Frost hardiness of *Eucalyptus delegatensis* R. T. Baker

by

Madge

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Abstract

A major factor limiting the growth of *E. delegatensis* is low temperature. This is important both in the natural distribution of the species and as a plantation species both within Australia and elsewhere. This project deals with aspects of the frost hardiness of *E. delegatensis* including the seasonal variation, genetic variation and comparison with other species of eucalypts.

The diffusate electrical conductivity method for measuring frost hardiness of plant tissue was adapted and developed for use with an air-filled frost chamber. The major adaptation was the addition of an ice nucleation agent, silver iodide, to tissue samples during test freezing to prevent supercooling. A thorough evaluation showed that this method is sensitive enough to detect differences of 0.3°C and would be useful for screening large numbers of plants for breeding.

Seven provenances of *E. delegatensis* planted in two provenance trials (planted in 1979) at Tarraleah and Myrtle Bank, Tasmania, were tested for frost hardiness. Plants at Tarraleah were tested at approximately six week intervals throughout 1984, while at Myrtle Bank they were tested three times during the hardening phase. Seasonal differences in frost hardiness ranged from 2.4°C for the Bicheno provenance (the least hardy) to 4.6°C for the Ben Lomond provenance (the most hardy) at Tarraleah. The maximum hardiness reached ranged from -6.0°C for the Bicheno provenance to -8.6°C for the Ben Lomond provenance at Tarraleah while at Myrtle Bank the range was from -4.7°C for the Bicheno provenance to -7.7°C for the Ben Lomond provenance. The same ranking of provenances at maximum frost hardiness was obtained at both trials.

Laboratory simulation of hardening conditions with night temperatures of 12, 4 and 0°C showed that colder night temperatures resulted in greater development of frost hardiness for all provenances tested. The ranking of provenances for frost hardiness corresponded to the field trial.

A field trial in the Esperance Valley, Tasmania, (planted in 1983) had two provenances each of *E. delegatensis*, *E. nitens*, *E. regnans* and *E. globulus* planted at altitudes of 60, 240, 440 and 650 m. One provenance of *E. grandis* was planted at the 60 and 240 m sites and one provenance of *E. pauciflora* at the 440 and 650 m sites. All species were tested for

frost hardiness in March and August of 1985. There was no significant difference between species or provenances in March. In August the only species with a significant difference between provenances was *E. delegatensis*. Significant differences between species were measured in August, when the species ranked in decreasing order of frost hardiness as follows: *E. delegatensis* = *E. nitens* > *E. pauciflora* > *E. globulus* > *E. grandis* > *E. regnans*. It was found that the lowest minimum temperatures occurred at the 60 m site followed by the 650 m site then the 440 m site with the 240 m site having the highest minimum temperatures. The frost hardiness of the plants tested also followed this pattern with the greatest development of frost hardiness at the 60 m site. Growth of the plants corresponded to the altitudinal sequence with most height and diameter growth at the 60 and 240 m sites which experienced the warmest maximum temperatures.

The development of a reliable method of testing plant tissue for frost hardiness is important for plant breeding, since both seedlings and established plants can be tested. Testing of established *E. delegatensis* showed that there is significant variation in frost hardiness within the species and that there is no significant interaction between frost hardiness and site within Tasmania. Frost hardiness development in *E. delegatensis* is a response to low night temperatures and is independent of day temperature and growth rate. *E. delegatensis* compared favourably with other commercially planted species of eucalypts in terms of frost hardiness. It was shown that it is possible to achieve good growth of eucalypts on a frost-prone site provided suitable provenances are planted.

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CHAPTER 1

General Introduction

The distribution of frost hardiness in the genus *Eucalyptus* and its variation within a species is assuming increasing importance particularly in its native environment. This has come about with a requirement for more efficient regeneration of cut-over native forests and the establishment of fast-growing eucalypt plantations. The establishment of plantations in marginal areas for the species overseas has also engendered much interest in frost tolerance of the species.

In Tasmania a severe problem of poor growth has arisen in the high altitude forest dominated by *E. delegatensis* which have been clearfelled. Keenan and Candy (1983) and Webb *et al* (1983) report observations referred to as "growth check" of *E. delegatensis* and describe the regeneration as trees having poor growth, rounded bushy crown shape, little apical dominance and heavy branching from the stem. These trees also have smaller leaf size, thicker leaf cuticles and greater leaf sclerophylly than faster growing trees. Webb *et al* (1983) suggested that frost is a major factor in causing growth check.

The successful establishment of eucalypt plantations in Australia and overseas has demanded stricter selection criteria for choice of species and provenances. Tibbits (1986) reported that in Tasmania the area of eucalypt plantations has increased from 500 ha in 1970 to 11420 ha in 1984. The total area of plantations in Australia was 50105 ha in 1983. It is interesting to note the change in species emphasis since the earlier plantations were established. In this respect, provenance trials of a number of species (Tibbits, 1986) have been and remain essential for the selection of suitable material for commercial plantations.

Eucalypts are planted overseas because of their good growth rates compared with other hardwoods (Potts and Potts, 1986) but there have been setbacks. In France, for example, there are approximately 400 ha of eucalypt plantations which were severely damaged by frosts in the winter of 1984-85 (Potts and Potts, 1986). Such high levels of damage are of course unacceptable and as a result the breeding of more frost resistant eucalypts by selection of frost hardy individuals (Marien, 1983) and by hybridization is being pursued (Cauvin, 1983). Plantations of *E. grandis* in the U.S.A. have inadvertently combined good growth with a high susceptibility to severe frosts (Franklin and Meskimen, 1983) and the immediate question arises about requirements for hardening in the genus.

Frost resistance of trees has been studied extensively in the Northern Hemisphere. In general it has been found that species originating from higher latitudes are more frost resistant than those from lower latitudes but within this wide spectrum of response there is considerable seasonal variation. As a satisfactory basis for study it has been hypothesised by Weiser (1970) that there are three distinct stages in development of frost hardiness.

1. Stage 1. Stimulation by shortening daylength or critical photoperiod.
2. Stage 2. A period of low temperatures usually defined in terms of a critical night temperature which induces further hardening.
3. Stage 3. A period of very low temperatures (-30 to -50°C) which induce even greater hardening which is quickly lost if warmer temperatures are experienced by the plant.

The relative importance of the above has been considered for conifers and all three stages participate in the hardening process (Aronsson, 1975; Greer and Warrington, 1982). For example, short daylength is more important for hardening than low temperature for *Pinus silvestris* and *Picea abies*, in that plants would harden under short day/warm temperature conditions but would not harden under long day/ low temperature conditions. Aronsson (1975) also found that dehardening was a much faster process than hardening and that this was more dependent on temperature than on daylength. In *Pinus radiata*, a critical photoperiod of less than 11 hours was required for low-temperature hardening and only night temperatures less than 5°C were effective. Repeated exposure to nondamaging frosting treatments produced a maximum frost hardiness as low as -19°C (Greer and Warrington, 1982).

The three stages of hardening are by no means as well defined in the eucalypts. Studies of frost hardening have shown that night temperatures of 4°C or lower are necessary to stimulate hardening (Eldridge, 1968; Harwood, 1980; Paton, 1980) but very little work has been done to examine the role of photoperiod and that has been inconclusive. In this respect Eldridge (1968) reported that *E. regnans* from high altitudes suffered less frost damage when grown under short photoperiods than when grown under long photoperiods. However no response was found for *E. regnans* originating from low altitudes but similar latitudes.

Several authors have reported altitudinal clines in frost hardiness of eucalypts viz. Grose, (1960) for *E. delegatensis*, Boden (1958) for *E. fastigata*, Ashton (1958) and Eldridge (1969) for *E. regnans*. Thus it would be anticipated that there may be considerable variation between provenances. In a comprehensive study Rook *et al* (1980) in New Zealand measured frost hardiness of thirty-eight provenances of *E. regnans* at three different times of the year. They found that the range in frost hardiness between provenances was approximately 2.0°C in autumn, 2.5°C in winter and 1.5°C in spring and that the most frost tolerant provenances would survive a temperature 2.5°C lower in winter than in spring.

Since *E. delegatensis* occurs naturally at altitudes overlapping those of *E. regnans* and extends to higher altitudes (Boland *et al*, 1984), it would be expected to show at least as much variability in frost hardiness within the species as *E. regnans* and be more frost hardy on average. Measurement of seasonal and genetic variation in *E. delegatensis* is necessary to determine whether it is possible to select suitable material for regenerating frost prone sites and so avoid growth check. In addition, selection criteria for plantations should combine good growth rate as well as a suitable level of frost resistance for the site.

In this thesis the genetic and physiological aspects of frost hardiness of *E. delegatensis* are examined. Firstly a non destructive method of testing frost hardiness was developed so that established provenance and species trials could be sampled. Two provenance trials of *E. delegatensis* in Tasmania were sampled to establish the range of seasonal and genetic variation in frost hardiness within the species. Controlled environment chambers were used to test the effect of temperature on hardening and dehardening of *E. delegatensis* seedlings. Finally, the frost hardiness of *E. delegatensis* was compared with that of other *Eucalyptus* species at four sites at different altitudes and the effect of site maximum and minimum temperatures on growth rate and frost hardiness were evaluated.

CHAPTER 2

Determination of frost hardiness using diffusate electrical conductivity

Introduction

Frost hardiness may be measured either on whole plants or on samples of plant tissue. Ashton (1958) frosted whole seedlings of *E. regnans* artificially in a refrigerator. A scoring system was used to assess frost hardiness in which the proportion of each leaf damaged was estimated for all the leaves present and the number of buds killed were counted. A damage score was calculated for the whole plant. Similarly Rook *et al* (1980) ranked 38 provenances of *E. regnans* for frost hardiness by subjecting them to both natural and artificial frosts and assessing the leaves visually for damage. This type of method has several disadvantages. Firstly, it requires a large number of plants which can only be tested once (Rook *et al* used over 4000 seedlings). Secondly, if the plants are to be artificially frosted in a chamber, the size of plant that can be tested is restricted by the dimensions of the chamber. Thus it is not possible to test established trees, a potentially valuable asset to tree breeders wishing to select for frost hardiness.

Methods have been developed for testing tissue samples and the most useful are electrical impedance (Van den Driessche, 1973; Timmis, 1976; Harwood, 1981) and electrical conductivity of diffusate (Dexter *et al*, 1932; Wilner, 1960; Green and Warrington, 1978; Eldridge *et al*, 1983; Raymond *et al*, 1986). Both have the advantage that the same plant can be sampled repeatedly and there is no limit to the size of the plant that can be tested. The main disadvantage is in relating the results back to the field. Small samples of plant tissue tend to supercool more than whole plants. Under certain conditions therefore, tissue samples may overestimate frost hardiness (Burke *et al*, 1976).

Electrical conductivity of diffusate was the preferred technique for this study as the measurement of electrical impedance requires special equipment, *viz*. an A.C. bridge (de Plater and Greenham, 1959). Following frosting and subsequent incubation of leaf samples in water any damage to cellular structure will be expressed in leakage of cellular components of which ionic constituents can be measured by the conductivity of the water. The use of this principle for the measurement of frost hardiness in *Eucalyptus* is described and its development to suit the equipment available, enhance its efficiency and avoid supercooling is considered.

Methods

1. Standard Procedure

A paper punch (6 mm diameter) was used to remove six discs (W.N. Tibbits, unpubl.) of leaf tissue into 25 cm^3 glass vials. As *Eucalyptus* is evergreen, leaf tissue was more convenient for sampling throughout the year than stem, bud or root tissue. Leaves also show a greater increase in conductivity in response to exposure to damaging temperature than stem or bud tissue of *Eucalyptus* and therefore provide a more sensitive test for assessing differences in frost hardiness (Webb *et al*, 1983). The six discs formed a single sample and resulted in readings of diffusate electrical conductivity in the range 10-200 μS (μSiemens) cm^{-1}. A small quantity (0.1 mg) of silver iodide (AgI) and 0.2 cm^3 of deionised water were added to the vials to prevent supercooling of the samples during frosting.

Artificial frosting was done in an air-filled freezing chamber (height 750 mm, width 500 mm and depth 520 mm, J. and A. Parr, Hobart, Australia). The chamber was painted black inside and had a freezing coil located 120 mm from the top with a mixing fan above it (Fig. 2.1). The rate of cooling was controlled electronically in the range 0.05°C min^{-1} to 1.05°C min^{-1} and a set temperature was controlled to within +/- 0.1°C. An aluminium tray located 25 mm above the floor of the chamber on wooden blocks was used to support up to 110 glass vials. Aluminium was used because its high thermal conductivity reduced minor fluctuations in temperature between vials. Temperature variation in the chamber was sensed and controlled by a transistor (Verster, 1972) during cooling and at the preset test temperature.

The vials were placed randomly on the aluminium tray. The temperature in the chamber was lowered rapidly at 1.05°C min^{-1}, until it reached 0.5°C and then the rate of cooling was reduced to 0.2°C min^{-1} to the test temperature. Samples were held at the test temperature for 90 minutes and then removed from the chamber and allowed to thaw at 3.0°C. Samples from each plant were subjected to a minimum of three test temperatures, usually 1°C apart.

When the samples had thawed, 8 cm^3 of deionised water, the minimum volume required for satisfactory immersion of the electrode, was added to the vials and they were incubated on a shaker for 18 h at room temperature. The electrical conductivity, Y_f ($\mu S\ cm^{-1}$), of the solution was read on a conductivity meter (Radiometer CDM3 with a cell type PP1042, Copenhagen, Denmark). The vials were then stood in boiling water for 10 minutes to kill the tissue. The samples were incubated on the shaker for 18 h and the electrical conductivity was remeasured (Y_k).

The results were calculated as the ratio of the conductivity of the frozen tissue (Y_f) to the conductivity of the killed tissue (Y_k) expressed in percentage terms.

(1) Relative Conductivity, $Y = Y_f/Y_k \times 100$

Relative conductivity was then plotted against temperature for each plant sampled (Fig. 2.2) and a lethal temperature (LT) was defined as the temperature at which Y = 50%. This definition of LT corresponds to that used by Green and Warrington(1978) for *Pinus radiata* and also to that used by Raymond *et al* (1986) for *E. delegatensis* and *E. regnans* where Y = 70% for LT and $Y(RC*) = ((Y_k - Y_f)/Y_k)^{0.5}$.

2. Evaluation

The effectiveness of AgI in the prevention of supercooling was evaluated by a comparison of samples with and without AgI added.

The variation of temperature in the chamber during cooling and at the steady state was monitored by six copper-constantan thermocouples (1.7 mm) suspended 10 mm above and diagonally across the aluminium tray at 60 mm intervals (Fig. 2.3). Thermocouples were also placed in selected vials to record tissue temperature.

The time required for incubation to obtain maximum values of Y_f was measured by sampling both frozen and killed tissue at four-hourly intervals for 20 hours. The samples were selected from seedlings with known differences in frost hardiness and frosted at -4°C. Sample preparation otherwise followed the standard procedure. An alternative to killing the tissue and repeating the electrical conductivity measurement is to oven dry the samples and calculate results on a dry weight basis (Dexter *et al*, 1932). To test the relationship between dry weight and Y_k, 67 samples were treated using the standard procedure. The tissue was oven-dried at 80°C for seven days before being weighed.

To estimate variability of results 9 samples taken from fully expanded leaves of one plant were tested using the standard procedure.

As eucalypts may have juvenile, intermediate and adult foliage simultaneously the standard procedure was used to compare juvenile and adult, juvenile and intermediate, and current and one-year-old adult leaves of two provenances of four year old *E. delegatensis* in a field trial at Tarraleah (Chapter 3).

Finally, to corroborate the definition of lethal temperature, discs from 32 seedlings (juvenile foliage) were tested using the standard procedure. The seedlings were then allocated to four groups and frosted as whole plants at one of the following temperatures, -4, -5, -6 or -7°C. The plants were observed for a period of six weeks, sufficient for symptoms of damage to develop, and scored for survival.

Results and Discussion

1. Supercooling

Addition of AgI to the vial before frosting increased the relative conductivity (Y). The difference between the seeded vials and control (without AgI) were statistically significant at temperatures close to the lethal temperature (Table 2.1). Thus supercooling, in the absence of AgI lowered LT from -4.4°C to -5.5°C. The values of Y at -3°C were not significantly different because this temperature is not cold enough to cause damage. At -6.0°C the temperature was low enough that plants were not able to supercool enough to avoid damage and although the value of Y was lower without AgI than with it, it was not a statistically significant difference.

Table 2.1. The effect of silver iodide during frosting on relative conductivity, Y. Frosting, to one of four temperatures, -3, -4, -5 and -6°C, and incubation followed the standard procedure. Each sample was replicated 10 times using fully expanded leaves from seedlings of *E. delegatensis*.

Temp (°C)	Relative conductivity, Y (Y_f/Y_k)%		
	Without AgI	With AgI	
-3	19.8	19.6	ns
-4	19.1	27.1	*
-5	21.7	76.1	***
-6	72.0	82.4	ns

Addition of AgI and water effectively eliminated a potential problem arising from the occurrence of supercooling of tissue samples in an air-filled chamber. Small plant samples tend to supercool more than whole plants such that the lethal temperature would be underestimated (Burke *et al*, 1976) though rapid intracellular freezing following supercooling may have the reverse effect (Olien, 1967). This is avoided in a liquid-filled chamber (Dexter, 1956; Raymond *et al*, 1986) where the usual method of overcoming the problem is to seed the samples with ice crystals when the bath temperature reaches -2 to -3°C. This was not possible in the air-filled chamber as the samples are not accessible during cooling, necessitating addition of a suitable ice-nucleator prior to cooling. Of those available, crystalline AgI is the most effective at the test temperatures (Lindow, 1983). Silver iodide is relatively inert chemically and biologically, does not alter the electrical conductivity of the solution and can be added to the sample before cooling begins. The small amount of water added to the samples ensured contact between the leaf tissue and the AgI and provided an observable indicator of freezing.

2. Frost chamber

The horizontal variation in temperature 10 mm above the aluminium tray during frosting did not exceed 0.8°C and was at times as small as 0.1°C (Fig. 2.3). The two thermocouples which had the greatest difference were consistently those at positions 1 and 5 (mean difference, 0.3°C) and the temperatures at other positions always lay between these two extremes. Changes in sample temperatures (Fig. 2.4) closely followed those of the chamber temperature although sample temperatures were always warmer (by up to 1°C) than the chamber temperature until the test temperature was reached. As this could take up to 30 min. after the chamber temperature had stabilised at the test temperature, samples were routinely held for 90 min. at the test temperature before removal.

Rates of cooling, rewarming and time held at the test temperature interact to determine the amount of damage to the plant tissue. The rate of cooling used in the standard procedure (12°C/h) lies within the range reported by other authors, which varies from 2°C/h (Emmert and Howlett, 1953 ; Paton, 1981) to over 200°C/h (Aronsson and Eliasson, 1970) with the most commonly used rates of cooling being in the range 4 to 6°C/h (Van den Driessche, 1973; Christersson, 1978; Harwood, 1980; Greer and Warrington, 1982; Raymond *et al*, 1986). The slower rates of cooling are generally intended to simulate field conditions.

Time held at the test temperature varies from very short i.e. the tissue reaches the test temperature and is immediately rewarmed (Van den Driessche, 1976; Harwood, 1981) to as long as 24 hours (Wilner, 1960). The 1.5 h used in this study is within the range of 1 to 6 hours more usually reported (Emmert and Howlett, 1953; Aronsson and Eliasson, 1970; Greer and Warrington, 1982; Raymond *et al*, 1986).

In this study, tissue was removed from the frost chamber and placed in a refrigerator at 3°C to rewarm. This relatively rapid rate of rewarming has also been used by Aronsson and Eliasson (1970) and Raymond *et al* (1986). The rates of cooling, rewarming and time at the test temperature are compromises between simulating what happens in the field and processing samples efficiently. The ultimate test of the procedure is whether it correlates with field observations and whether it is able to predict the effect of low temperature on whole plants (see below).

3. Incubation time

The electrical conductivity (Y_f or Y_k) of the solutions increased rapidly during the first 4 h of incubation and had reached at least 95% of maximum conductivity after 12 hours (Fig. 2.5). Although samples could have been read prior to 18 h, Hallam & Tibbits (1986) found that larger discs took longer to equilibrate than the 6 mm discs. It was decided to standardize on 18 hours, so that the test results would be comparable using any size of disc. Differences in conductivity between 12 and 20 hours were not statistically significant ($P<0.01$) and were independent of Y and LT. Hence over the range from relatively little to full ionic leakage, maximum conductivities are achieved in similar times after addition of water. Aronsson and Eliasson (1970) used incubation periods of 18 to 20 h and found that for periods of over 24 h increased conductivity readings were caused by bacterial contamination of the samples.

A linear relationship was found between Y_k and dry weight (Fig.2.6), but the degree of association ($r^2 = 0.66$) suggested that accurate estimates of frost hardiness require an estimate of Y_k rather than dry weight since Y_k is a direct measure of potential leakage of ions from the tissue.

4. Plant tissue

Testing 9 samples from the one plant resulted in a mean lethal temperature of -4.44°C with 95% confidence interval of -4.23 to -4.65.

There was no significant difference in lethal temperature between juvenile and adult leaves or juvenile and intermediate leaves of similar age (Table 2.2). A difference ($P<0.05$) in mean lethal temperature was found between one-year-old (-7.0°C) and new adult leaves (-5.7°C) of the Ben Lomond provenance.

Table 2.2. Comparison of mean lethal temperatures for different types of plant tissue. Leaves taken from two provenances of four year old *E. delegatensis* plants in the Tarraleah provenance trial (see Chapter 3). Values with similar letters are not significantly different ($P<0.05$, $n = 5$).

Provenance	Leaf form	Mean LT (°C)
Ben Lomond	one-year-old adult	-7.0 a
	one-year-old juvenile	-7.0 a
	new adult	-5.7 b
Upper Howqua	one-year-old juvenile	-5.3 c
	one-year-old intermediate	-5.5 c

Different plant tissues have been used for frost testing. Timmis (1976) showed for Douglas Fir that stem, bud and leaf tissue all followed essentially the same pattern of frost hardening. Webb *et al.*(1983) showed that bud tissue of *E. delegatensis* is the most sensitive to frost followed by leaf tissue and then stem tissue. Leaf tissue has an advantage over stem, bud and root tissue in that it is easy to remove with minimal damage to the plant and it is easy to subsample. The results showed that the different leaf forms of *E.delegatensis* do not give a significantly different result when tested by the method described. Recently developed leaves may be more frost sensitive than older leaves however and this should be taken into account when samples are selected.

