dispersed in the capsule will not begin to break dormancy until it is released from the capsule and desiccation will be delayed by wet, cold conditions. Seed or capsules may fall onto snow and will melt their way down through the snowpack to arrive at the soil surface. At this stage free seed (1) will have imbibed and begun to break dormancy whereas seed in the capsule (3) must be released from the capsule before dormancy can be broken. Germination of seed (1) in the snowpack is unlikely because the rate of breaking dormancy and the rate of germination are very slow at the prevailing temperatures (-0.5-0.5°C). Desiccation and release of seed from the capsule (3) will occur after the snow has melted and the breaking of dormancy and germination of this seed will depend on the sequence of conditions after seed release. If the thaw occurred in early spring the chances of the seed (3) becoming seedlings is greater.
than if the snow persisted until early summer. At this stage the soil temperatures will be too high to break dormancy and may be high enough to induce dormancy - the seed may not germinate until the following spring and is unlikely to produce seedlings (Section 7.2).
8.3 Mechanism of dormancy

This section reviews the evidence for the mechanism of dormancy of *E. pauciflora* seed. The basic cause of dormancy is the inability of the embryonic axis to overcome the constraints against growth which are acting upon it (Bewley & Black 1982). The restraint of the embryo may be achieved by constraints on the potential for growth of the embryo or by the strength of the covering structures of the seed or both. Three aspects of seed dormancy are commonly studied which seek either to find experimental treatments which can break dormancy, or to associate dormancy with particular structures (seed coat, cotyledons), or to elucidate the biochemical events, and thereby the mechanism, which governs the breaking of dormancy.

Bewley & Black (1982) reviewed many studies which have searched for the mechanisms controlling seed dormancy in a range of species and have concluded that

> it is apparent that many of the changes [in metabolism] which have been described are non-specific either to conditions of cold temperatures or to dormant seeds. The true nature of the effects of cold stratification on dormancy breaking remains to be elucidated.

There are no reports in the literature of studies on the mechanism of the dormancy of *E. pauciflora* or of any other eucalypt species; however, several studies have examined the conditions necessary for, and the structures involved in, the breaking of dormancy in *E. pauciflora* and *E. delegatensis*.

The embryo of *E. pauciflora* is able to grow when the seed coat has been removed (Boden 1957, Bachelard 1967). The group of eucalypte-
which includes *E. pauciflora* (Subgenus *Monocalyptus*, Section *Renantharia*, Series *Obliquae*) has a seed coat which has an inner integument with a distinctive structure which was described by Gauba & Pryor (1958)

[the inner integument] occurs immediately below the outer integument. In ripe seeds the median cuticle delimiting the two integuments is resorbed. In the genuine Renantherae the inner integument is two-layered, being formed of both epidermal layers alone. The cells are tabular and without intercellular spaces.... [the inner integument] is suberized throughout, the walls not nearly being impregnated with fatty substances..... the walls are in addition more or less impregnated with tannin-like material.

Pryor (1954), Boden (1957) and Bachelard (1967) noted that most of the eucalypts of the colder areas (*E. delegatensis*, *E. pauciflora*, *E. stellulata*, to name a few, see Table 8.1), which had dormant seeds, belonged to this group although not all of them do (for example *E. perriniana*, *E. glaucesens*). Not all of the members of the group exhibit seed dormancy (for example *E. sieberi*). The dormancy of the seeds must therefore be associated with more subtle differences than the gross anatomy of the seed coat.

Grose (1963) showed that the presence of the inner integument was sufficient to prevent germination since seed of *E. delegatensis* did not germinate when the inner integument was intact but germinated readily when both the inner and outer integuments were removed. This observation shows that the presence of the inner integument is sufficient to prevent germination, but does not show that the cause of dormancy resides entirely in the inner integument. The mechanism behind the action of the inner integument in inhibiting germination has not been investigated. Removal of the seed coat may not remove all of the constraints on growth present in the seed of this species since
Grose (1963) also found that the rate of growth of excised *E. delegatensis* embryos was lower than those which had germinated without interference.