5. Whole plants vs discs

The survival of whole trees subjected to test temperatures between -4 and -7°C was similar to that anticipated from leaf discs. The mean lethal temperature determined by the leakage of solutes from leaf discs for the 32 plants was -5.34°C with 95% confidence interval of -5.21 to -5.47°C. Probit analysis of data obtained by the frosting of whole plants (Table 2.3) gave the regression -

Probit = -6.16 - 1.231 Temp.

with LD_{50} = -5.005 and 95% confidence interval -4.416 to -5.561, where LD_{50} is the lethal dose (temperature) which would kill 50% of the seedling.

The mean LT from leaf discs and its 95% confidence interval for tissue samples thus lie within the 95% confidence interval for the results from the testing of whole plants. The means therefore are not significantly different.

Table 2.3. Results of testing whole plants of *E. delegatensis* which had previously been tested using the standard procedure. Thirty-two seedlings were assigned to four different test temperatures and scored for survival six weeks after frosting.

Test temp. (°C)	Proportion killed
-4	1/9
-5	4/8
-6	7/8
-7	7/7

As there is no evidence of recovery of the whole plants which had not initially survived the test temperature, a value of relative conductivity, Y < 50% for leaf discs would appear to be an acceptable measure of potential survival of eucalypts in the field following a frost. Using stem sections, Green and Warrington (1980), also found that values of Y =/> 50% were followed by death of seedlings of *Pinus radiata*. Similarly Raymond *et al* (1986) found that in the absence of post-frost recovery, measured values of Y, given a suitable range of test temperatures, could be used to predict leaf survival of eucalypts.

Conclusions

The modifications to the electrical conductivity method of Dexter *et al* (1932) have resulted in a reliable procedure for measuring frost hardiness of *Eucalyptus* species. Variation in results has been shown to be small and the results obtained by frosting whole seedlings are not significantly different from those obtained by testing leaf discs.

Fig. 2.1 The frost chamber with its door removed to show the cooling coil in the top and the tissue samples in glass vials on an aluminium tray supported on blocks on the floor of the chamber. To the left of the chamber is a chart recorder which plots the temperature inside the chamber.

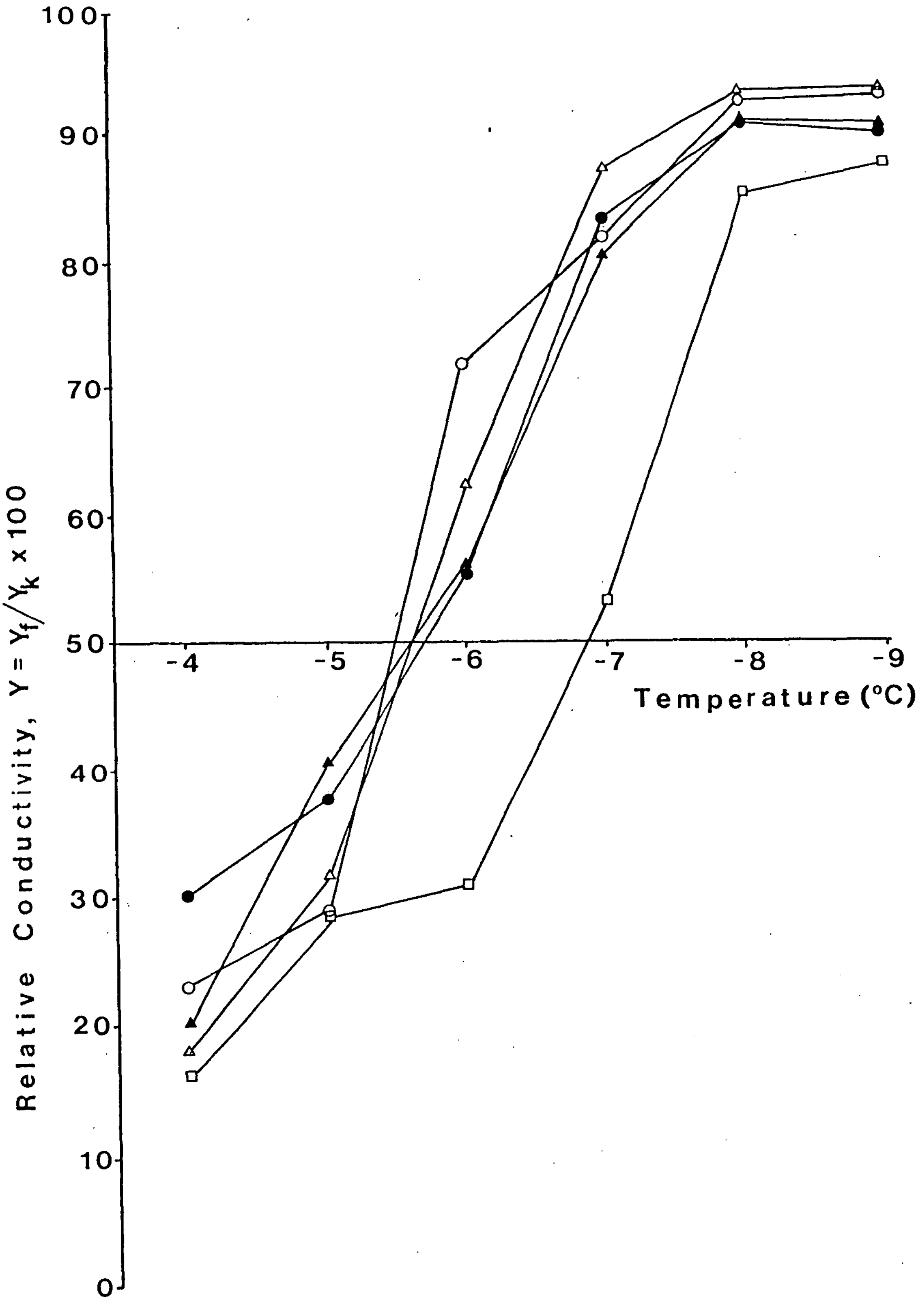


Fig 2.2 Relative Conductivity, Y, versus test temperature for five trees of the Upper Howqua provenance from the Tarraleah Provenance Trial, sampled in May 1984.

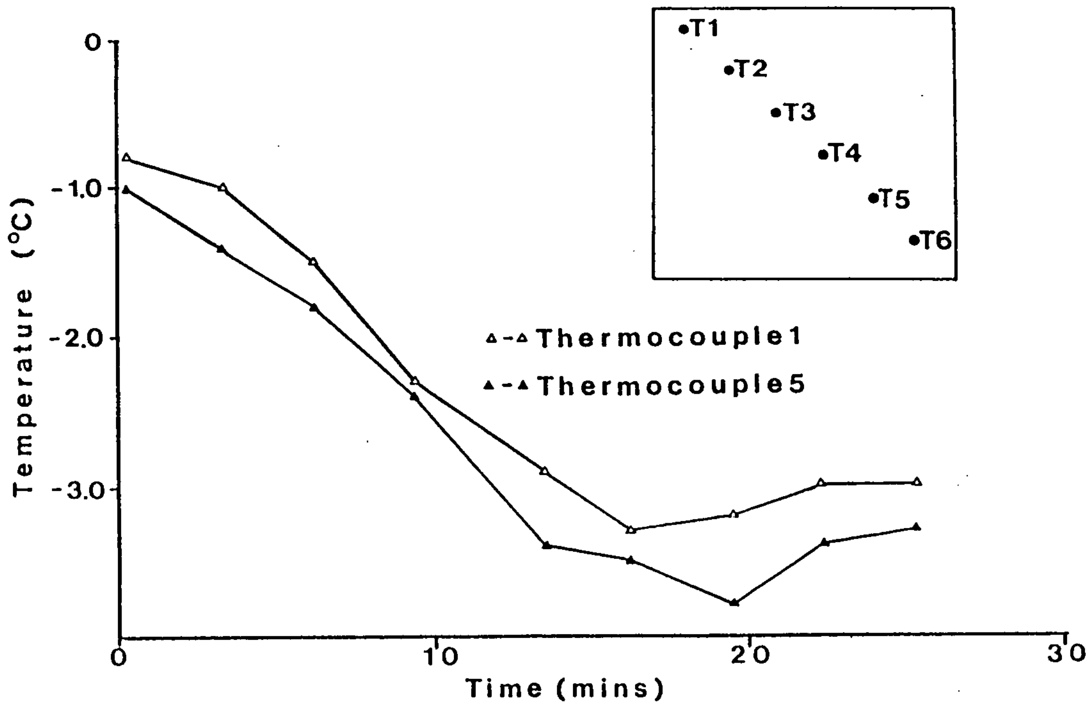


Fig. 2.3 The horizontal variation in temperature in the frost chamber 10 mm above the aluminium tray during cooling to -3.0°C. The thermocouples were 60 mm apart (see inset, T1 to T6). Thermocouples 2,3 4 and 6 gave readings intermediate to thermocouples 1 and 5.

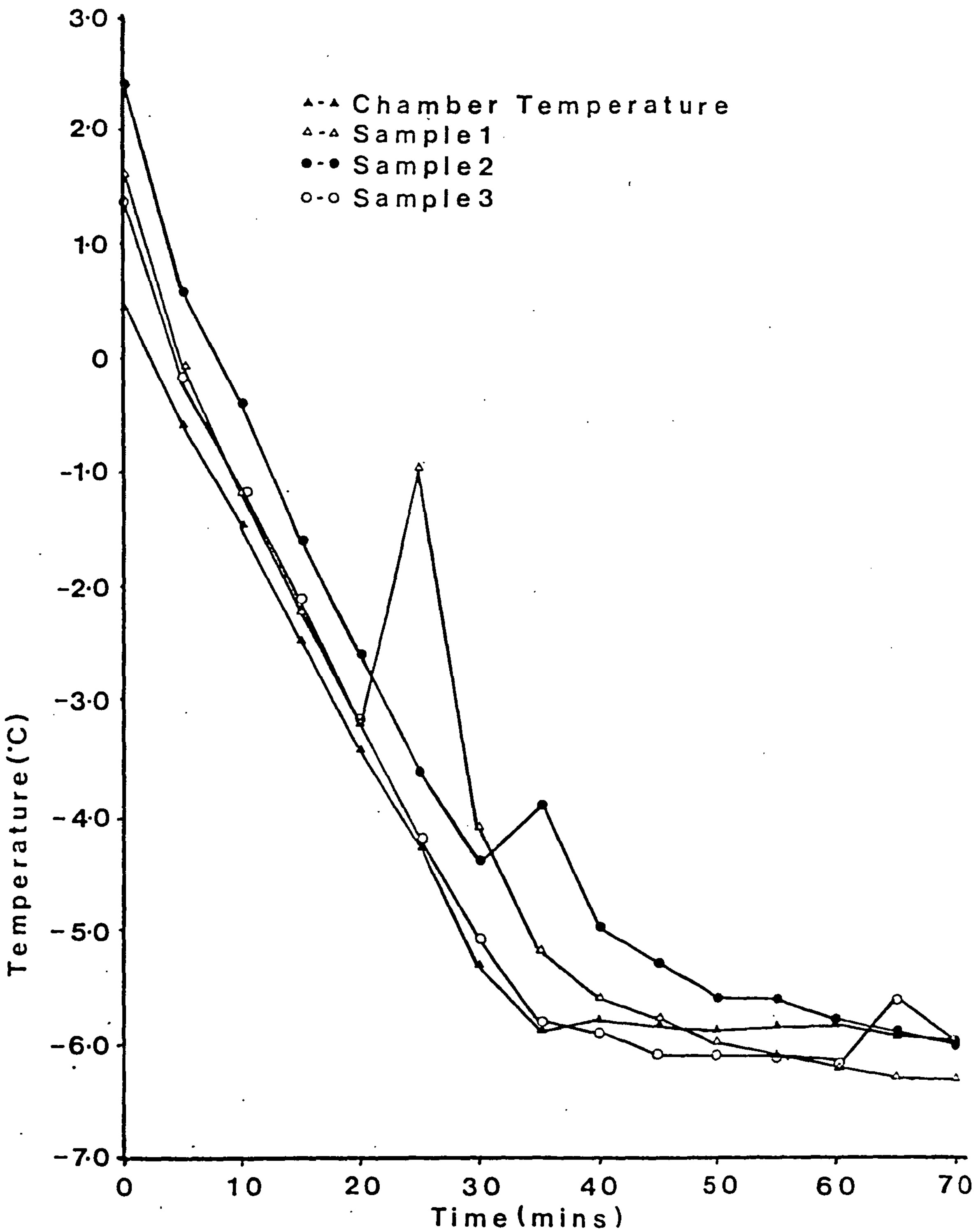


Fig. 2.4 The temperature of the frost chamber and three samples during cooling at 0.2°C/min to a test temperature of -6.0°C. The sudden increases in sample temperature are exotherms due to release of heat when the samples freeze.

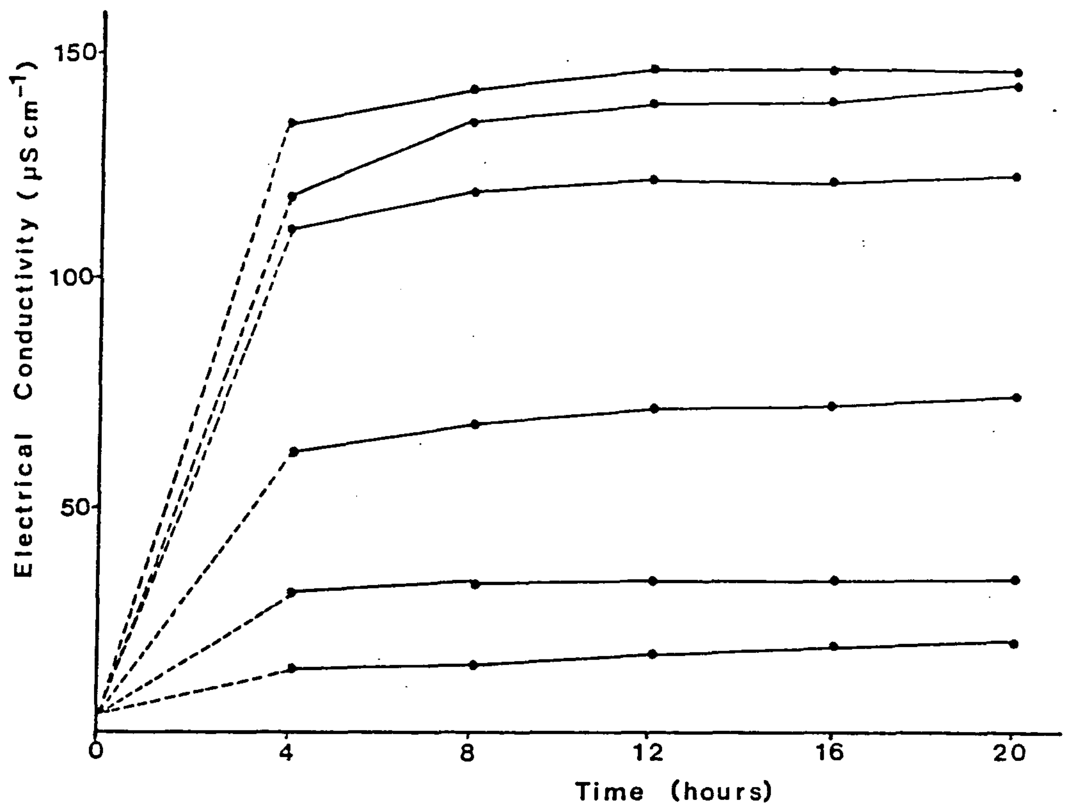


Fig. 2.5 The electrical conductivity of six samples of differing frost hardiness tested at -4.0°C, measured at four-hourly intervals during incubation.

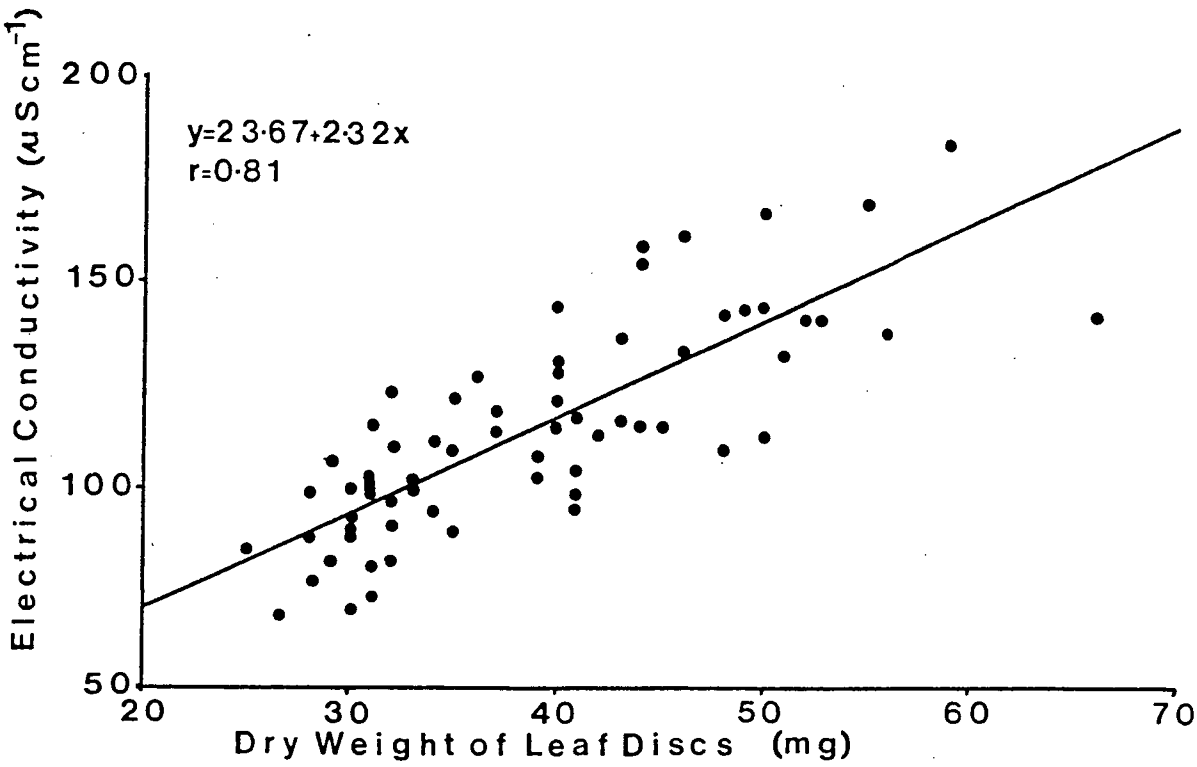


Fig. 2.6 The relationship between electrical conductivity and dry weight of leaf discs for 67 samples.

CHAPTER 3

Seasonal and genetic variation in frost hardiness of *E.delegatensis* in the field

Introduction

Variation in frost hardiness of eucalypts has only rarely been measured for more than one provenance of a species over a complete year (Harwood, 1980). Either frost hardiness has been measured for a single provenance of several different species throughout the year or, where more than one provenance of a species has been studied, frost hardiness has only been measured on two or three occasions during the year (e.g. Menzies *et al*, 1981; Rook *et al*, 1980).

Menzies *et al.* (1981) determined frost hardiness by artificially frosting seedlings of *Eucalyptus saligna, E. regnans* and *E. fastigata* at monthly intervals. They were able to show that frost hardiness increased to a peak in midwinter and that the three species differed in their resistance to frost at all times of the year. *E. saligna* was the least frost hardy and *E. fastigata* the most frost hardy as would be expected from their natural distributions.

Secondly, frost hardiness for 38 provenances of *E. regnans* was determined at three different times of the year, autumn, winter and spring (Rook *et al*; 1980). The range of frost hardiness among provenances was greatest in winter, about 2.5°C, and there was a statistically significant interaction between season and provenance such that the Tasmanian provenances were ranked higher in frost hardiness in autumn than in winter and spring and the Victorian provenances ranked higher in winter and spring than in autumn. Thus the variation in frost hardiness even within a species can have a strong genetic and seasonal basis. A preliminary study suggested that large seasonal differences in frost hardiness between provenances were also a feature of *E. delegatensis* (Webb *et al*, 1983).

In the present study an experiment was designed to follow the cycle of frost hardening and dehardening throughout a full year for seven different provenances of *E. delegatensis*. Since *E. delegatensis* occurs naturally over a wide range of altitudes in Tasmania (200-1200 m), Victoria (900-1400 m) and New South Wales (900-1500 m), provenances originate from sites which experience different mean, maximum and minimum temperatures. It was hypothesised that the provenances selected for testing would show measurable differences in their genetic ability to survive low temperatures. The results presented express the cycle of frost hardening as the change in lethal temperature for each provenance throughout the year.

Materials and Methods

1.Test material

During 1978 and 1979, the Genetics Section of CSIRO Division of Forest Research, in collaboration with the State Forestry Commissions of Tasmania and New South Wales, Australian Newsprint Mills Ltd and Australian Pulp and Paper Manufacturers, planted provenance trials of *E. delegatensis* at four sites in Tasmania (Tarraleah, Myrtle Bank, Parrawe and Diddleum Plains) and one site, Pilot Hill, in New South Wales (Fig.3.1). Each trial consists of an 8x8 balanced latin square with 64 provenances represented in each of nine replicates. Each replicate is divided into eight incomplete blocks of eight treatments. The layout of these trials was described in detail by Bell(1979). The collection sites of the provenances are shown in Fig. 3.1.

Two of the Tasmanian trials were sampled, Tarraleah (Fig. 3.2) every 4 to 8 weeks from February 1984 until March 1985, and Myrtle Bank (Fig. 3.3) on three occasions, in February, June and July 1984.

Table 3.1. Ranked adjusted means of provenances for frost damage as assessed in 1980 in the Pilot Hill field trial (from Boland and Dunn, 1985). An asterisk indicates a provenance chosen for testing.

Provenance Number	Provenance Name	State	Adjusted mean	
55	Bicheno	Tas.	2.03464	*
53	Middle Peak	Tas	1.91481	
39	Dazzler Range	Tas.	1.76925	
57	Bendover Hill	Tas.	1.75556	
36	Plateau Rd.	Tas.	1.72361	
35	Hartz Mtn.	Tas.	1.70556	
54	Lake Tooms	Tas.	1.7	
56	Fingal Tier	Tas.	1.63148	
51	Russell River	Tas.	1.60185	
40	Cluan Tier	Tas.	1.57151	
50	Lake Pedder	Tas.	1.55185	
72	Eaglehawk Tier	Tas.	1.49074	
45	Heemskirk River	Tas.	1.48148	
49	Mt Dromedary	Tas.	1.38889	
38	Ben Nevis	Tas.	1.35926	*
41	Maggs Mtn.	Tas.	1.31852	
73	Misery Plateau	Tas.	1.27963	
25	Razorback Spur	Vic.	1.27778	
37	Ben Lomond	Tas.	1.23148	*
47	Miena	Tas.	1.21481	
4	Mt Bogong	N.S.W.	1.21481	
46	King William Saddle	Tas.	1.20185	
27	Royston River Rd.	Vic.	1.17963	
42	Guildford	Tas.	1.16296	
29	Mt Macedon	Vic.	1.12963	
75	Mt St Gwinear	Vic.	1.11481	

23	Big Hill	Vic.	1.11111	
48	Tunbridge Tier	Tas.	1.111	
62	Mt Ellery	Vic.	1.10105	*
10	Youngal	N.S.W.	1.1	
28	Lake Mountain	Vic.	1.0963	
43	Yellow Marsh Rd.	Tas.	1.09259	
9	Cascade	N.S.W.	1.09259	
44	Luina	Tas.	1.08889	*
7	Mt Nurenmerenmang	N.S.W.	1.07778	
13	Dargals Range	N.S.W.	1.07407	
19	Pilot Hill	N.S.W.	1.07407	
24	Big Ben	Vic.	1.06667	
12	The Pinnacle	N.S.W.	1.06667	
16	Yarrangobilly	N.S.W.	1.06667	
60	Bulls Head	A.C.T.	1.06296	
14	Mt Flinders	N.S.W.	1.06296	
21	Beecher Hill	Vic.	1.06296	
18	Bald Hill	N.S.W.	1.05926	
31	Mt Useful	Vic.	1.05556	
22	Mt Buffalo	Vic.	1.05556	
34	Mt Baldhead	Vic.	1.04444	
30	Ada River	Vic.	1.04074	
5	Clear Creek	N.S.W.	1.03704	
1	Yaouk Bill Range	N.S.W.	1.03704	
8	Mt Black Jack	N.S.W.	1.02778	
3	Smokers Flat	N.S.W.	1.02222	
20	Mt Wills	Vic.	1.02222	
33	Mt Ewen	Vic.	1.02222	
26	Upper Howqua	Vic.	1.01852	*
11	Geehi	N.S.W.	1.0	
2	Leura Gap	A.C.T.	1	
6	The Granites	N.S.W.	1	
61	Mt Delegate	N.S.W.	1	
15	Peppercorn Hill	N.S.W.	1	
17	Gungarlin River	N.S.W.	1	*

Selection of provenances for testing was made using the data on frost damage in 1980 from the Pilot Hill trial in New South Wales (Table 3.1). It is particularly noticeable from this table that the Tasmanian provenances are listed as the 16 least hardy provenances. The provenances selected for testing were chosen to represent the full range of frost hardiness indicated by this ranking, while also being from a range of altitudes and a mixture of coastal and more continental sites. The Bicheno provenance was chosen because it was the least frost hardy and came from a low altitude coastal site in Tasmania. The Gungarlin R. provenance was the most hardy and was from a high altitude, continental site in New South Wales. The Ben Nevis provenance was a less hardy one from an intermediate altitude in Tasmania. The Ben Lomond provenance was selected because it came from the highest altitude in Tasmania and was ranked as much less frost hardy than would have been expected. The Upper Howqua provenance represented an intermediate altitude, continental site in Victoria. The Luina provenance was selected because it was the most frost hardy of the Tasmanian provenances while also coming from a low altitude, coastal site. The Mt. Ellery provenance was selected because it was the only mainland provenance from a coastal site.

An initial test sampling was done in August 1983 in which six of the seven provenances selected were tested. These provenances were rated 1, 2 or 3 according to whether the lethal temperature was in the range -8 to -9°C, -9 to -10°C or lower than -10°C respectively (Table 3.2). Sampling was done from one replicate in each trial, replicate 2 at the Tarraleah trial (Fig. 3.4) since this replicate was located entirely on level ground unlike the other replicates which were unevenly sloped. At Myrtle Bank, replicates 9A and 9B (Fig. 3.5) were sampled as these were located on a relatively even slope.

Table 3.2. *E. delegatensis* provenances selected for testing of frost resistance. The hardiness rating is the result of a test sampling in August 1983.

No. Provenance	State	Altitude of Collection(m)	Hardiness Rating
44 Luina	Tas.	450	1
55 Bicheno	Tas.	450	1
38 Ben Nevis	Tas.	820	3
37 Ben Lomond	Tas.	1220	2
6 Upper Howqua	Vic.	1000	2
62 Mt. Ellery	Vic.	1150	*
17 Gungarlin R.	N.S.W.	1200	2

1 = lethal temperature, -8 to -9°C
2 = lethal temperature, -9 to -10°C
3 = lethal temperature, lower than -10°C
* = provenance not tested in August 1983

2.Temperature Measurement.

At Tarraleah, maximum-minimum thermometers were placed near each provenance at a height of 350 mm above the ground (Fig 3.4). At the centre of the block, near the Upper Howqua provenance (26), additional maximum-minimum thermometers were suspended at 830 mm and at 1550mm above the ground. At Myrtle Bank which was not visited as frequently, three maximum-minimum thermometers were located near the provenances which were sampled (Fig 3.5). All thermometers were fastened to wooden stakes in a vertical position and located so that they faced South. Wooden flaps attached to the stakes shielded the thermometers from direct sunlight. Daily maximum and minimum temperatures recorded by the Meteorological Bureau from standard mercury-in-glass thermometers in a Stevenson Screen were available from Tarraleah Village approximately 5 km away for comparison with site temperatures (Fig. 3.2). Tarraleah village is at the same altitude and has the same aspect as the field trial.

3. Experimental procedure.

Six leaves were taken from each of 5 trees of each provenance and stored in a refrigerator overnight (Raymond *et al*, 1986). The next day one disc was punched from each leaf and these six discs were combined to make a sample. Each sample was replicated once. Samples were tested at up to a maximum of 6 temperatures using the standard procedure described in Chapter 2.

Results

1. Temperature Variation at Tarraleah.

The maximum-minimum thermometers showed that there was very little variation in minimum temperatures over the area of the provenances sampled, (Table 3.3(a)). The maximum difference between the thermometers on the dates when measurements were recorded was 3°C but they were usually within 1°C of each other.

Table 3.3(a) Minimum temperatures (°C, missing values are indicated by *) recorded at Tarraleah at a height of 350 mm above the ground.

Period	Provenance Number							Range	Mean
	44	55	38	37	26	17	62		
28/4/84 - 28/5/84	-4	-4	-3	*	-4	-4	-3	1	-3.7
28/5/84 - 25/6/84	-7	-7	-6	*	-7	-6	-7	1	-6.7
25/6/84 - 24/7/84	-6	-8	-7	*	-7	-7	-7	2	-7.0
24/7/84 - 20/8/84	-7	-6	-6	-6	-6	-6	-7	1	-6.3
20/8/84 - 1/10/84	-3	-6	-4	-6	-3	-6	-4	3	-4.6
1/10/84 - 29/10/84	-1	-1	-1	-1	-1	-1	-1	0	-1.0
29/10/84 - 26/11/84	-2	-4	-4	-4	-4	-4	-5	3	-3.9
26/11/84 - 14/1/85	1	1	2	2	2	1	1	1	1.4
14/1/85 - 5/3/85	1	0	1	0	0	0	0	1	0.3

The suspended thermometers also showed very little vertical stratification of minimum temperature with temperatures usually being within 1°C of each other (Table 3.3(b)). The lowest minimum temperature recorded at Tarraleah during 1984 was -8°C.

Table 3.3(b) Minimum temperatures (°C) recorded at different heights at Tarraleah .

Period	Height above the ground		
	350 mm	830 mm	1550 mm
28/4/84 - 28/5/84	-4	-3	-3
28/5/84 - 25/6/84	-7	-6	-6
25/6/84 - 24/7/84	-7	-6	-6
24/7/84 - 20/8/84	-6	-6	-6
20/8/84 - 1/10/84	-3	-3	-3
1/10/84 - 29/10/84	-1	-1	-1
29/10/84 - 26/11/84	-4	-3	-4
26/11/84 - 14/1/85	2	3	1
14/1/85 - 5/3/85	0	2	2

2. Seasonal Variation at Tarraleah

Hardening commenced in April 1984. Maximum hardiness and minimum temperatures (mean and lowest for the month) were recorded in the period between July and August (Fig 3.6). The greatest difference between maximum and minimum hardiness measured was 4.6°C for Ben Lomond provenance (Fig. 3.7(b) and the smallest difference was 2.4°C for Bicheno provenance (Fig. 3.7(a).

The measurements of frost hardiness in August 1983 demonstrated that the maximum hardiness varies from year to year depending on the minimum temperatures experienced (Fig. 3.6). The Bicheno provenance was 3°C hardier in August 1983 than in August 1984, while the Ben Lomond provenance was 1°C hardier in August 1983 than in August 1984. There was also variation in the summer level of minimum hardiness from year to year. The winter and spring of 1983 had colder minimum temperatures than the winter and spring of 1984 and this resulted in the trees dehardening less in the spring of 1983 than in 1984 (Fig 3.6).

3. Genetic Variation at Tarraleah

All provenances tested showed the same seasonal trends of frost hardiness (Fig. 3.7 a,b and c), but different rates of hardening and dehardening showed that some provenances e.g. Ben Lomond, Gungarlin R. (Fig. 32.7 b) were able to respond to falling and rising minimum temperatures to a greater degree than others e.g. Bicheno (Fig. 3.7 a). The difference between mean lethal temperatures of the most hardy and least hardy provenances at maximum hardiness was 2.7°C.

The Bicheno provenance proved to be the least frost hardy provenance at all times of the year, especially in winter when little hardening occurred, the mean lethal temperature in July being -6.0°C. Thus the minimum temperature in June 1984 at Tarraleah (Fig 3.6) was too cold for this provenance to grow successfully and there was a high incidence of frost damage. High mortality of this provenance was also observed following the very low minimum temperatures in July 1983. Although the Mt Ellery provenance also only lowered its mean lethal temperature to -6.0°C (Fig 3.7(a)) it hardened earlier in the year and thus did not suffer as much damage.

The Ben Lomond provenance was the most frost hardy with a mean lethal temperature of -8.6°C in August. Although the Gungarlin R. provenance reached the same level of hardiness (-7.9°C) as the Ben Lomond provenance in July 1984(Fig. 3.7(b), the Ben Lomond provenance continued to harden during August while the Gungarlin R. provenance had already begun to deharden (-6.7°C). The next most hardy provenance was the Luina provenance (Fig. 3.7(c)), with a mean lethal temperature of -7.2 in July, followed by the Upper Howqua provenances (Fig. 3.7(c)), and the Ben Nevis provenance (Fig. 3.7(a)) which both had mean lethal temperatures of -6.9°C in July.

4. Temperature Variation at Myrtle Bank

Minimum temperatures were only recorded for two time periods (Table 3.4). These showed a maximum of 1°C variation over the experimental site. The lowest minimum temperature recorded at Myrtle Bank for 1984 was -4°C.

Table 3.4 Minimum Temperatures recorded at the Myrtle Bank (°C) provenance trial.

Period	Upper	Middle	Lower
4/6/84 - 31/7/84	-4	-4	-3
31/7/84 - 3/2/85	-4	-4	-4

5. Genetic Variation at Myrtle Bank

Lethal temperatures for the provenances sampled at Myrtle Bank are plotted with the Tarraleah results in Fig 3.7(a,b and c). In all cases the trees at Myrtle Bank did not reach the same level of hardiness as at Tarraleah. The range of values of mean lethal temperatures at maximum hardiness at Myrtle Bank were from -4.7°C for the Bicheno provenance to -7.7°C for the Ben Lomond provenance. The values for lethal temperature at maximum hardiness for the two sites were highly correlated ($r = 0.94$, Fig 3.10).

6. Frost hardiness at Pilot Hill, NSW

It is interesting that the ranking of the provenances for frost hardiness is different in the Tasmanian trials than at the New South Wales trial (Table 3.5) The Tasmanian provenances appeared hardier in Tasmania than in New South Wales. Victorian provenances hardened less effectively at the Tasmanian sites. Ben Lomond provenance which was the most frost hardy provenance in Tasmania was less hardy than the Gungarlin R, Upper Howqua, Luina and Mt Ellery provenances at Pilot Hill.

Table 3.5. Frost hardiness ranking at the Tasmanian provenance trials compared with the New South Wales trial (see Table 3.1).

	Tasmania	New South Wales
Least Hardy	Bicheno	Bicheno
	Mt Ellery Ben Nevis Upper Howqua	Ben Nevis Ben Lomond Mt Ellery
	Luina	Luina
	Gungarlin R.	Upper Howqua
	Ben Lomond	Gungarlin R.

Discussion

1. Seasonal Variation in Frost Hardiness

The annual cycle of hardening and dehardening in *E. delegatensis* was very similar to that reported by Menzies *et al* (1981) for *E. saligna*, *E. regnans* and *E. fastigata*. They also found that hardening began in April and continued until June for *E. saligna* whereas in the more hardy species, *E. regnans* and *E. fastigata*, and *E. delegatensis* in this study, hardening continued until July and August. Thus variation in ability to harden exists both between and within species through different responses to low temperature (Fig.3.7(a,b and c)). Thus Ben Lomond provenance, the hardiest provenance, was able to significantly decrease the lethal temperature between July and August (Fig.3.7(b)), presumably in response to the low minimum temperature experienced during this period (-6^{o}C, Table 3.3(b)). For all other provenances differences in hardening were entirely due to their ability to harden between April and July (Fig 3.7 (a,b and c)) as in some instances, significant dehardening occurred (Ben Nevis and Gungarlin R. provenances, Fig.3.7(a and b)) between July and August in spite of low minimum temperatures. These differences cannot be explained from the present data but a photoperiodic response by some provenances is a possibility (Eldridge,1969).

It is apparent from the data that *E. delegatensis* does not reach the the same level of hardening every winter or the same level of dehardening every summer (Fig. 3.6). Minimum temperatures of -10, -13 and -11°C were recorded during the period 30 June to 1 July 1983 by the Meteorological Bureau at Tarraleah Village compared with a minimum of -6°C in 1984. These minimum temperatures in 1983 confirm the low mean lethal temperatures measured in August 1983 (Fig. 3.6) since the hardiest provenance in 1984 (Ben Lomond) would have been killed by a temperature of -8.6°C, (Fig. 3.6). Observations showed that only some trees of the more frost sensitive provenances were killed in 1983. Sakai (1956) reported that for a fixed time period, the effectiveness of hardening increases with decreasing temperature for artificially hardened mulberry twigs. The difference between the results from Tarraleah and Myrtle Bank can also be considered in this way. The lowest minimum temperature recorded in 1984 for Tarraleah was -8°C while for Myrtle Bank it was -4°C. Since the rankings of maximum hardiness for provenances was the same for both sites, the difference in level of maximum hardiness may be simply due to the difference between minimum temperatures experienced by the two sites. Since there is no apparent interaction between the ranking of provenances and site within Tasmania for frost hardiness, it may be reasonable to use the results from provenance trials when selecting provenances to plant at other sites within the state.

2. Genetic Variation

Altitudinal clines for frost hardiness have been reported for *E. delegatensis* by Grose(1960), for *E. fastigata* by Boden(1958) and for *E. regnans* by Ashton(1958) and Eldridge(1969). It was therefore anticipated that the different provenances of *E. delegatensis* used in this experiment would show differences in ability to frost harden. In this study the maximum difference in winter between the most (Ben Lomond) and least hardy (Bicheno) provenances was found to be 2.7°C in 1984, a difference which would be very important when selecting for survival at low temperatures. At the Tarraleah trial only the Bicheno provenance (the least hardy) suffered from frost damage nearly every winter (Fig. 3.9) other more hardy provenances have suffered little or no damage at this site.

Comparing the data from Tarraleah and Myrtle Bank it can be seen that measurement of frost hardiness in field trials does not necessarily show the maximum genetic potential for frost hardiness. Unless the measurements are done during an extremely cold winter or on more extreme sites, only relative hardiness of the provenances will be obtained. It was however encouraging that the ranking of provenances was the same at both sites, a result which suggests that the testing procedure adopted is adequate for selecting suitable provenances for planting, at least in Tasmania.

It appears from the data that preceding temperature is the main factor controlling development of frost hardiness. In temperate species in the Northern Hemisphere it is generally accepted that daylength is also a major factor controlling the development of frost hardiness, with short days and cold temperatures both being necessary to induce maximum hardiness (Weiser, 1970; Aronsson,1975, Glerum,1976). Eldridge (1968) has also observed that *E. regnans* seedlings which were artificially frosted suffered less damage if they had been grown under short rather than long daylengths. Daylength would not be a factor when comparing the hardening response at Tarraleah with Myrtle Bank since they are only 1^{o} latitude apart,though between provenance differences may be in some way related to daylength. Daylength may also account for the difference in provenance ranking between the Tasmanian trials and the New South Wales trial, as the difference in latitude between Tarraleah and Pilot Hill is 7^{o}. If Tasmanian provenances are more sensitive to daylength than the Victorian and New South Wales provenances, hardening would be more effective in Tasmania than when planted further north (Table 3.5). The role of daylength in the hardening of *Eucalyptus* requires further study.

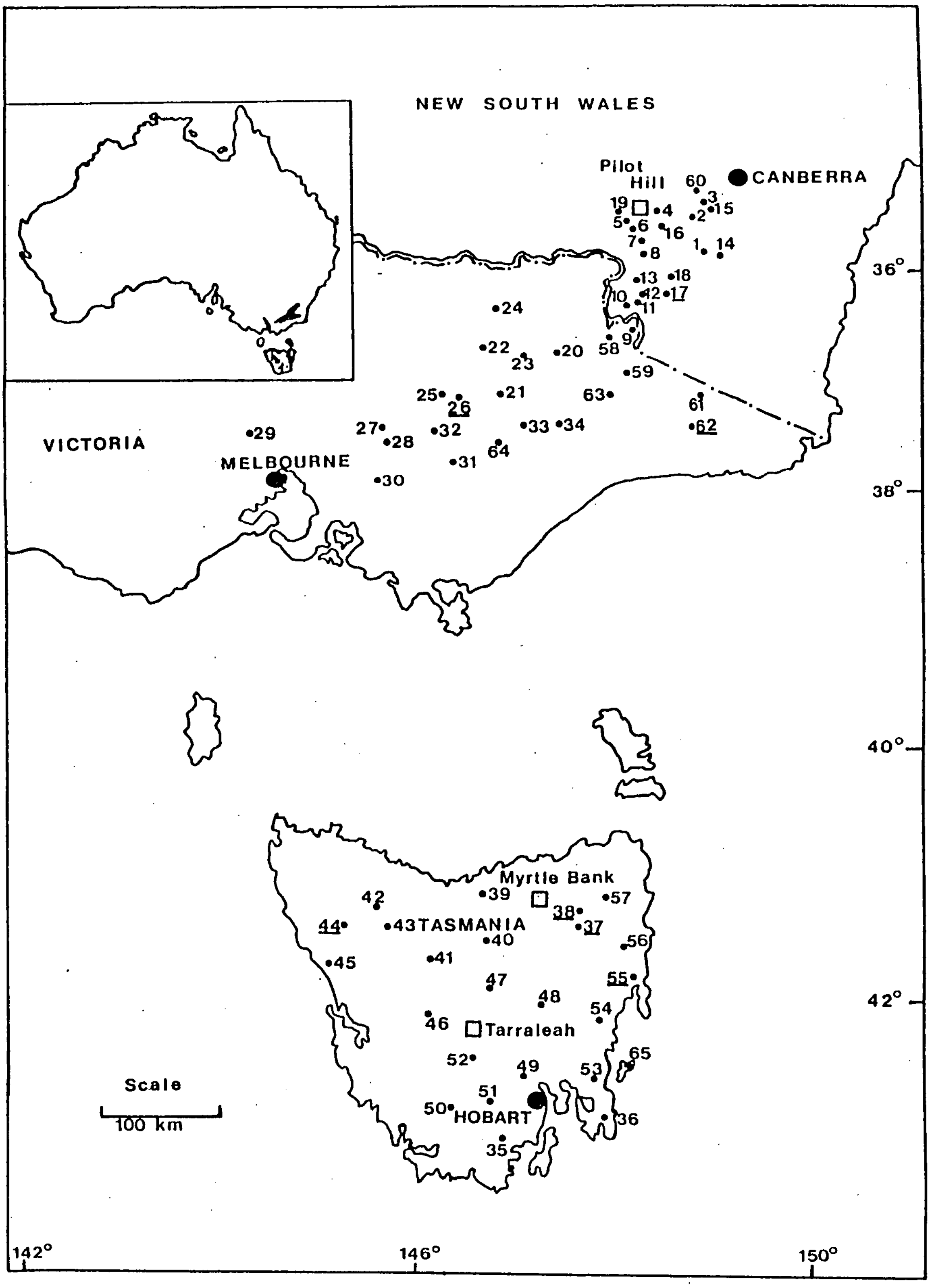


Fig. 3.1 Distribution of seed collection sites for provenance trials. Underlined numbers indicate the provenances sampled and □ indicate locations of provenance trials used in this study. Inset map shows the natural distribution of E. delegatensis. (from Boland et al, 1980).