Grose (1963) postulated that the dormancy of *E. delegatensis* was mediated by a restriction of gas exchange by the seed coat which leads to a limitation of oxygen supply to the embryo. He based this assertion on the stimulation of germination in dormant seed by atmospheres which were high in oxygen. Bachelard (1967) disagreed with this view and maintained that the dormancy of both *E. delegatensis* and *E. pauciflora* seed was the result of mechanical restraint imposed on the embryo by the seed coat. The evidence tendered by Bachelard (1967) was that germination never occurred when the seed coat could resist the elongation of the embryo (cutting the seed coat on the sides compared to cutting the coat at either the micropylar or cotyledonary end) although the embryo was partly exposed to the atmosphere. Bachelard's observations do not exclude the limitation of gas exchange as a limiting process in the breaking of dormancy of intact seeds because they do not provide evidence of changes in either the strength of the seed coat or the strength of the embryo in the breaking of dormancy by stratification. If the dormancy of *E. pauciflora* was mediated by the restraint of the seed coat then concurrent studies of the changes in the strength of the seed coat and the strength of the embryo as dormancy is broken, such as those of Esashi & Leopold (1972) with Xanthium seeds, may lead to further insights into the mechanism of dormancy. Such an experimental approach would be of little use if the breaking of dormancy is mediated through changes in the permeability of the seed coat to oxygen as Grose (1963) suggests.

The studies reported in this thesis have set out to describe the
responses of the seed to its environment and are important in describing the responses which a mechanism of dormancy must explain. The main aspects of this response are listed below:

1. The strength of innate dormancy of seed increases with increasing altitude of collection.

2. Larger seeds have a weaker dormancy.

3. Dormancy is induced at temperatures above 9°C in both stratified and unstratified seed with measurable responses occurring in a period of 5 days or more at 15°C.

4. Dormancy is broken at temperatures below 9°C (optimum temperature of 5.5°C) with measurable changes in a period of 10 days or more.

5. The rate at which dormancy is broken begins to decline at a water potential of -4 bars and is negligible at a water potential of -10 bars.

6. The dormancy of individual seeds is not associated with their water content.

7. The range of temperatures which are suitable for germination increases as the dormancy of the seed is broken.

8. As the dormancy of a seed sample is broken the rate of germination increases.

9. Soaking seed in solutions of gibberellic acid will break dormancy
10. The dormancy of seed is associated with the seed coat (Boden 1957, Bachelard 1967) and possibly only the inner integument (Grose 1963).

11. Treatment of seed in atmospheres high in oxygen breaks dormancy (Grose 1963).

Many of the responses listed above could be attributed to either increases in the force exerted by the embryo or to a weakening of the seed coat or both and it would be pointless to speculate on the detailed mechanism of dormancy in the absence of information on changes in these characters.
8.4 Regeneration of Eucalyptus pauciflora

Regeneration niche was defined by Grubb (1977) as an expression of the requirements for a high chance of success in the replacement of one individual by a mature individual of the next generation. The chances of success during the regeneration of Eucalyptus have been associated, in the first instance, with the occurrence of disturbances such as fire and flood, and then with seasonal changes in the suitability of the climate for regeneration (Florence 1981).

The chances of successful regeneration of eucalypts from many environments are closely linked to seasonal conditions (Cremer et al. 1978), which places an emphasis on mechanisms which cue the timing of germination to favourable seasonal conditions. The timing of germination is critical for the survival of the seedling because it is the stage when the seedling is least tolerant of environmental extremes of temperature and moisture availability yet is in an environment near the surface of the soil, which is potentially most extreme and variable.

The studies in this thesis have shown that the regeneration niche of E. pauciflora includes a requirement for a period of cold moist conditions which brings about a delay in the timing of germination. This delay is likely to be associated with increased survival of individuals since emergence in spring reduces the chances of exposure of a newly emergent seedling to the lethal environmental conditions of summer and winter whilst giving the seedling the longest possible growing season before it is exposed to the rigours of the subsequent winter. Reciprocal transplants of seed between altitudes have shown that the critical element of the seed dormancy of E. pauciflora is the
balance between the strength of the dormancy and the capacity of the environment to break dormancy, since this determines the effectiveness of dormancy in timing germination.