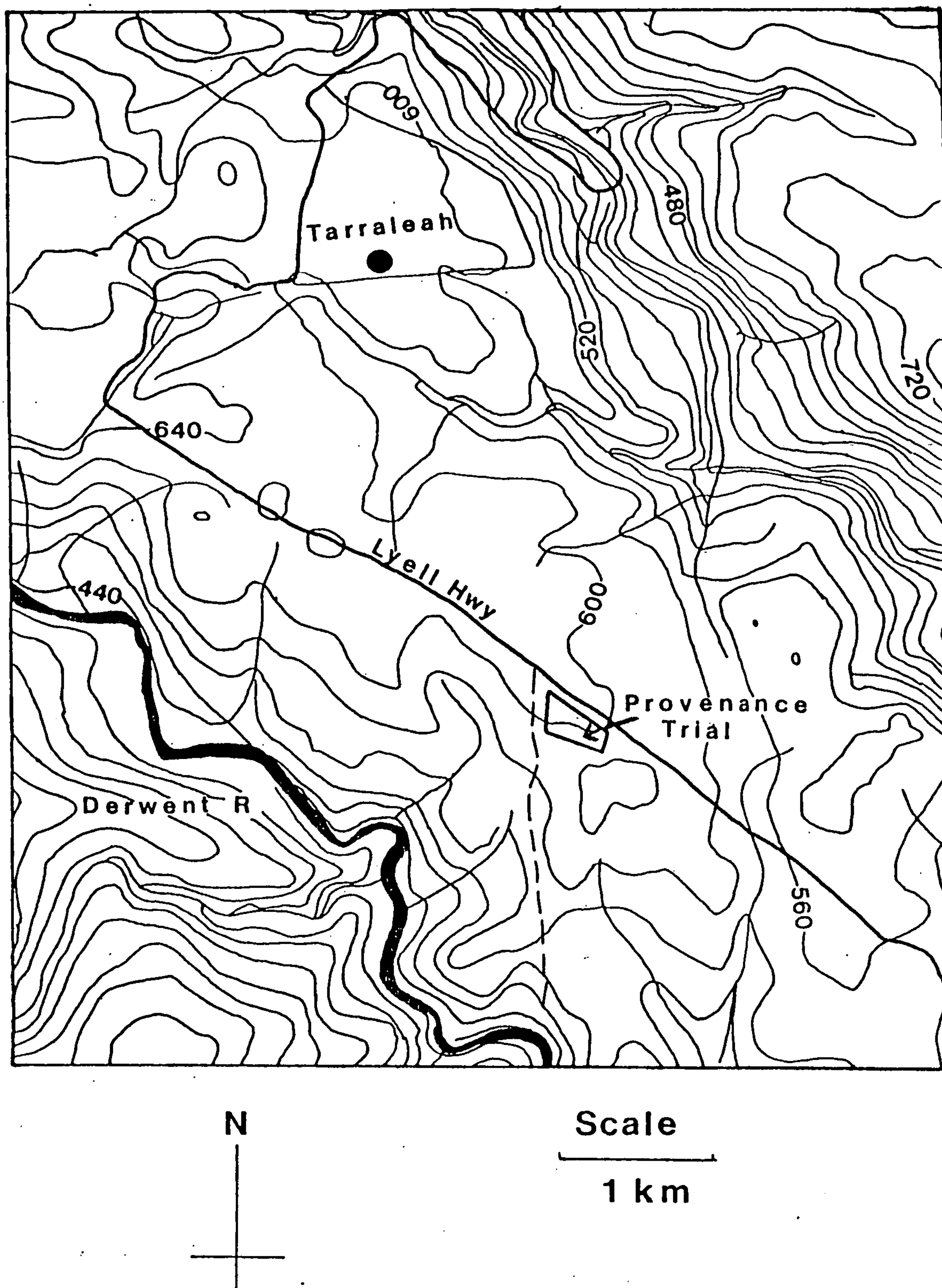


Fig. 3.2 The location of the Tarraleah provenance trial (Lat. 42°20', Long. 146°26'). Contour lines are at 40 m intervals.

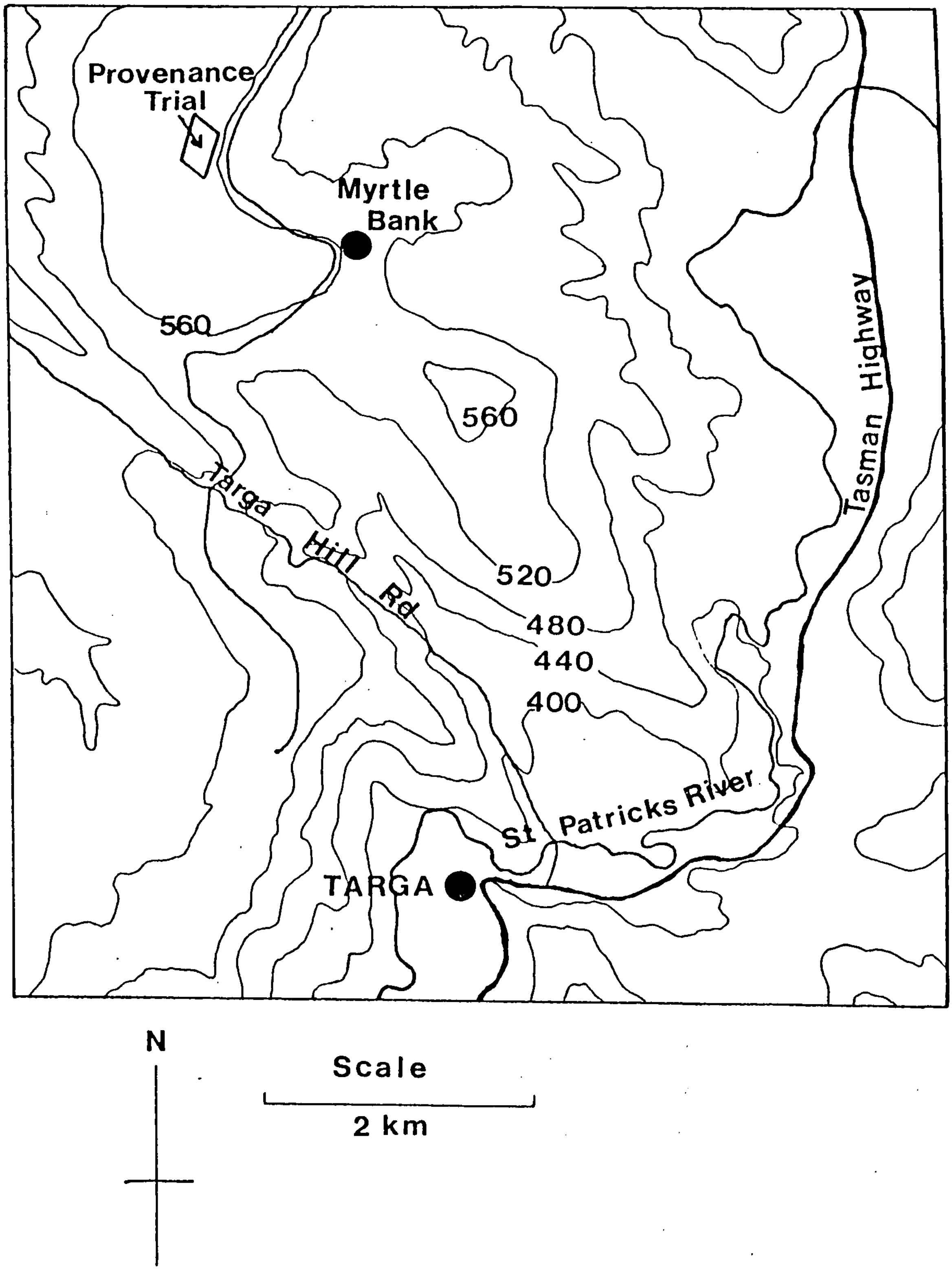


Fig. 3.3 The location of the Myrtle Bank provenance trial (Lat. 41°27', Long. 147°21'). Contour lines are at 40 m intervals.

31	37 T	22	65	51	T 38	52	43	59	28
55	40	15	6	27	49	50	12	7	39
59	62 T	28	17 T	73	24	58	30	71	8
48	61	72	48	T 55	26 T	3	8	29	44
45	53	31	19	75	13	44 T	54	63	51
52	24	23	18	39	20	9	32	64	17
25	25	47	14	2	45	57	46	56	45
54	5	70	41	42	10	33	11	35	61

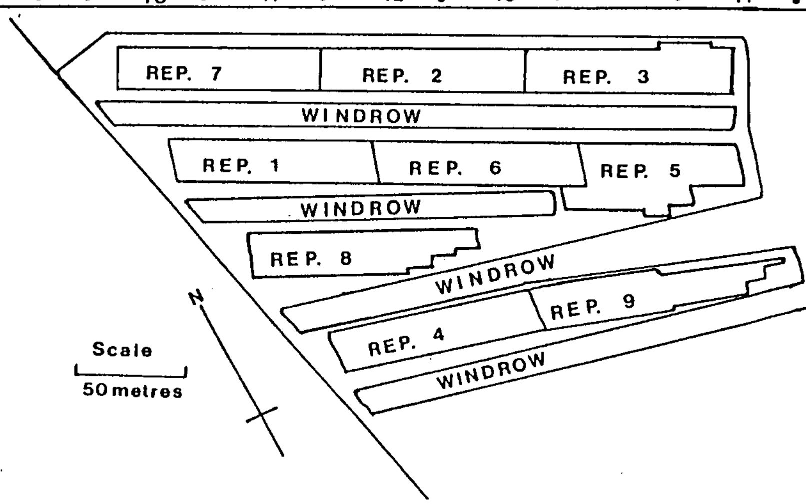


Fig. 3.4 The layout of replicates at the Tarraleah provenance trial. The positions of provenances in Rep. 2 are shown. Underlined numbers indicate the provenances which were sampled in this study. T indicates the locations of maximum-minimum thermometers.

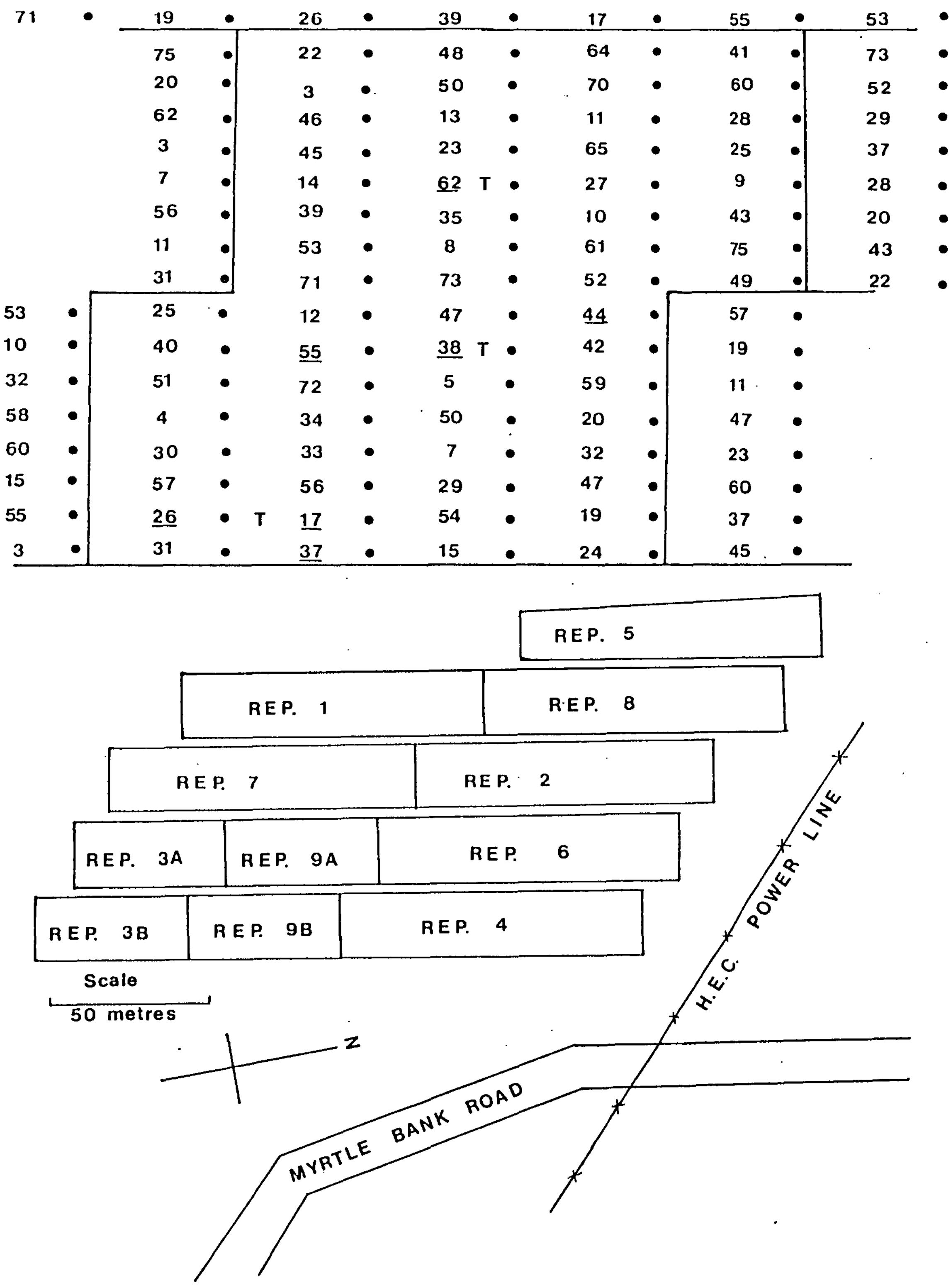


Fig. 3.5 The layout of replicates in the Myrtle Bank provenance trial. The positions of provenances in Rep. 9A and B are shown. Underlined numbers indicate provenances which were sampled in this study. T indicates locations of maximum-minimum thermometers.

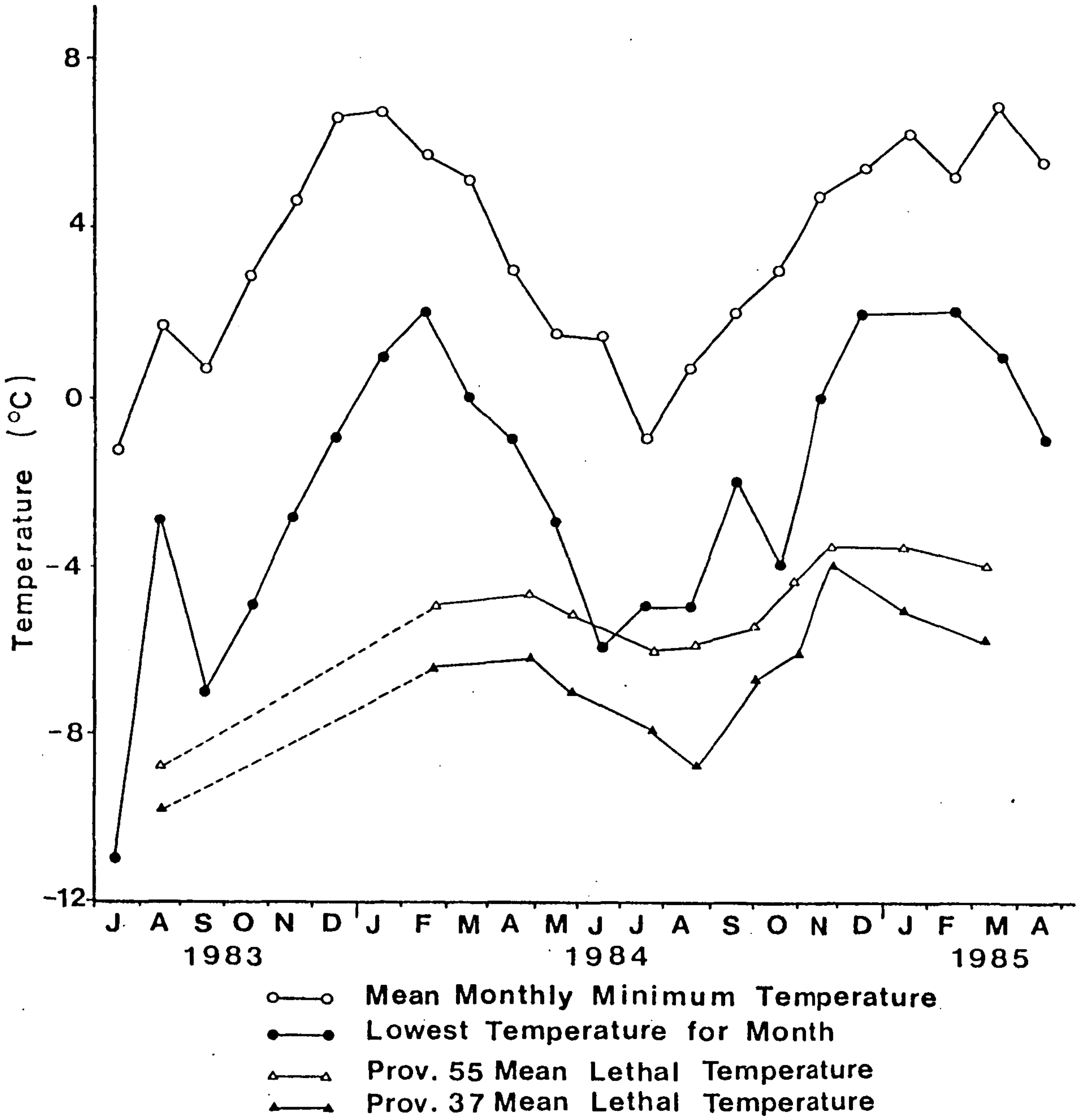


Fig. 3.6 Seasonal variation in mean lethal temperature of Bicheno (55) and Ben Lomond (37) provenances at Tarraleah provenance trial. Mean monthly minimum temperature and extreme minimum for the month are from the Meteorological Station at Tarraleah Village.

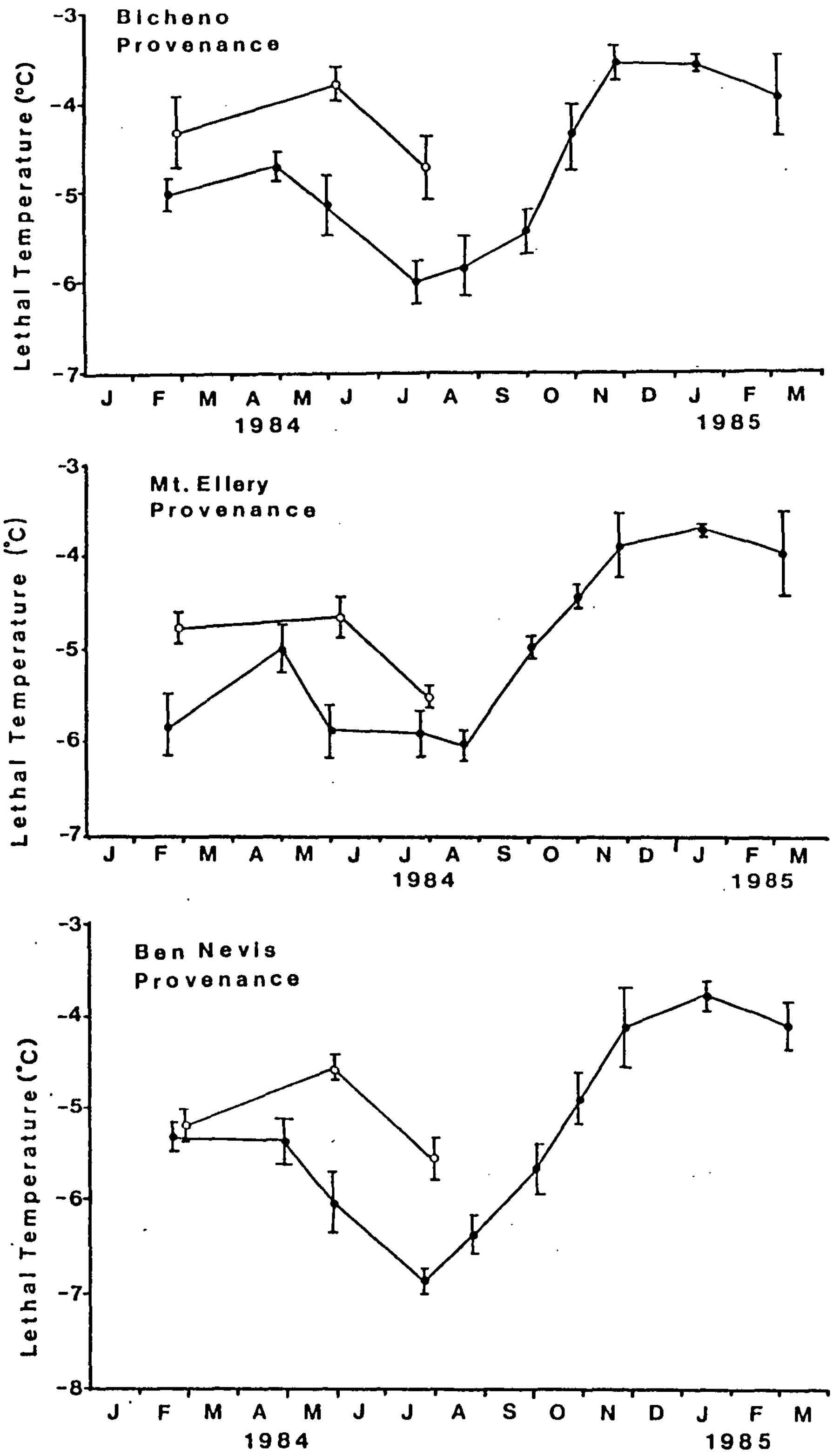
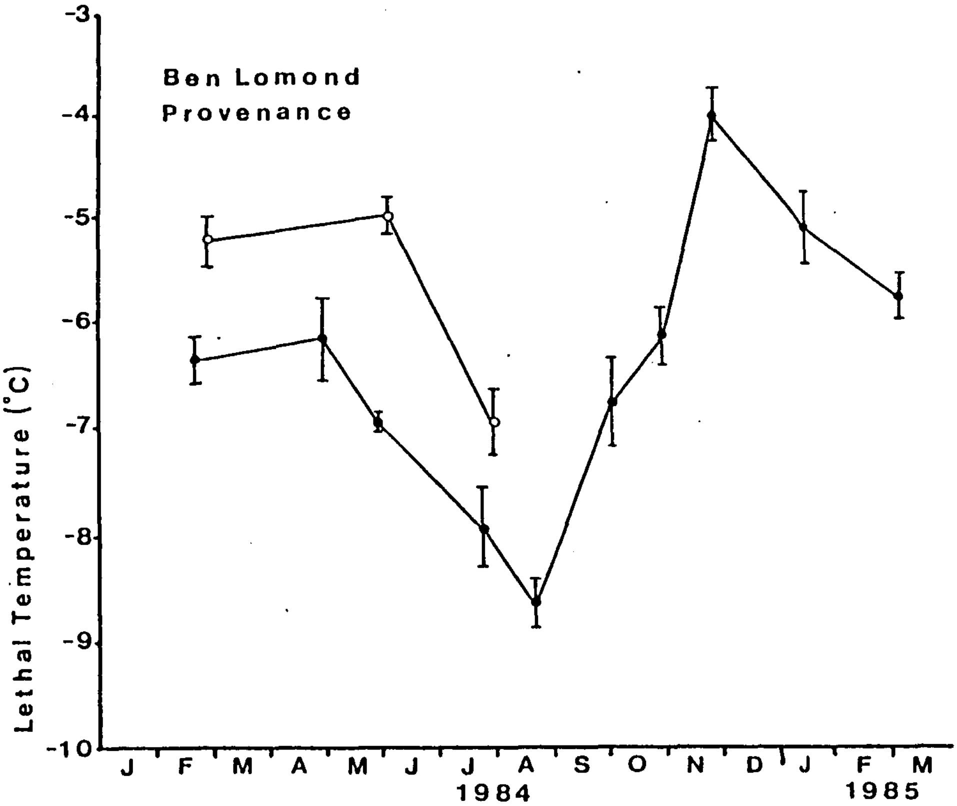


Fig. 3.7(a) Seasonal variation in mean lethal temperature for Bicheno, Mt. Ellery and Ben Nevis provenances at Tarraleah (●——●) and Myrtle Bank (○——○). I represents the standard error of the mean of five trees.



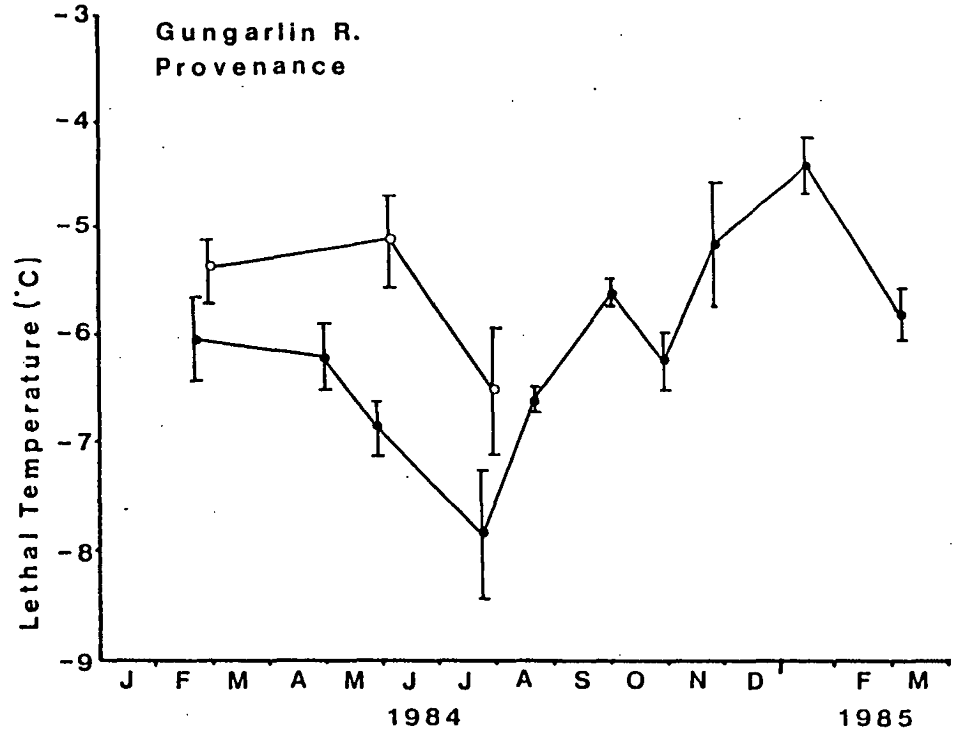


Fig. 3.7(b) Seasonal variation in mean lethal temperature for Ben Lomond and Gungarlin R. provenances at Tarraleah (●——●) and Myrtle Bank (○——○). I represents the standard error of the mean of five trees.

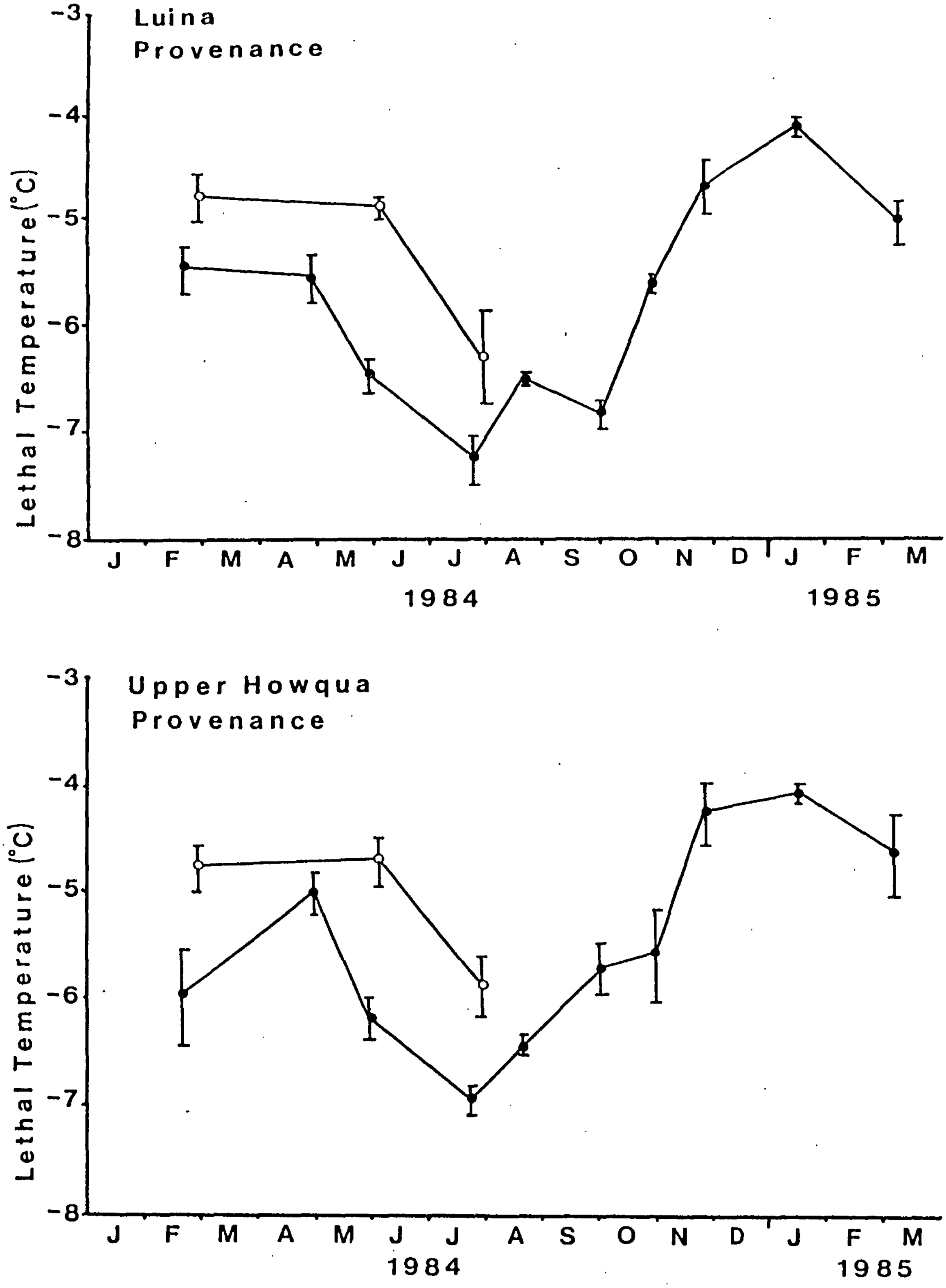
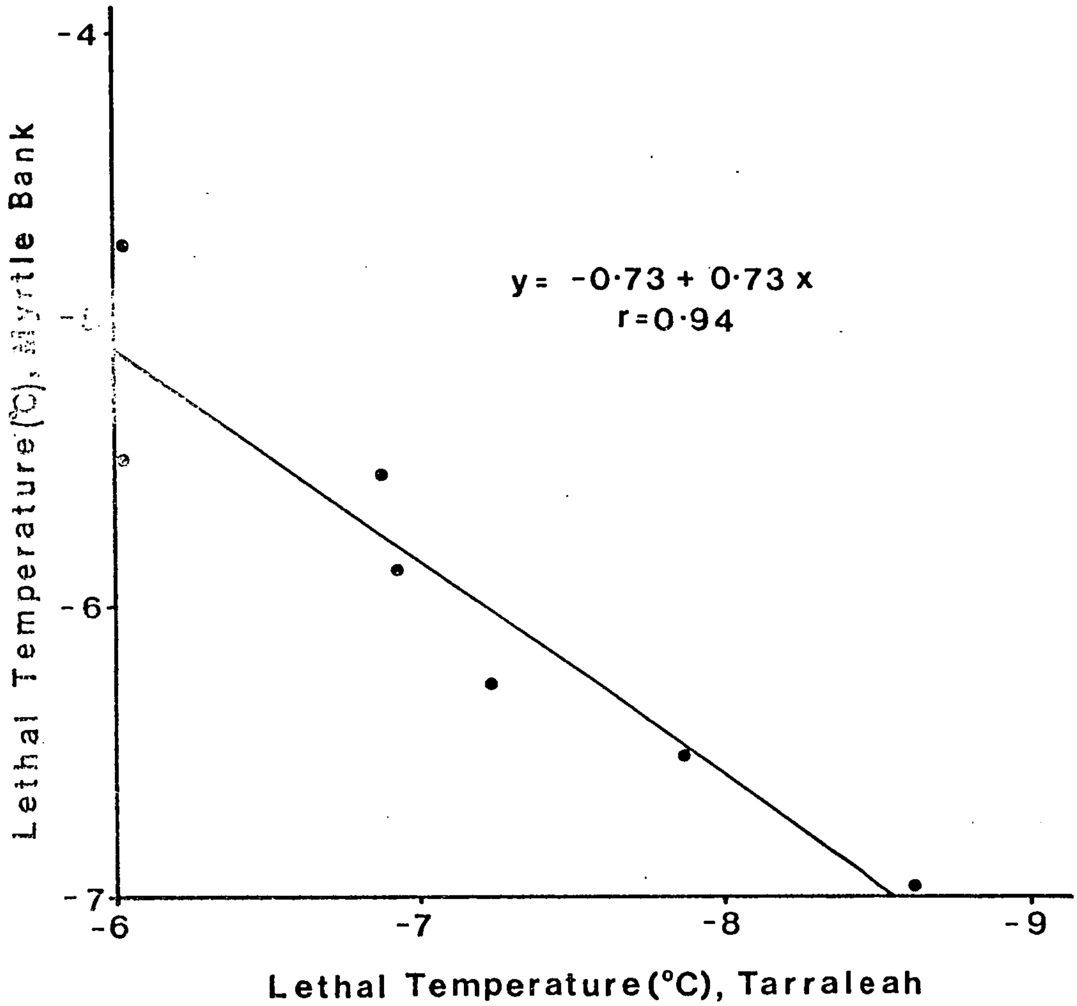


Fig. 3.7(c) Seasonal variation in mean lethal temperature for Luina and Upper Howqua provenances at Tarraleah (●——●) and Myrtle Bank (○——○). I represents the standard error of the mean of five trees.

Fig. 3.8 A tree of the Bicheno provenance at the Tarraleah provenance trial showing severe frost damage. This occured during a period of unusually low minimum temperatures (-10, -13 and -11°C) in the winter of 1983.

<u>Fig. 3.9</u> A 5-year-old tree of the Bicheno provenance at the Tarraleah provenance trial. Its main stem has been killed by frost and it has resprouted from the base of the stem.



<u>Fig. 3.10</u> The relationship between mean lethal temperature (n=5) at maximum hardiness of seven provenances at the Tarraleah and Myrtle Bank provenance trials.

CHAPTER 4

Hardening of seedlings in controlled environment chambers

Introduction

Differences in frost hardiness, expressed in terms of lethal temperature between summer and winter, of provenances of *E.delegatensis* ranged from 2.4°C for the least hardy (Bicheno) to 4.6°C for the most hardy (Ben Lomond, see Chapter 3). The main seasonal changes during the hardening period were decreasing day and night temperatures and shortening photoperiod. It has been demonstrated for other *Eucalyptus* species in controlled environments that low night temperatures are necessary for satisfactory development of winter frost hardiness. For example, Eldridge (1969) showed that a night temperature of 4°C was more effective than 7°C in inducing hardiness of seedlings of *E. regnans*. Similarly, Harwood (1980) showed that 4°C nights were more effective in stimulating the development of frost hardiness of *E. pauciflora* than 9°C nights.

The duration as well as the intensity of cold nights is also important. Harwood (1980) found that 3-4 weeks of nights at 4°C hardened the seedlings to -8.5°C while 7-8 weeks of nights at 4°C resulted in seedlings surviving temperatures of -10.0°C. Paton (1980) suggested that hardening is a much faster process than reported above and that exposure of seedlings to a constant temperature of 2°C for two days increased the frost hardiness of *E. viminalis* to survive temperatures of -6.5°C which would previously have killed them.

The facilities available for the measurement of temperature in the provenance trial at Tarraleah were not adequate to evaluate hardening and dehardening responses to an integrated measure of temperature. Experiments, described in this chapter, were therefore designed to measure the effect of different temperatures in controlled environments on the rate of frost hardening of *E.delegatensis* and their rate of dehardening when the hardened plants were placed in warm conditions.

Materials and Methods

Seeds were supplied by the CSIRO, Division of Forest Research Seed Centre. Seeds were stratified by placing them on moist vermiculite in a cold room at $4^{o}C$ for one month. They were then placed in the glasshouse (day $23^{\circ}C$/night $17^{\circ}C$) under natural light to germinate, pricked into individual 60 mm by 150 mm plastic pots in a standard potting mix (see Tibbits and Reid, 1986) and kept in the glasshouse until they had produced sufficient leaves that sampling would only remove a small proportion of the total number (height, 500 - 800 mm). Leaf tissue was tested for frost hardiness as described in chapter 2. The origins of the provenances used in these experiments are the underlined numbers shown in Fig.3.1. The growth chambers in which the plants were hardened had a combination of incandescent and fluorescent lights with an average intensity of 200 μ moles of quanta m^{-2} sec^{-1}. This was a low light intensity relative to natural light, however the plants were able to continue to grow in this environment.

1. Experiment 1

To test whether it was possible to artificially frost harden seedlings in growth chambers, seedlings of two Tasmanian provenances, Guildford (42) and Maydena (52) were subjected to the following day (10 h)/night (14 h) temperature regimes: 14/10 and 8/$4^{o}C$ with 10 hour photoperiods for 53 days. Nine seedlings of each provenance were treated at each temperature. Leaves from individual seedlings were tested separately.

2. Experiment 2

Seedlings of four provenances, Gungarlin R. (17), Ben Nevis (38), Luina (44) and Bicheno (55) were grown in the glasshouse until they were approximately 500mm high. Thirty seedlings of each provenance were selected for uniformity of size and six of each were placed in growth cabinets with 10 hour photoperiods and the following day/night temperature regimes: 16/12, 12/8, 8/4, 4/0 and +2/-2 ^{o}C. These were chosen to have a $4^{o}C$ difference between day and night. Using the method described in chapter 2 they were tested for frost hardiness before treatment and after 3 days and then at 1, 3 and 5 weeks of treatment. Samples for frost testing were bulked for each treatment i.e. samples of 6 discs were made up of one disc from a leaf of each seedling of a provenance within a treatment.

3. Experiment 3

Seedlings of six provenances, Gungarlin R. (17), Ben Lomond (37), Ben Nevis (39), Upper Howqua (26), Luina (44) and Bicheno (55) were grown in the glasshouse until they were approximately 600 mm high. Twenty-four seedlings of each provenance were selected for uniformity of height and six of each were placed in growth cabinets with 8 hour photoperiods and the following day/night temperature regimes: 12/12, 12/4, 12/0 and 4/4°C. These were tested using the method described in chapter 2 at 0, 2, 4, 6, 8 and 10 weeks of treatment. At 10 weeks the seedlings in the 12/0°C regime were moved in with the ones in the 12/12°C treatment and these two groups of seedlings were retested after 4 weeks to determine whether dehardening was occurring. As in experiment 2, samples were combined within each provenance in a treatment.

4. Experiment 4

Seedlings of 3 provenances, Gungarlin R. (17), Ben Lomond (37) and Bicheno (55) were grown in the glasshouse until they were approximately 800 mm high. Five seedlings of each provenance were moved to growth cabinets with 9 hour photoperiods and day/night temperature regimes of 12/12, 12/4 and 12/0°C. They were tested for frost hardiness after 0, 2, 4, 6, 8 and 10 weeks of treatment. All plants were then moved into the 12/12°C treatment and tested after further periods of 2 and 4 weeks had elapsed. Five extra seedlings of Ben Lomond provenance were placed in the 12/0°C regime at week 0 and tested for frost hardiness after 10, 12 and 14 weeks of treatment. Each seedling was tested individually.

Results

1. Experiment 1

Seedlings of both provenances showed significantly greater frost hardiness in the 8/4°C treatment than in the 14/10°C treatment (Table 4.1) and it was concluded that the controlled environment facilities available were adequate to induce hardening and to distinguish the ability of provenances to harden and deharden. In this instance the Guildford provenance developed significantly greater frost hardiness than Maydena provenance.

Table 4.1. Mean lethal temperatures for Guildford and Maydena provenances (42 and 52) after 53 days at 14/10 and 8/4°C. Analysis of variance gave the result that all values were significantly different ($P<0.01$).

Provenance	Treatment	
	14/10	8/4
Maydena	-3.8	-5.3
Guildford	-4.3	-6.5

2. Experiment 2

The lowest temperature regimes, 4/0 and +2/-2°C, which were expected to stimulate the greatest amount of hardening were found to be unsatisfactory, as low temperatures during the day caused the seedlings to wilt. This problem has also been reported by Tibbits and Reid (1986) who suggested that it may be caused by low root temperatures, as the wilting did not occur in these treatments if the roots were kept warmed to approximately 8°C. These treatments were discontinued prior to the three week sampling, when the plants in the 4/0 and +2/-2°C treatments had deteriorated to the extent that further measurements were not possible. The 16/12, 12/8 and 8/4°C treatments were continued for 5 weeks until cabinet breakdowns forced the ending of the experiment. During this time no significant hardening had occurred (Table 4.2).

Table 4.2. The mean lethal temperatures for seedlings of provenances Gungarlin R. (17), Ben Nevis (38), Luina (44) and Bicheno (55) treated at five different day/night temperature combinations over 5 weeks.

Day No.	Provenance No.	Day/Night Temperature (^oC)				
		16/12	12/8	8/4	4/0	+2/-2
0	17	-4.3	-4.4	-4.5	-4.4	-4.2
	38	-4.2	-4.5	-4.5	-4.4	-4.4
	44	-4.3	-4.5	-4.7	-4.4	-4.5
	55	-4.4	-4.4	-4.7	-4.3	-4.5
3	17	-4.2	-4.0	-4.3	-4.1	-4.3
	38	-4.2	-4.3	-4.3	-4.2	-4.4
	44	-4.0	-4.3	-4.3	-4.5	-4.7
	55	-4.3	-3.5	-4.5	-4.4	-4.3
7	17	-4.3	-4.4	-4.5	-4.1	-4.4
	38	-4.5	-4.3	-4.2	-4.4	-4.3
	44	-4.5	-4.1	-4.4	-4.3	-4.3
	55	-4.4	-4.2	-4.3	-4.4	-4.4
21	17	-4.3	-4.6	-4.7		
	38	-4.4	-4.1	-5.1		
	44	-4.4	-4.3	-4.6		
	55	-4.2	-3.7	-4.2		
35	17	-4.2	-4.4			
	38	-4.2	-4.3	-4.5		
	44	-3.5	-4.4	-4.2		
	55	-3.5	-3.5	-4.2		

3. Experiment 3

Over the 10 weeks, the six provenances all developed increased frost hardiness (Fig 4.1. (a and b)). The 12/0°C treatment had induced the most frost hardiness at the eighth week in all but one case (Bicheno provenance). The mean lethal temperatures of plants in the 12/4 and 4/4°C treatments were intermediate between those in the 12/12 and 12/0°C treatments over the first eight weeks (Table 4.3).

Table 4.3. Means of all lethal temperatures measured over the first 8 week period for each provenance at each day/night temperature treatment.

Day/night Temp. (°C)	Provenance Number 17	37	38	26	44	55
12/12	-5.1	-5.1	-4.8	-4.8	-4.5	-5.0
12/4	-5.3	-5.5	-5.4	-5.2	-5.8	-5.3
4/4	-5.3	-5.8	-5.5	-5.0	-5.6	-5.1
12/0	-5.9	-6.0	-5.7	-5.8	-6.0	-5.5

Analysis of variance gave a significant difference between provenances ($P<0.01$), between day/night temperature treatments ($P<0.001$), between sampling times ($P<0.001$) and the treatment-time interaction ($P<0.001$). The provenance-treatment and provenance-time interactions were nonsignificant.

The Ben Lomond and Gungarlin R. provenances hardened the most and the Bicheno and Luina provenances the least with the Ben Nevis and Upper Howqua intermediate (Fig.4.1(a and b)). The Bicheno and Upper Howqua provenances reached maximum hardening at eight weeks but the others continued to harden up to ten weeks although the rate of hardening of the Luina provenance appeared to be decreasing.

The four weeks dehardening resulted in all of the provenances except the Ben Lomond and Bicheno provenances dehardening to within 0.3°C of the original 12/12°C treatment.

4 Experiment 4

The Bicheno provenance showed a high degree of variability at the beginning of the experiment, but at 4 weeks the seedlings in the 12/0°C treatmnent were over 1°C hardier than the other treatments. All seedlings except ther Ben Lomond ones in the 12/0°C treatment (Fig. 4.2) dehardened to some extent over the first 2 weeks. This may have been a response to the low light levels in the growth chambers.

After 4 weeks, seedlings of all provenances in the 12/0°C treatment were significantly hardier ($P<0.05$) than in the 12/12 or 12/4°C treatments (Table 4.4). At 6 weeks, mean lethal temperatures of seedlings of Gungarlin R. and Ben Lomond provenances were significantly different at each night temperature. For the Bicheno provenance the 12/4°C treatment was not significantly different to the 12/12°C treatment for the duration of the experiment apart from the initial value at week 0.

Table 4.4. Mean lethal temperatures of Ben Lomond (37), Gungarlin R.(17) and Bicheno (55) provenances which were treated to night temperatures of 12, 4 and 0°C and sampled at two week intervals.

Week No.	Provenance No.	Treatment (Day/night temperature °C) 12/12	12/4	12/0
Hardening Phase				
0	37	-3.96 aD	-4.32 aD	-3.88 aD†
	17	-4.08 aD	-4.38 aD	-4.48 bD
	55	-3.66 aD	-4.16 aE	-4.36 bE
2	37	-3.38 aD	-3.38 aD	-4.30 aE
	17	-3.36 aD	-3.58 aD	-4.22 aE
	55	-3.28 aD	-3.36 aD	-4.10 aE
4	37	-3.96 aD	-4.18 bD	-5.32 aE
	17	-4.10 aD	-4.44 bD	-5.26 aE
	55	-3.96 aD	-3.50 aD	-5.12 aE
6	37	-4.14 aD	-4.80 bE	-5.42 abF
	17	-4.66 bD	-5.18 bE	-5.74 bF
	55	-4.08 aD	-4.18 aD	-5.24 aE
8	37	-4.85 bD	-4.98 bD	-5.98 bE
	17	-4.68 bD	-5.64 cE	-6.06 bE
	55	-3.96 aD	-4.30 aD	-5.44 aE
10	37	-4.68 bD	-4.82 aD	-5.76 bE
	17	-4.48 abD	-5.68 bE	-6.24 cF
	55	-4.02 aD	-4.44 aD	-5.16 aE

Dehardening Phase				
12	37	-4.64 bD	-5.14 bE	-5.52 abE
	17	-4.82 bD	-5.50 bE	-5.86 bE
	55	-4.00 aD	-4.32 aD	-5.22 aE
14	37	-4.62 bD	-5.02 bD	-5.58 abE
	17	-4.86 bD	-5.56 cE	-5.76 bE
	55	-4.10 aD	-4.30 aD	-5.18 aE

† Different upper case letters indicate a significant difference ($P<0.05$) between treatments within a provenance. Diferent lower case letters indicate a significant difference ($P<0.05$) between provenances within a treatment. The 5% least significant difference for assessing pairwise differences between means is lsd = 0.47.