_Eucalyptus pauciflora_ is one of sixteen species of _Eucalyptus_ in which innate seed dormancy has been recorded (Table 8.1). Of the species which possess innate seed dormancy twelve occur in cold, higher altitude environments of southern Australia which receive snow. Most of these species are classified in _Monocalyptus_, Section _Renantheria_, Series _Obliquae_ (MAK). The requirement for a period of cold, moist conditions as part of the regeneration niche of these species is presumed to minimize germination in autumn and promote it in spring as it does in _E. pauciflora._

The timing of germination has been shown to be critical for maximizing the survival of individuals up to the stage of seedling emergence in _E. pauciflora_, however regeneration may still be prevented at a later stage in plant development.

The regeneration of _E. pauciflora_ in the absence disturbances such as fire is rare (Slatyer pers. comm.). The limitations to regeneration in the absence of fire in _Eucalyptus regnans_ and _E. incrassata_ were discussed in Chapter 1 in which it was shown that the increased chances of successful regeneration are associated with transient changes in resources following fires in these species. Fire has been associated with increased chances of successful regeneration through increases in light and the 'ashbed effect' Pryor (1963). Fire also results in a plethora of changes in the soil which include increases in nutrient availability, changes in the composition of the soil microflora (Renbuss _et al._ 1972), and the removal of allelopathic substances.
General discussion:

TABLE 8.1
Species of *Eucalyptus* which have innate seed dormancy

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>REQUIREMENT</th>
<th>CLASSIFICATION</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subgenus Monocalyptus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>amygdalina</td>
<td>stratify 4 weeks</td>
<td>MATEH</td>
</tr>
<tr>
<td>delegatensis</td>
<td>stratify 6,8,10 weeks</td>
<td>MAKBE</td>
</tr>
<tr>
<td>kybeanensis</td>
<td>stratify 6 weeks</td>
<td>MAKKA</td>
</tr>
<tr>
<td>mitchelliana</td>
<td>stratify 6 weeks</td>
<td>MAKLA</td>
</tr>
<tr>
<td>pauciflora</td>
<td>stratify 4 weeks</td>
<td>MAKHA</td>
</tr>
<tr>
<td>regnans</td>
<td>stratify 3 weeks</td>
<td>MAKCA</td>
</tr>
<tr>
<td>stellulata</td>
<td>stratify 3 weeks</td>
<td>MAKMA</td>
</tr>
<tr>
<td><strong>Subgenus Symphyomyrtus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>behriana</td>
<td>light</td>
<td>SUDGA</td>
</tr>
<tr>
<td>camaldulensis</td>
<td>light</td>
<td>SNEEPA</td>
</tr>
<tr>
<td>flocktoniae</td>
<td>stratify 4 weeks</td>
<td>SIT:T</td>
</tr>
<tr>
<td>glaucescens</td>
<td>stratify 6 weeks</td>
<td>SPINH</td>
</tr>
<tr>
<td>microcarpa</td>
<td>light</td>
<td>SUL:DB</td>
</tr>
<tr>
<td>microtheca</td>
<td>light</td>
<td>SUDPF</td>
</tr>
<tr>
<td>nitens</td>
<td>stratify 3 weeks</td>
<td>SPITG</td>
</tr>
<tr>
<td>perriniana</td>
<td>stratify 3 weeks</td>
<td>SPINN</td>
</tr>
<tr>
<td>spathulata</td>
<td>light</td>
<td>SICDA</td>
</tr>
</tbody>
</table>

1 from Appendix 2, Boland et al. (1980)
2 Pryor & Johnson (1971)

The requirement for disturbance in the regeneration niche does not detract from the critical importance of the many other processes which ensure that the regenerating plant has a high chance of survival. The timing of germination, mediated by seed dormancy, has been shown to be one such process in *Eucalyptus pauciflora.*
8.5 Relationship to other studies

The results of studies reported in this thesis and the studies of Grose (1960) on *E. delegatensis* are very similar. Both show that control of the timing of germination, through the processes of induction and breaking of dormancy and germination and the processes appear to have a similar response to temperature in the two species.