Maximum hardiness was reached at week 8 for the Ben Lomond and the Bicheno provenances but not for the Gungarlin R. provenance, However the values at weeks 8 and 10 were not significantly different (Fig.4.2). During the experiment some hardening of seedlings in the 12/12°C treatment occurred, however this was small (less than 0.8°C) compared with the 12/0°C treatment.

During the 4 weeks of dehardening after week 10 some but not complete dehardening occurred. Seedlings of Ben Lomond provenance which were left in the 12/0°C treatment after the other plants were removed continued to harden (Fig.4.2).

Discussion

The seedlings in Experiment 1 are the same provenances that were planted in the Esperance Valley (Chapter 5). The results from the growth chambers agree with those obtained by sampling from the field trial, in which Guildford provenance developed greater frost hardiness than Maydena provenance.

Although Experiment 2 was abandoned after 5 weeks, it did demonstrate that even at low day and night temperatures (4/0 and +2/-2°C) *E. delegatensis* does not develop significant, measurable increases in frost hardiness within days as reported by Paton (1980) for *E. viminalis*. It is not surprising that no measurable hardening occurred in the other treatments (16/12, 12/8 and 8/4°C) in five weeks since treatments with 4°C nights in Experiments 3 and 4 also showed little or no hardening in that period.

Experiment 3 showed that colder nights result in greater frost hardiness and demonstrated that day temperature is not important in the development of frost hardiness since there was no significant difference ($P > 0.05$) between the 12/4 and the 4/4°C treatments. The provenances were ranked for maximum frost hardiness essentially in the same order as at the Tarraleah field trial. Dehardening was found to be faster than hardening as was expected from the results of Aronsson (1975) and Harwood (1981). In this experiment ten weeks hardening at 12/0°C followed by four weeks dehardening at 12/12°C resulted in four of the six provenances dehardening to within 0.3°C of the plants in the original 12/12°C treatment. This suggests that dehardening is approximately twice as fast as hardening for *E. delegatensis*.

This experiment was repeated (Experiment 4) using samples from individual seedlings but fewer provenances. Experiment 4 confirmed that frost hardening is a relatively slow process in *E. delegatensis*, requiring a minimum of two weeks of cold nights (i.e. 0°C) to achieve 1°C of hardening. Maximum hardening rates occurred at between two and four weeks of treatment at 12/0°C for the three provenances. All three provenances had a maximum hardening rate of 0.07°C/day which was low compared with reported rates for other species (Table 4.5).

Table 4.5. Rates of frost hardening of different species (from Greer,1983)

Source	Species	Day/Night Temp. (°C)	Rate of hardening (°C day^{-1})
Timmis & Worrall (1975)	*Pseudotsuga menziessii*	2.0/1.5	-0.07 to -0.14
Christersson (1978)	*Pinus silvestris*	2.0/2.0	-0.5
"	*Picea abies*	2.0/2.0	-0.8
Greer (1983)	*Pinus radiata*	14.5/1.3	-0.182
"	" "	14.5/-1.0	-0.286
Harrison *et al* (1978)	*Cornus stolonifera*	?/-5.0	>-4.0
Paton (1980)	*Eucalyptus viminalis*	2.0/2.0	-2.0?
Harwood (1981)	*Eucalyptus pauciflora*	18/4	-0.25
Tibbits and Reid (1986)	*Eucalyptus nitens*	13/3	-0.07
Hallam (this study)	*Eucalyptus delegatensis*	12/0	-0.07

The two Tasmanian provenances (Ben Lomond and Bicheno) appeared to respond differently to the New South Wales one (Gungarlin R.) in that the 12/4°C treatment had more effect on mean lethal temperature for the Gungarlin R. provenance than the others. This suggests that the Tasmanian provenances require colder nights to stimulate the development of frost hardiness.

All the experiments described in this chapter were done using short photoperiods (8-10 h) corresponding to winter daylengths in Tasmania. Further work on the control of frost hardiness is required to determine whether photoperiod is important for frost hardening of eucalypts. The difference in relative frost hardiness of *E. delegatensis* provenances planted in New South Wales and in Tasmania may be due Tasmanian provenances being adapted to respond to shorter daylengths (Chapter 3). Short days are known to be necessary for the development of frost hardiness in many Northern hemisphere species (Aronsson, 1975; Christersson, 1978; Greer and Warrington, 1982). Harwood (1981) reported that long days did not prevent development of frost hardiness in *E. pauciflora*, although short days may enhance it as reported for high altitude provenances of *E. regnans* (Eldridge, 1969). Eldridge did not find a significant response in low altitude provenances of *E. regnans*.

In these experiments it was only possible to test the effect of fixed night temperatures. In attempting to simulate field conditions it would be more appropriate to test the effect of a sequence of progressively colder nights with correspondingly shortening daylength. Greer and Warrington (1982) attempted this with *Pinus radiata* by shortening the daylength at 11 day intervals and decreasing the night temperature at 21 day intervals. They also imposed a series of nonlethal frosts on some plants. Plants which were treated with a final series of night temperatures of 2°C plus a series of frosts to -4°C were found to have hardened to -19°C compared to those with 2°C nights but no frosts which only hardened to -11°C. Unfortunately they did not have a treatment without the sequential drop in temperature for comparison.

Experiment 3 showed that for most provenances dehardening was virtually complete within four weeks which is consistent with reports that dehardening is a faster process than hardening (Aronsson, 1975; Paton, 1979; Harwood, 1981). In the field a period of cold nights (<4°C) may be interrupted by one or more warmer nights (>4°C) which, depending on the number of warm nights, may cause the plant to begin dehardening. The minimum number of warm (>4°C) nights required to stimulate dehardening thus may be critical for plant survival in the field and requires further investigation.

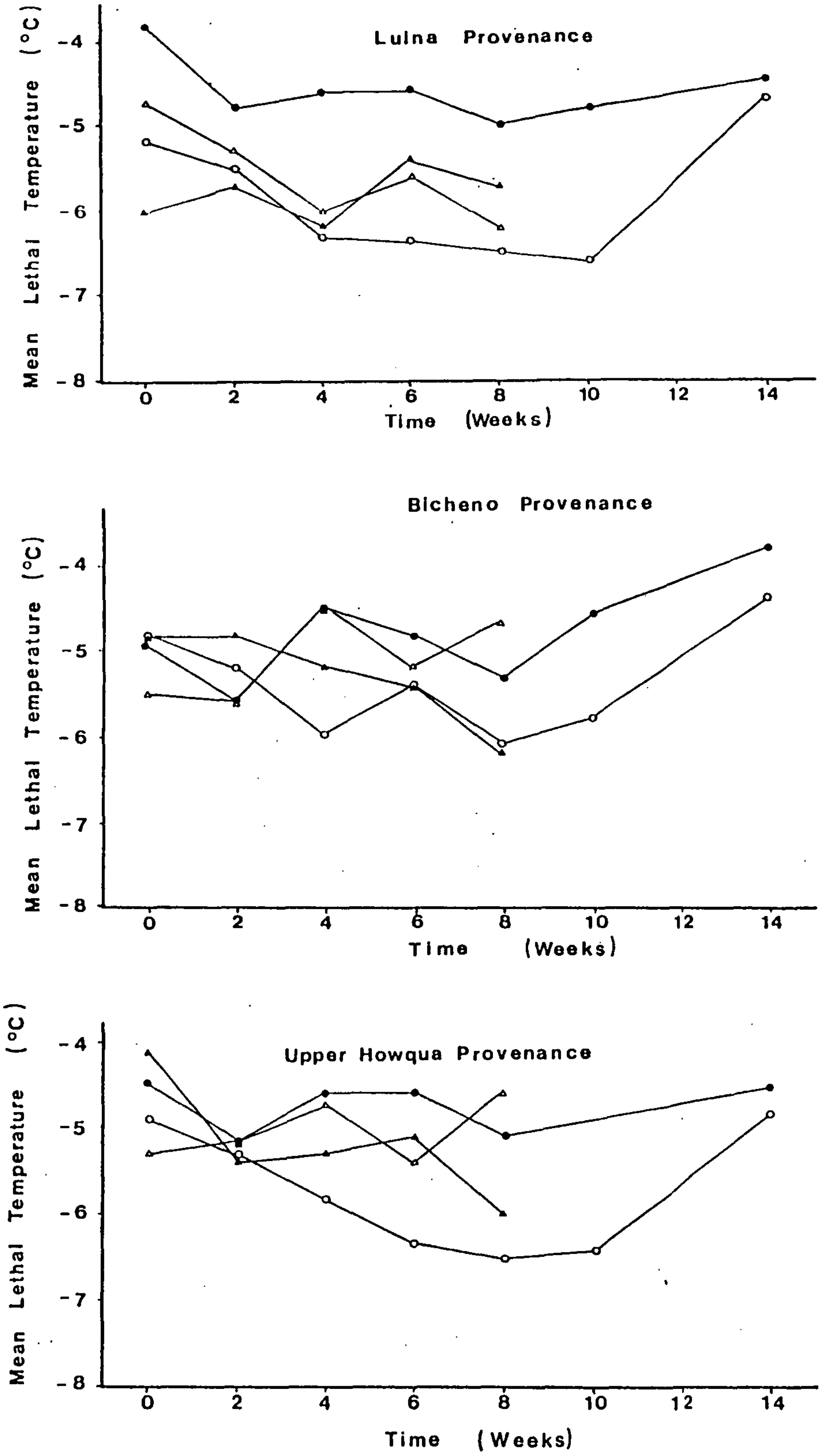


Fig. 4.1(a) Change in mean lethal temperature over 10 weeks of the following day/night temperature regimes, 12/12°C (●—●), 12/0°C (○—○), 12/4°C (▲—▲) and 4/4°C (△—△). At week 10, the seedlings in the 12/0°C treatment were moved into the 12/12°C treatment.

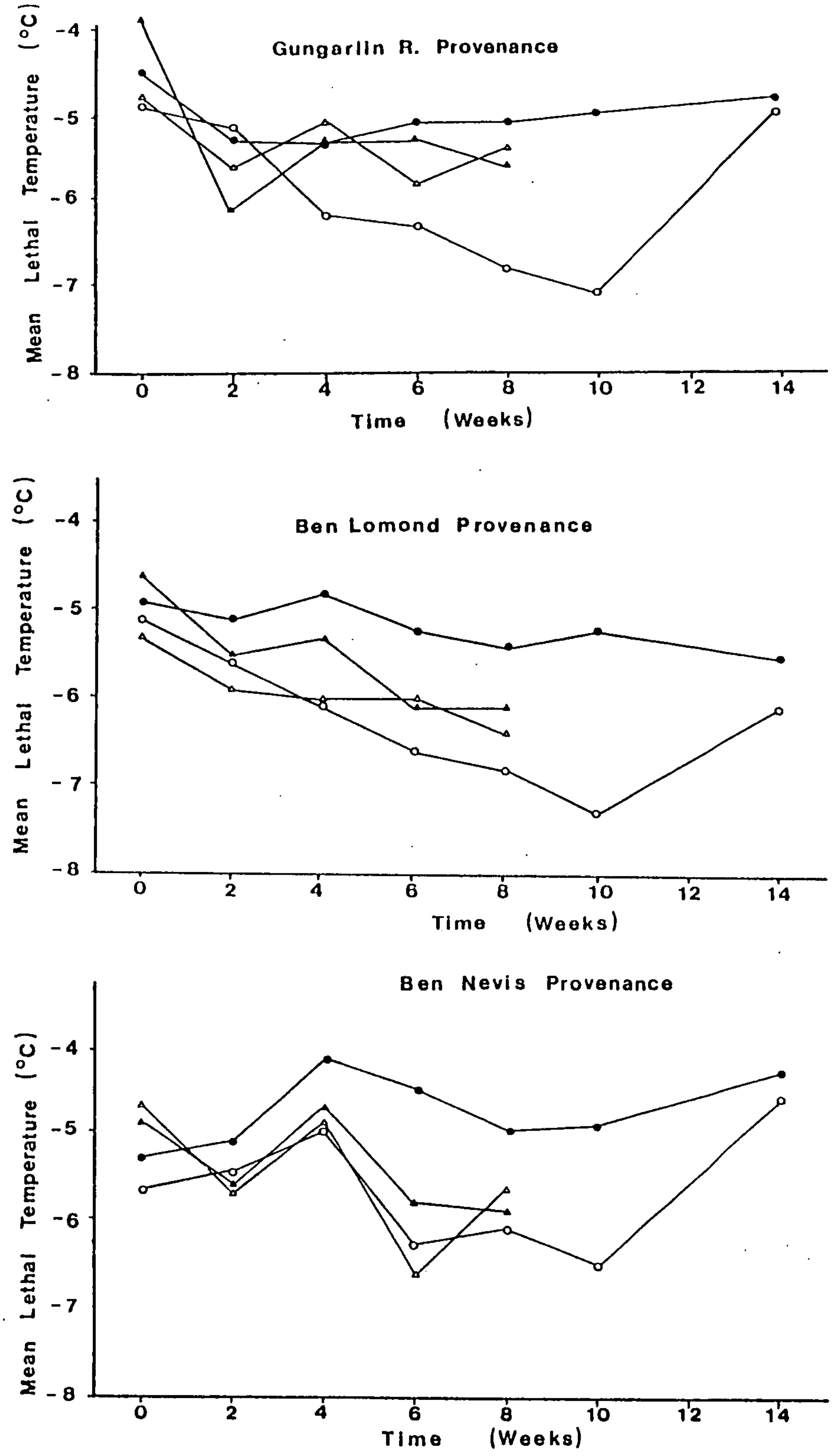
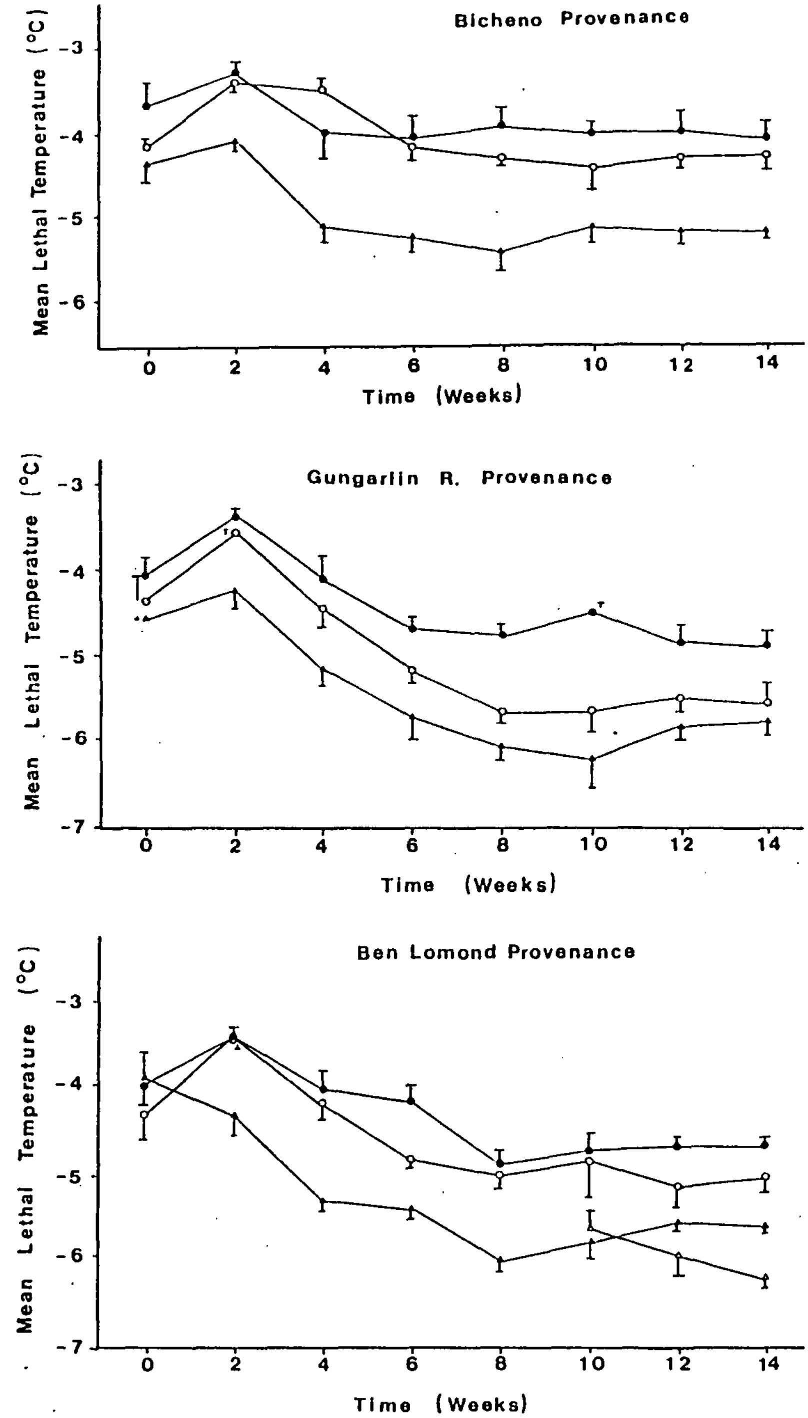


Fig. 4.1(b) Change in mean lethal temperature over 10 weeks of the following day/night temperature regimes, 12/12°C (●—●), 12/0°C (○—○), 12/4°C (▲—▲) and 4/4°C (△—△). At week 10 the seedlings in the 12/0°C treatment were moved into the 12/12°C treatment.



<u>Fig. 4.2</u> Change in mean lethal temperature over 10 weeks of the following day/night temperature regimes, 12/12°C (●——●), 12/4°C (○——○) and 12/0°C (▲——▲ and △——△). At week 10, the seedlings from the 12/4°C and 12/0°C treatments were moved into the 12/12°C treatment. Seedlings in the 12/0°C treatment indicated by △——△ were left in the 12/0°C treatment for a further 4 weeks.

Ⅰ represents the standard error of the mean of 5 seedlings.

CHAPTER 5

Frost hardiness of *E. delegatensis* compared with other species

Introduction

Comparative studies of the frost hardiness of different *Eucalyptus* species have been done previously but these have generally been limited to observations of damage following a frost in the field (e.g. Mullin and Barnes, 1977; Davidson and Reid, 1985). In the latter study, species ranking according to hardiness following a severe frost at Snug Plains, Tasmania was *E. gunnii* > *E. coccifera* > *E. johnstonii* =/> *E. delegatensis* > *E. pulchella* and their natural distribution was closely related to the distribution of minimum temperature.

Other attempts have been made to rank the species for hardiness based on their performance when planted on more severe sites overseas (Martin, 1948; Linnard, 1969; Hunt and Zobel, 1978). Marien (1979) has tested several *Eucalyptus* species in France, including *E. delegatensis*, to select frost hardy species for plantations. In New Zealand, Menzies *et al* (1981) found that *E. fastigata* was more frost hardy than *E. regnans* which was more frost hardy than *E. saligna* with the differences being greatest in winter. However both Harwood (1980) and Paton (1981) have examined the relative hardiness of species and their seasonal variability using artificial frosting.

It is important to know which species of *Eucalyptus* can be grown on different sites both locally and overseas particularly where low temperature is a feature of their growth cycle. In this study, samples were taken from two provenances of *E. delegatensis*, *E. regnans*, *E. globulus* and *E. nitens* and a single provenance of *E. grandis* and *E. pauciflora* which had been planted at four different altitudes in the Esperance Valley. These experiments form part of a wider study into the response of each eucalypt species to different climatic conditions.

It was anticipated that frost hardiness of all species would follow the altitudinal sequence with lowest mean lethal temperature at the highest site (650 m). Further, based on the results of Martin (1948), Linnard (1969) and McKimm and Flynn (1979) it was expected that *E. pauciflora*, *E. nitens* and *E. delegatensis* would be significantly more frost hardy than *E. regnans*, *E. globulus* and *E. grandis*. Results from Chapter 3 suggested that there would also be differences between provenances of each species.

Materials and methods

1. Experimental sites

Four experimental eucalypt plantations have been established in the Esperance valley (Fig. 5.1). The sites, numbers 1, 2, 3 and 4 are at altitudes of 60 m (Fig. 5.2(a)), 240 m (Fig. 2(b)), 440 m (Fig 5.2(c)) and 650 m (Fig. 5.2(d)), respectively. Two provenances each of *E. regnans*, *E. nitens*, *E. globulus* and *E. delegatensis* have been planted at each site. One provenance of *E. grandis* has been planted at sites 1 and 2 and one provenance of *E. pauciflora* has been planted at sites 3 and 4. All trees were planted in August 1983. Each site has a meteorological station with maximum and minimum thermometers in a Stevenson Screen which were read weekly.

2. Experimental material

The seedlings were planted in blocks at 2 m x 2 m spacings, with a central group for nondestructive sampling and a buffer area of 4 rows of plants (Fig. 5.2). All destructive sampling including the removal of leaves for frost testing was taken from the buffer trees.

The species belong to two groups within the genus, *E. regnans*, *E. delegatensis* and *E. pauciflora* belong to the informal subgenus

Monocalyptus and *E. globulus*, *E. nitens* and *E. grandis* belong to the informal subgenus *Symphyomyrtus* (Pryor and Johnson, 1971). The species and provenances were chosen because of their different early growth patterns, resistance to stress and performance in the field. The origins of these species are shown in Table 5.1.

Table 5.1. The locality and altitude of seed collected for planting at the four experimental sites in the Esperance Valley.

Species	Code	Origin	Altitude(m)
E. regnans	A1	Moogara,Tas	550
	A2	Traralgon Ck,Vic	500
E. globulus	B1	Geeveston,Tas	200
	B2	Otways,Vic	125
E. delegatensis	C1	Guildford,Tas	580
	C2	Maydena,Tas	650
E. nitens	D1	Mt.Toorongo,Vic	900
	D2	Bendoc,Vic	1070
E. pauciflora	E	Ben Lomond,Tas	500
E. grandis	F	Coffs Hbr,NSW	135

3. Experimental procedure

Samples from all provenances for the measurement of frost hardiness were taken on 11 and 18 March 1985 and again on 12 and 19 August 1985. Six leaves were taken from each of four trees of each provenance and tested using the electrical conductivity method described in Chapter 2. Each sample was replicated once. The heights of all the trees in the central block of each species were measured in August 1985 to give a mean height for each provenance of each species.

4. Treatment of results

To find out whether frost hardiness can be predicted from temperature records, the relationship between weekly minimum temperatures from the Stephenson screens and mean lethal temperature was examined. A thermal time value, t_{th}, was calculated for each site by summing the differences between the weekly minimum temperatures for the period 18th March and 12th August 1985 and a threshold minimum temperature of 5°C. Thus,

$$t_{th} = \Sigma(5 - t_{min}) \quad (^{o}C \text{ week})$$

The threshold temperature was set at 5°C because previous studies have shown that night temperatures of less than 5°C are required for commencement of hardening whereas dehardening commences at 6°C (Paton, 1980; Harwood, 1980; Tibbits and Reid, 1986).

Results

1. Seasonal Response

Analysis of variance showed that the mean lethal temperature measured in March was significantly higher than in August for all species ($P < 0.001$) (Fig. 5.4). The provenances of *E. regnans* had the smallest differences in lethal temperature between seasons of all the species and the Traralgon Crk provenance at Site 2 had a higher lethal temperature by 0.5°C in August than in March. However this difference was not statistically significant. For both provenances at the other sites the difference in mean lethal temperature ranged from 0.8°C to 2.1°C.

The provenances of *E. delegatensis* behaved differently to each other. The Guildford provenance had a minimum seasonal difference of 2.3°C at site 2 and a maximum seasonal difference of 3.3°C at site 4. The Maydena provenance had a minimum difference of 1.6°C at site 4 and a maximum difference of 2.4°C at site 1.

For *E. globulus* and *E. nitens* the difference in mean lethal temperature in March and August at all sites was in the range 1.6 to 2.4°C and 2.0 to 3.3°C respectively.

For *E. pauciflora* the difference in mean lethal temperature was 2.4°C at site 3 and 1.9°C at site 4 while for *E. grandis* the difference was 1.7°C at site 1 and 1.3°C at site 2.

2. Species differences

In March, the distribution of mean lethal temperatures was such that analysis of variance gave no significant differences between species or between sites ($P>0.05$). In contrast, there were significant differences in mean lethal temperatures in August (Fig. 5.4).

The least hardy species was *E.regnans* (Fig.5.5). Although its mean lethal temperatures had not been significantly different to the other species in March, in August it consistently had the highest mean lethal temperatures at every site. The highest mean lethal temperature in August was -4.5°C for the Traralgon Crk provenance at site 2 while the lowest was -7.0°C for the Moogara provenance at site 1.

Taking each species in order of ranking, the next most hardy species was *E. grandis* with a mean lethal temperature of -7.2°C at site 1 and -6.3°C at site 2, followed by *E. globulus* which had its highest lethal temperature, -6.0°C for the Otway provenance at site 2 and its lowest at -7.9°C for the Geeveston provenance at site 1. *E. pauciflora* at sites 3 and 4 ranked between *E. globulus* and *E. nitens* with a mean lethal temperature of -7.5°C at site 3 and -7.4°C at site 4. *E. nitens* had its highest mean lethal temperature, -7.2°C, at site 2 for Mt Toorongo provenance and its lowest, -8.1°C, at site 1 for Bendoc provenance.

E. delegatensis was the only species to have a significant difference between provenances in August. For the less frost hardy provenance, Maydena, mean lethal temperature was in the range -6.6 to -8.1°C and for Guildford provenance, -7.3 to -8.7°C. The mean lethal temperature of the more frost hardy provenance, Guildford, was not significantly different from that of the *E. nitens* provenances.

3. Site differences

There was an altitudinal sequence of mean maximum temperatures with decreasing temperatures at increasing altitudes (Table 5.2). Mean minimum temperature however, did not follow this altitudinal sequence because of topography. Site 1 (60 m) is in a frost hollow and experienced similar temperature minima to site 4 (650 m) although its temperature maxima were similar to site 2 (240 m) (Table 5.2). Site 3 (440 m) experienced minimum and maximum temperatures intermediate to sites 2 and 4.

Table 5.2 Temperature data for the four experimental sites in the Esperance Valley from 12 August 1984 based 12 August 1985 on weekly maxima and minima in Stevenson Screens.

	Site			
	1	2	3	4
No. of weeks min. temp.<4°C	38	26	36	41
Coldest temp.(°C)	-2.9	-0.2	-1.7	-2.0
Mean minimum temp (°C)	2.0	4.0	2.7	2.1
No. of weeks max. temp. >15°C	44	36	30	24
Warmest temp.(°C)	29.6	32.1	31.2	29.5
Mean maximum temp (°C)	20.8	19.8	18.1	16.3

The level of frost hardiness developed during winter for all species corresponded in general to the pattern of minimum temperatures experienced at the four sites. Lowest mean lethal temperatures were measured at sites 1 (60 m) and 4 (650 m), highest mean lethal temperatures occurred at site 2 (240 m) and mean lethal temperatures at site 3 (440 m) intermediate (Fig. 5.4).

Height and diameter growth corresponded to the pattern of maximum temperatures which were greatest at sites 1 and 2 (Table 5.3). *E. globulus* and *E. nitens* grew best at site 2, while *E. regnans* and *E. delegatensis* and *E. grandis* grew best at site 1 (Tables 5.3 and 5.4).

Table 5.3 Mean tree heights (cm) for each provenance and species at the experimental sites measured on 13 August 1985 (age 2 years ,n = 60).

Species	Provenance	Site			
		1	2	3	4
E. regnans	A1	305	185	137	122
	A2	376	218	112	82
E. globulus	B1	281	357	225	108
	B2	397	405	262	133
E. delegatensis	C1	291	202	146	163
	C2	240	109	110	123
E. nitens	D1	311	325	256	239
	D2	290	298	241	188
E. pauciflora	E	-	-	70	127
E. grandis	F	270	242	-	-

Table 5.4. Mean diameter (cm) of stems at 1.3 m height for each provenance and species at all sites on 13th August 1985 (age 2 years, n = 60).

Species	Provenance	Site 1	Site 2	Site 3	Site 4
E. regnans	A1	1.933	0.67	*	*
	A2	2.88	1.04	*	*
E. globulus	B1	1.95	2.98	1.44	*
	B2	3.33	3.42	1.82	*
E. delegatensis	C1	1.88	0.79	*	*
	C2	1.18	*	*	*
E. nitens	D1	2.85	2.60	1.82	1.54
	D2	1.76	1.78	1.21	0.63
E. pauciflora	E	-	-	*	*
E. grandis	F	1.65	1.32	-	-

* denotes mean height less than 1.3 m.

5. Relationship between mean lethal temperatures and screen minimum

Since *E. delegatensis* was the only species with a significant difference in mean lethal temperature between provenances in August, two regressions, with four data points in each, were calculated for this species. For each of the other species one regression was calculated using the data points for both provenances (eight points). For *E. nitens* a poor correlation ($r^2 = 0.40$) was obtained. The regression was repeated omitting the data from site 1, this increased r^2 to 0.78. Using this regression a value for mean lethal temperature at site 1 was calculated to be -8.5°C. This was 0.7°c lower than the measured value. The thermal time values (°C weeks) were 85 for site 1, 49 for site 2, 73 for site 3 and 75 for site 4.

The regression coefficients of the linear regressions calculated for mean lethal temperature against thermal time are shown in Table 5.5. Regressions for all species except *E. nitens* gave significant r^2 (P<0.01) and for *E. nitens* omitting site 1 data (P<0.05). Thus within the limits 49 to 85°C weeks temperature data can be used to predict mean lethal temperatures for these species. This allows the hardening regime as well as the extreme minimum at this site to be taken into account.

Table 5.5. Regression coefficients and r^2 for mean lethal temperature (Y) against thermal time (X) where Y = aX + b and n = number of points.

Species	Provenance	a	b	r^2	n	
E. regnans		-2.78	-0.046	0.76	8	
E. globulus		-4.10	-0.042	0.85	8	
E. delegatensis	Guildford	-5.88	-0.032	0.97	4	
	Maydena	-5.51	-0.022	0.96	4	
E. nitens		-6.37	0.020	0.40	8	
E. nitens		-5.53	-0.035	0.78	6	*

* data from site 1 omitted

Discussion

1. Seasonal response

The trees were sampled in March and August to obtain maximum seasonal differences in hardiness. Since all species had similar mean lethal temperatures in March those with the largest seasonal difference in mean lethal temperature were also those which became most hardy. However at Tarraleah significant differences between provenances of *E. delegatensis* were present even in summer (Chapter 3) and this clearly occurs between species in other studies (e.g. Menzies *et al*, 1981).

Although there was no significant difference in mean lethal temperature between species at the Esperance sites in March, the difference was highly significant in August (P<0.001). *E. regnans* showed the smallest seasonal response varying from no significant difference for the Traralgon Crk provenance at site 2 to 2.1°C for the Moogara provenance at site. The largest seasonal differences (3.3°C) were shown by the Guildford provenance of *E.*

delegatensis and the Bendoc provenance of *E. nitens*. To rank species in order of their frost hardiness it is necessary to sample when differences are greatest, which is usually in late winter (Rook *et al*, 1980; Menzies *et al*, 1981).

The seasonal difference in mean lethal temperature of *E.delegatensis* at Tarraleah (reported in Chapter 3) ranges from 2.4^{o}C for the Bicheno provenance to 4.6^{o}C for the Ben Lomond provenance. At the Esperance trial the minimum difference was 1.6^{o}C for Maydena provenance at site 4 and the maximum difference is 3.3^{o}C for Guildford provenance at site 4. The somewhat larger seasonal difference at Tarraleah appeared to be due to the plants dehardening more in summer rather than hardening more in winter.

2. Species Response

a. *E. regnans*

The lowest mean lethal temperature for *E. regnans* was -7.0^{o}C at site 1 whereas at site 4 the mean lethal temperature was -6.5^{o}C for Moogara provenance and -6.2^{o}C for Traralgon Crk provenance (Fig 5.4). Site 4 was the only site however, at which *E. regnans* suffered frost damage mainly to the young leaves although some trees have been killed. This was at first sight a surprising result since the minimum screen temperature for site 4, -2.0^{o}C, was greater than site 1, -2.9^{o}C. The leaves at site 4 remain wetter for longer periods and windspeeds are higher than at site 1. As a result leaves possibly experience lower temperatures than indicated by the screen temperatures. Secondly, sampling trees at site 4 which had survived frosts in winter 1984 may have led to an underestimation of mean lethal temperature for the population, as it was of course only possible to remove discs from the hardier trees which remained.

Nevertheless, the range of mean lethal temperatures in August across all sites, -7.0 to -4.5^{o}C, are in close agreement with those of Rook *et* al(1980). In this study seedlings of the most to least frost hardy seedlings were expected to survive frosts of -7.0 to -5.5^{o}C. It is interesting to note that Moogara was one of the most frost hardy (see also Menzies *et al*, 1981) while Traralgon Creek was one of the least frost hardy provenances (see Tables 7 and 12 in Rook *et al*, 1980). The extent to which tolerance to low temperature contributes to growth as well as survival is not clear, but at the Esperance sites, the more productive provenance at low elevation was Traralgon Creek while that at high elevation which are exposed to low mean temperature as well as low minimum temperatures, was Moogara (Table 5.3 and 5.4). Griffin *et al* (1982) categorised the Moogara provenance as frost hardy and fast growing, while the Traralgon Creek provenance was frost tender and fast growing (Fig. 2 in Griffin *et al* ,1982) based on results from provenance trials in Australia. An inherent resistance to low temperature where this is important for survival may result in better growth of that provenance at cooler sites than of a provenance which is less frost resistant. In *E. regnans*, perhaps not surprisingly, the provenances with the most frost resistance came from the upper altitudinal limits of the species (Ashton,1958; Eldridge, 1968; Rook *et al*, 1980). The gain in rates of biomass production which can be made by planting a particular provenance will be determined by the microclimate at the site.

b. *E. grandis*

The *E. grandis* was only planted at sites 1 and 2 because it was thought that it would not be frost hardy enough to survive at the higher altitude sites. The plants which were sampled proved to be slightly more frost hardy than those of *E. regnans* at both sites with a mean lethal temperature of -7.2°C at site 1. Many trees at site 1 have had extensive leaf and stem damage (Fig 5.8) due to frost although the recorded screen minima (minimum -2.9°C) are not as low as the measured lethal temperatures. The reasons for this apparent anomaly are not clear. In general specific differences in the frost tolerance of eucalypts tends to follow their natural distribution (Menzies *et al*,1981) and *E. grandis*, a native of New South Wales and southern Queensland from some studies would appear to be no exception (Hunt and Zobel, 1978; Meskimen and Franklin, 1978). There is evidence however that given suitable preconditioning, i.e. night temperatures of 4°C or lower for several weeks before the frost, *E. grandis* may not be damaged by temperatures as low as -8.8°C (in Canberra, Burgess, 1983) and -8.5°C (in laboratory conditions, Paton,1980). At the

Esperance, the leaves have developed a red coloration (Fig 5.9) which can persist throughout the year, a clear indication that secondary pigments are being synthesised in response to low temperature stress (see also Paton 1980). The development of frost resistance may not be sufficient to prevent damage to the leaves should there be a sudden frost following warm conditions. Further reduction in lethal temperature may occur subsequently but symptoms of leaf damage would still develop.

c. *E. globulus*

The provenances of *E. globulus* planted were from low altitude sources (125 and 200m), but they were able to harden more than those of *E. regnans* which came from sources at higher altitude (550 and 500m) (Fig. 5.5). *E. globulus* planted at site 4 has been badly damaged and some trees have been killed. Branches of this species appear to be very brittle at this altitude and many of the trees have been broken off or have lost branches through a combination of snow and strong winds.

d. *E. pauciflora*

This species did not harden as much as was expected from Harwood(1981), only reaching a mean lethal temperature of -7.5°C whereas Harwood reported that it was possible to harden *E. pauciflora* down to -14°C. There are two possible reasons for this large difference. First that the *E. pauciflora* from Ben Lomond (500 m altitude) in Tasmania may not be capable of hardening to the same degree as the provenance Harwood used which came from an altitude of 1260 m. Second, the provenance used may be capable of greater hardening if it is exposed to lower minimum temperatures. *E. pauciflora* grew better (Table 5.3) at site 4 than at site 3 in contrast to the other species which all grew better at lower altitudes.

e. *E. nitens*

E. nitens was, with *E. delegatensis*, the most frost hardy species at all sites. There was no evidence that *E. nitens* was more frost hardy than *E. delegatensis* although this has been suggested by Martin (1983). The height and diameter growth of *E. nitens* was better than that of *E.*

delegatensis at all sites with the difference increasing at the higher altitude sites (Table 5.3). Although there was no significant difference in frost hardiness between provenances of *E. nitens*, height and diameter growth of Mt Toorongo provenance was greater than for Bendoc provenance at all sites.

Beadle and Turnbull (1986) have suggested that the greater early growth of this *Symphyomyrtus* eucalypt compared with *E. delegatensis* from the *Monocalyptus* group is related to its ability to produce greater leaf area during the early growth period. Its success at both low and high altitudes in this experiment suggests that it can also maintain growth over a wide range of temperatures. It is interesting to note (C.R.A.Turnbull, pers. comm.) that *E. nitens* has been able to keep growing during the winter months while *E. delegatensis* has not. *E. nitens* is increasingly being used to replace *E. delegatensis* in Tasmania because of its better early growth and equivalent resistance to frost.

f. *E. delegatensis*

This was the only species with a significant difference in mean lethal temperature between provenances. The Guildford provenance was more frost hardy than the Maydena provenance although both provenances originate from similar altitudes, 580 and 650 m. In general, frost resistance of this species increases with altitude while conversely height growth is inversely related to altitude of origin (Marien, 1983). At the Esperance sites, the more frost resistant provenance also had the higher growth rate. Artificial hardening of seedlings of these two provenances in growth chambers (Chapter 4) gave similar results in that there was a significant difference in mean lethal temperature with Guildford provenance having a lower mean lethal temperature than Maydena provenance.

The frost hardiness of these two provenances was not measured at Tarraleah. The mean lethal temperatures measured at the Esperance sites were in the same range as for the *E. delegatensis* provenances at Tarraleah (Chapter 3). The least frost hardy provenance (Bicheno) had mean lethal temperatures of -5.9°C in August 1984 and -4.0°C in March 1985, while for the most hardy provenance (Ben Lomond) they were -8.6°C in August 1984 and -5.8°C in March 1985. Although there were lower minimum temperatures at Tarraleah (-8.0°C) this did not result in greater frost hardening. Possibly the temperatures leading up to the frosts were more suitable for development of frost hardiness at the Esperance sites.

The species can be ranked in order of decreasing frost hardiness as follows: *E. delegatensis* = *E. nitens* > *E. pauciflora* > *E. globulus* > *E. grandis* > *E. regnans*. It must be emphasised that the mean lethal temperatures presented here are specific to the temperature conditions that applied prior to the test and may change if different hardening temperatures occur. It is also possible that the species ranking for frost hardiness may change slightly if they are tested under different conditions.

3. Site Response

There was a significant difference in mean lethal temperatures between sites (P>0.001). Lowest mean lethal temperatures were measured at sites 1 (60 m) (-6.4°C) and 4 (650 m) (-6.3°C) and were not significantly different from each other. The highest mean lethal temperature was measured at site 2 (-5.5°C) and at site 3 it was intermediate (-6.1°C). The measured mean lethal temperatures corresponded to the pattern of minimum temperatures (Table 5.2) at the four sites.

Height and diameter growth (Tables 5.3 and 5.4) of all species (except *E. pauciflora*) were greatest at sites 1 and 2 corresponding to the highest maximum temperatures (Table 5.2).

It is apparent from this data that temperature conditions which promote growth are not necessarily incompatible with the development of frost hardiness, since plants became as frost hardy at site 1 as they did at site 4. This is supported by the data in Chapter 4 which demonstrated that day temperature did not influence development of frost hardiness. This shows that both good growth and productivity are possible on quite frost prone sites provided suitable provenances are selected.

4. Relationship between mean lethal temperature and thermal time

Evaluating the suitability of sites for new planting of eucalypts requires not only some measure of their potential for growth but also for survival. The use of "^{o}C week" to predict the mean lethal temperature is one approach. Provided a suitable threshold temperature is selected it may give satisfactory results, although the model was not tested independently.

The relationship between thermal time and mean lethal temperature was strongly linear over the range of the data (49 to 85 ^{o}C weeks) for all species except *E. nitens*. Using the regression equation which omitted data from site 1 a mean lethal temperature of -8.5^{o}C was calculated for site 1. This was 0.7^{o}C lower than the measured value and suggests that *E. nitens* did not harden as much as expected at site 1. This may have been caused by the species having already started to deharden in August at this site. It has already been noted that in contrast to the other species this species continues to grow during winter (C.R.A. Turnbull, pers. comm) and thus it may be able to respond more quickly to rising temperatures and commence dehardening earlier.

It is apparent that these calculated relationships between thermal time and mean lethal temperature are linear sections in a hardening curve. Since mean lethal temperatures in March do not correspond to the zero calculated from the regression, the curve before 49^{o}C weeks must be nonlinear. Similarly with increasing thermal time beyond 85 ^{o}C weeks the rate of hardening would be expected to level off as it reached the maximum capability of the plant to harden. *E. regnans* does not appear to be able to respond to a thermal time of less than 49^{o}C weeks since its mean lethal temperature is the same at that time as it was in March. In contrast the other three species do appear to be able to respond to a smaller thermal time, since their mean lethal temperatures at 49^{o}C weeks are lower than they were in March. This also suggests that *E. regnans* is more likely than the other species to be damaged by frosts early in the winter when it has not received sufficient stimulus to begin hardening.

Better relationships between mean lethal temperature and thermal time should be obtained if they are calculated on a daily basis rather than on a weekly basis. If temperature records for a site to be planted were available this relationship could be used to determine whether a particular species would be likely to harden sufficiently to survive the minimum temperatures at the site i.e. it provides a technique for determining the suitability of hardening conditions for the species or provenance to be planted.

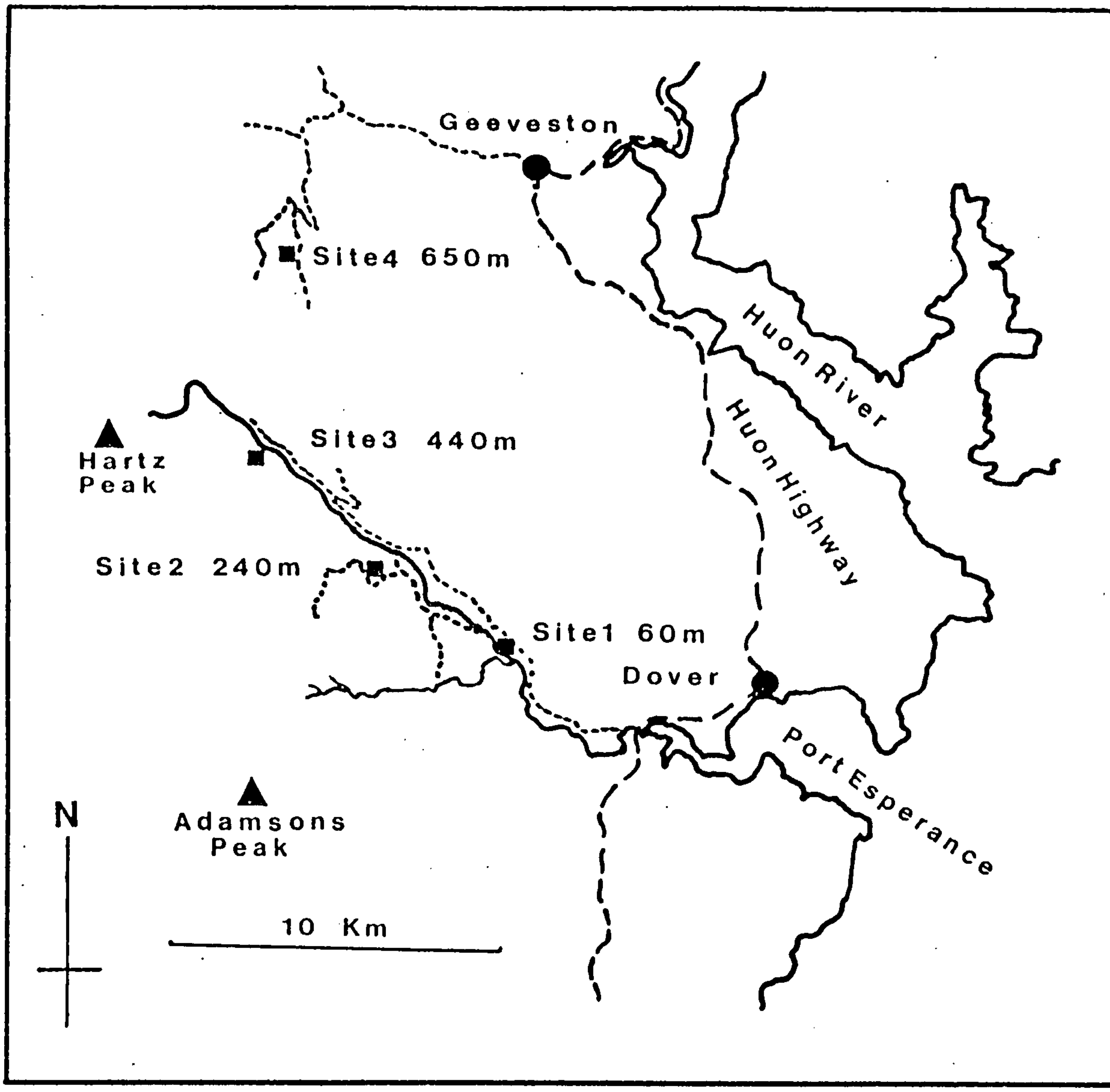


Fig. 5.1 Locations of the experimental sites in the Esperance Valley in Southern Tasmania (Lat. 43°,Long. 147°). Sites 1 to 4 (■) are at altitudes of 60, 240, 440 and 650 m respectively.