The apparatus (gradient plate) and statistical techniques used in the studies of the dormancy and germination of *E. pauciflora* seed permitted precise definition and analysis of the temperature responses of these processes in *E. pauciflora*. Temperature influences both the direction and rate of change in dormancy and these studies showed that the changes in dormancy are very sensitive to the temperature conditions. A change in temperature from 6°C, which favours the breaking of dormancy, to 9°C would result in the induction of dormancy. There was also an indication that the rate of induction of dormancy depends on temperature but this was not fully investigated.

The responses noted in constant temperature studies were consistent with changes in dormancy observed in seed exposed to field conditions, however there is the need for further work on the action of variation in temperature over a range which spans the 6°C to 9°C region.

Comparison of field studies showed that seed dormancy changed in response to changes in weather conditions in both *E. delegatensis* (Grose 1960) and *E. pauciflora*; these changes acted to prevent seed from germinating in autumn and winter and cue germination to spring.
Dormancy mechanisms which cue germination to a favourable time of year have been described by many workers (Karssen 1980, Levins 1969). The investigations reported in this thesis show that dormancy has survival value in *E. pauciflora*. The variation in the strength of dormancy of seed from different altitudes may be considered adaptive and the response of dormancy to environmental conditions provides the means by which the germination of the seed is synchronized with the environment.
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THE REGENERATION OF

Eucalyptus pauciflora Sieb. ex Spreng.

FROM SEED

by

Douglas Graham Abrecht

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

February 1985
BIBLIOGRAPHY


Pryor L.D. (1963) Ash-bed growth response as a key to plantation establishment on poor sites. Aust. For. 27, 48-51


Slatyer R.O., Cochrane, P.M. & Galloway, R.W. (1985) Duration and extent of snow cover in the Snowy mountains and a comparison with Switzerland. Search 15, 11-12


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APPENDIX 1

LOCATION AND ALTITUDE OF SEED SOURCES

<table>
<thead>
<tr>
<th>SITE NAME</th>
<th>MAP</th>
<th>ALTITUDE (m)</th>
<th>LATITUDE °</th>
<th>LONGITUDE °</th>
<th>GRID REF.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waste Point</td>
<td>WP</td>
<td>960</td>
<td>36 21</td>
<td>148 36</td>
<td>FV430762</td>
</tr>
<tr>
<td>Sawpit Creek</td>
<td>SC</td>
<td>1240</td>
<td>36 21</td>
<td>148 34</td>
<td>FV404763</td>
</tr>
<tr>
<td>KR13</td>
<td>K13</td>
<td>1310</td>
<td>36 21</td>
<td>148 33</td>
<td>FV391759</td>
</tr>
<tr>
<td>KR15</td>
<td>K15</td>
<td>1510</td>
<td>36 21</td>
<td>148 29</td>
<td>FQ335758</td>
</tr>
<tr>
<td>Rennix Gap</td>
<td>RX</td>
<td>1580</td>
<td>36 22</td>
<td>148 30</td>
<td>FV355746</td>
</tr>
<tr>
<td>Smiggins Holes</td>
<td>SH</td>
<td>1730</td>
<td>36 24</td>
<td>148 26</td>
<td>FQ278723</td>
</tr>
<tr>
<td>Dicky Cooper Ck.</td>
<td>DC</td>
<td>1740</td>
<td>36 16</td>
<td>148 22</td>
<td>FQ234862</td>
</tr>
<tr>
<td>The Perisher</td>
<td>P</td>
<td>2000</td>
<td>36 25</td>
<td>148 23</td>
<td>FQ242697</td>
</tr>
<tr>
<td>Thredbo treeline</td>
<td>TT</td>
<td>1900</td>
<td>36 30</td>
<td>148 17</td>
<td>FV147560</td>
</tr>
<tr>
<td>Baker's Creek</td>
<td>BC</td>
<td>1890</td>
<td>36 18</td>
<td>148 22</td>
<td>FQ231822</td>
</tr>
</tbody>
</table>

1 Figure 1.1

2 Universal Grid Reference, Grid Zone 55H