Fig. 5.2(a) Site 1 (60 m) showing the Maydena provenance of *E. delegatensis* (mean height, 2.40 m, age 2 years).

Fig. 5.2(b) Site 2 (240 m) with the Guildford provenance of *E. delegatensis* (mean height, 2.02 m) in the foreground. The Traralgon Crk provenance of *E. regnans* (mean height, 2.18 m) can be seen in the background. (age 2 years)

Fig. 5.2(c) Site 3 showing the Maydena provenance of E. delegatensis (mean height, 1.10 m) in the foreground. The Bendoc provenance of E. nitens (mean height, 2.41 m) can be seen in the background. (age 2 years)

Fig. 5.2(d) Site 4 (650 m) showing the Guildford provenance of *E. delegatensis* (mean height, 1.63 m, age 2 years)

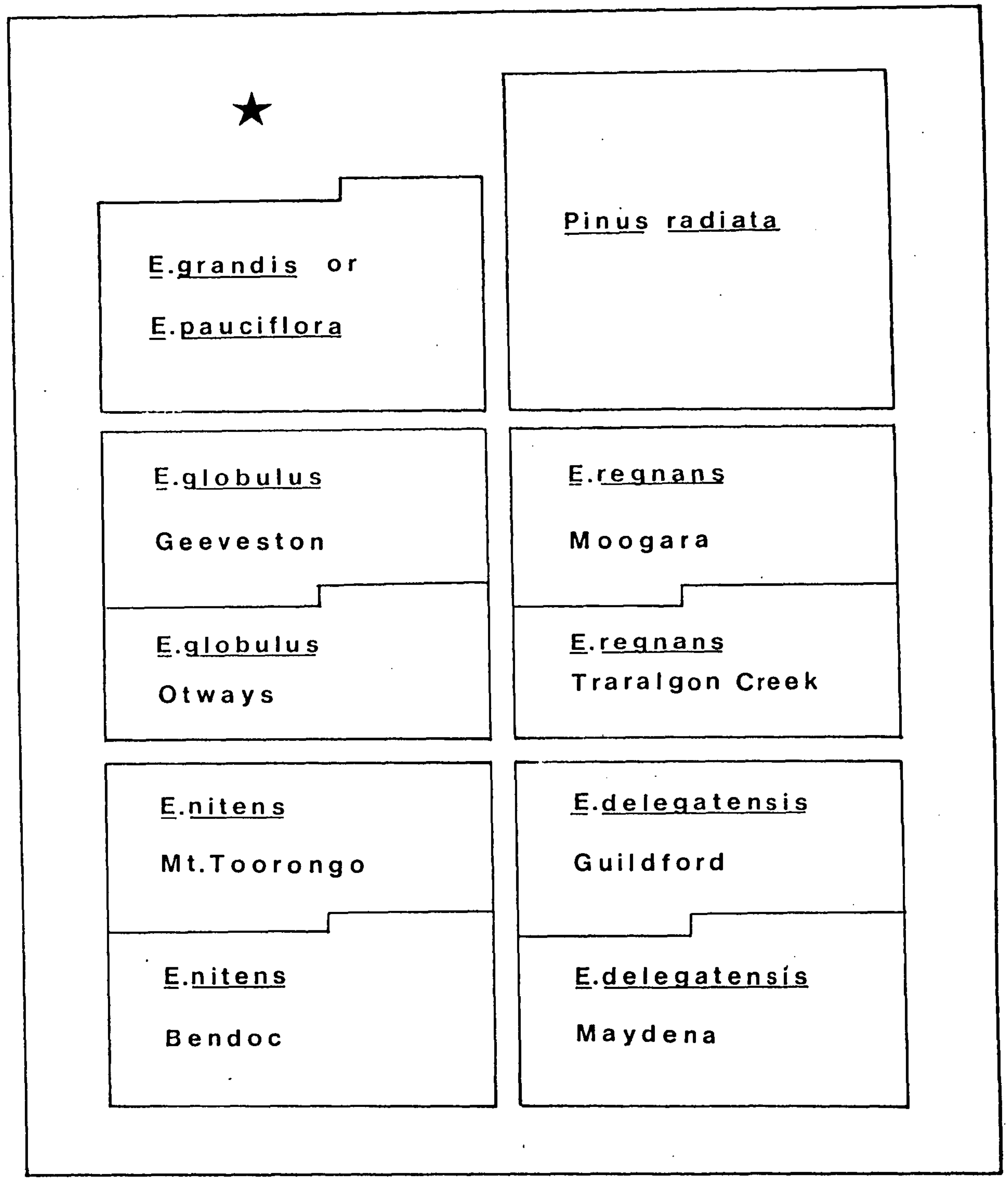


Fig. 5.3 The layout of species and provenances at the four sites in the Esperance Valley. ★ indicates the location of the meteorological instruments.

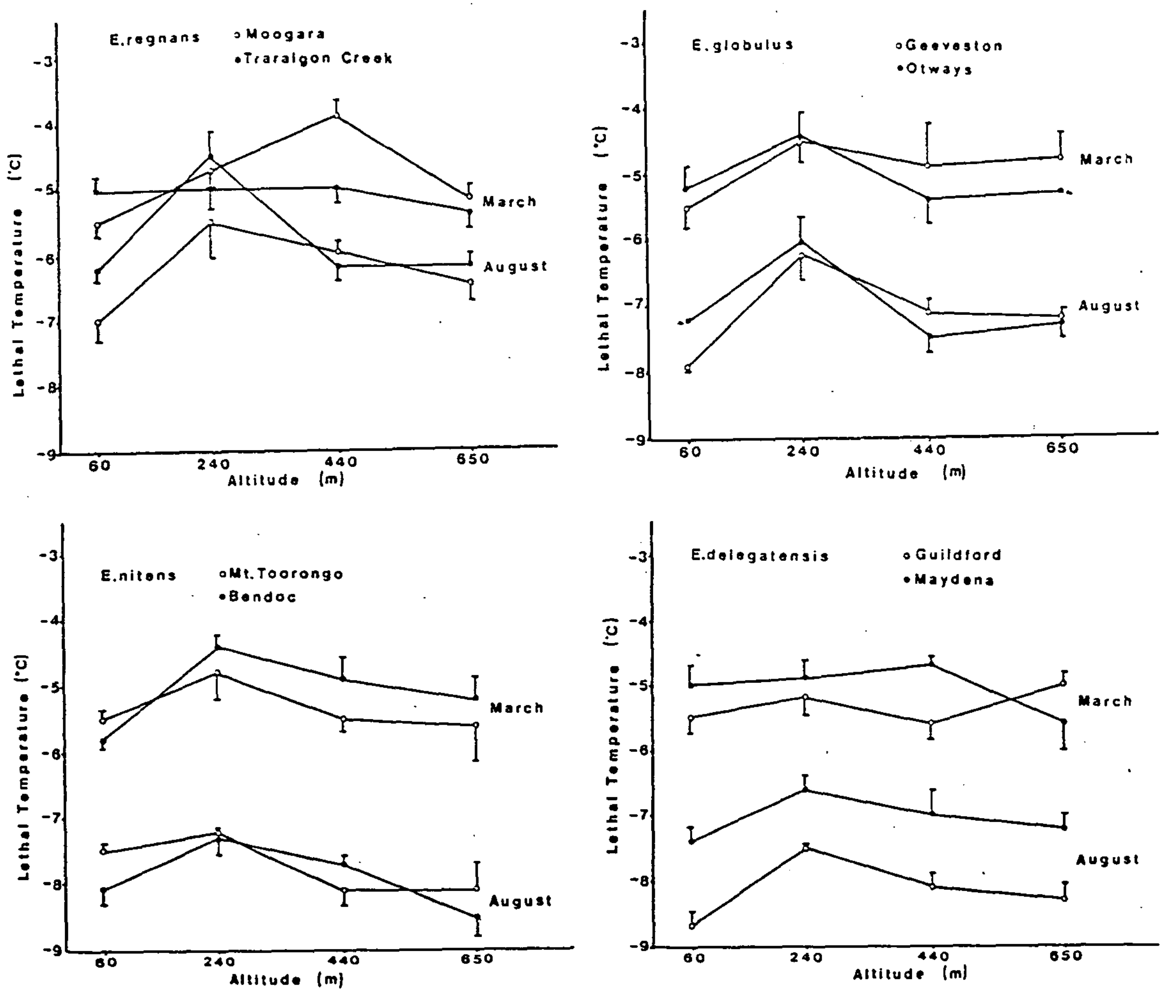


Fig. 5.4 The mean lethal temperature for each provenance of *E. regnans*, *E. globulus*, *E. nitens* and *E. delegatensis* in March and August 1985 at the four experimental sites. ⟙ represents the standard error of the mean of 4 trees.

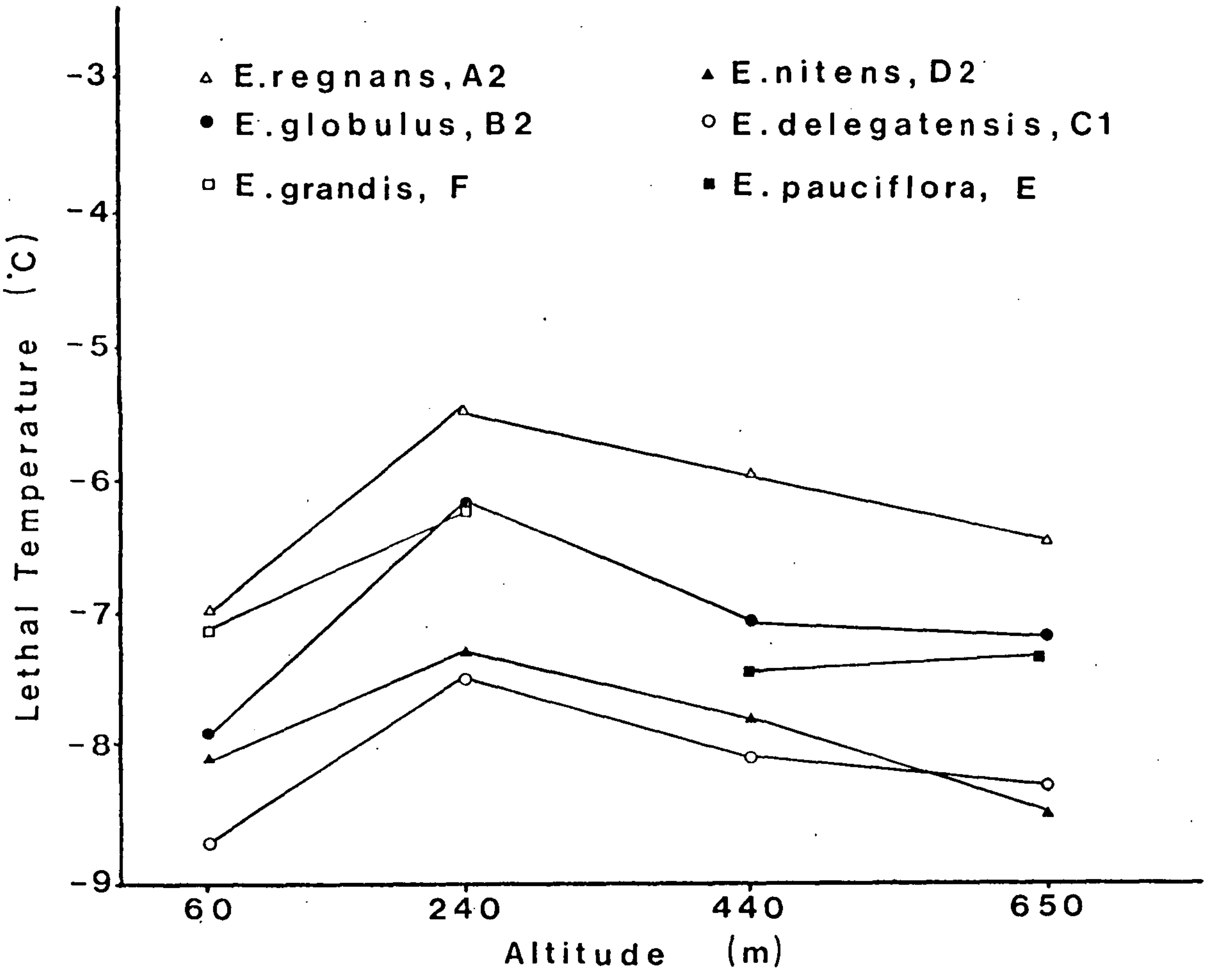


Fig. 5.5 The mean lethal temperature for one provenance of each species in August 1985.

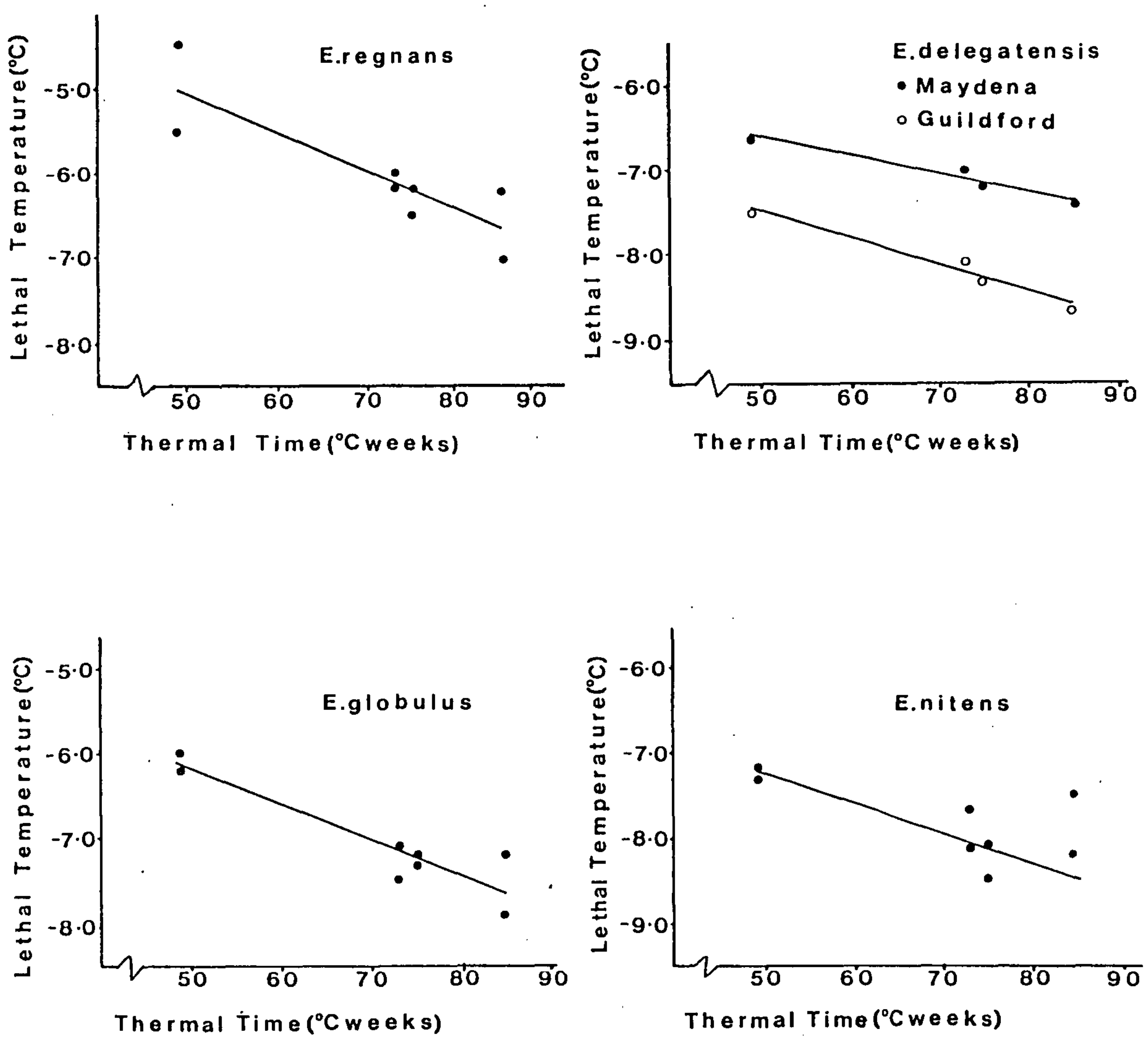


Fig. 5.6 The relationship between thermal time and lethal temperature for each species. Thermal times of 49, 73, 75 and 85 °C weeks correspond to sites 2, 3, 4 and 1 respectively. For *E. nitens* only, the points at 85°C weeks were not included in the regression.

Fig. 5.7 *E. regnans* at site 4 (650 m) with frost-damaged leaves.

Fig. 5.8 Frost damage to young leaves of E. grandis at site 1 (60 m)

Fig. 5.9 E. grandis at site 1 with red leaves which are probably due to low temperature stress.

CHAPTER 6

General Discussion

The development of a nondestructive method of testing plants for frost hardiness is an important asset for plant breeding. It means that seedlings in tree improvement programs can be tested without killing them and that established trees of any size such as those with superior growth rates in existing provenance trials can be tested. In this study the diffusate electrical conductivity method of Dexter *et al* (1932) has been modified for use with an air-filled frost chamber. Testing leaf tissue by this method and comparing the results with the frosting of whole seedlings (see Chapter 2) showed that this technique reliably predicts lethal temperatures.

While the above methodology can distinguish efficiently between species or provenances, sources of variation within trees must be reduced to a minimum. In this study the problem was avoided by taking six leaves from around the tree at a constant height and taking a disc from each leaf to form a combined sample. The problem of whether eucalypts frost harden as a whole plant or whether their leaves harden individually has not previously been investigated. Since cold air is known to stratify on still nights (Moore and Williams, 1976; Harwood, 1976; Davidson and Reid, 1985) some parts of the plant will experience lower temperatures than others. In the field gradation of damage with height on a tree is readily observable but it is not possible to say whether this gradation is due to the different temperatures experienced at different heights, causing different amounts of damage to leaves with the same degree of frost hardiness, or whether the leaves themselves are to some extent frost hardy to different temperatures. Timmis and Worrall (1975) found that for climatically 'split' Douglas fir that a branch in a cold (2°C) environment increased its frost hardiness but did not transmit this to branches in a warm environment (20°C). Conversely, branches in the warm environment did appear to transmit a factor which prevented full hardening of branches in the cold environment.

In this study (Chapter 2, Table 2.2) it was found that one year old leaves were more frost hardy than new leaves, as would be expected from field observations (see Chapter 5, Fig. 5.8). There was no significant difference in frost hardiness between the juvenile, intermediate and adult leaf forms of similar age of *E. delegatensis*. This is an important result since different individuals change their leaf form at different stages of growth and may bear both types of leaf concurrently.

Results from Chapter 3 showed that within Tasmania the ranking of seven provenances of *E. delegatensis* at maximum hardiness remained constant regardless of prior temperature conditions. Results from two sites at the same altitude, Tarraleah and Myrtle Bank were consistent, with the mean lethal temperature over all provenances being 1°C lower at Tarraleah in July than at Myrtle Bank, although the minimum temperatures reached at these sites differed by up to 4°C. Growth chamber results (see Chapter 4) also gave the same ranking of provenances although in this case the night temperatures were held constant throughout the hardening period in contrast to the field sites where the night temperatures showed an overall decline. At the Esperance sites (see Chapter 5) two provenances (Guildford and Maydena) of *E. delegatensis* maintained the same winter ranking at four different altitudes. Again the same ranking was found in growth chamber experiments with constant night temperatures. This result is useful since it suggests that there is no interaction between the ranking of provenances for frost hardiness and site within Tasmania. This is in agreement with Rook *et al* (1980) who reported that the provenance-site interaction for *E. regnans* at three sites in New Zealand was not statistically significant. They also found that the same rankings were obtained from artificial frosting as in scoring the damage by natural frosts in the field.

Transfer of results of frost hardiness testing to other latitudes or quite different climatic types (eg. more continental climates) may not be satisfactory, since the results of an assessment of frost damage (Boland and Dunn, 1985) in New South Wales for *E. delegatensis* did not give the same ranking of provenances. It is particularly noticable that Tasmanian provenances did not perform as well relative to mainland provenances when planted in New South Wales as they did when planted in Tasmania (a difference of 7° latitude). The reasons for this difference are not clear though it is tempting to suggest that a response to photoperiod is being observed by the Tasmanian provenances at Tarraleah and Myrtle Bank which was not triggered to the same extent at Pilot Hill in New South Wales. Without further experiment however there is little evidence in the literature to support this contention. A response to photoperiod was observed but only for high altitude provenances of *E. regnans* (Eldridge, 1968) but in a comprehensive study of 38 provenances of this species, including three from high altitude (>800 m) there was no significant difference in their ranking for frost resistance at sites located over a range of 8° latitude in N.Z.

A second explanation for differences in ranking in Tasmania with NSW may relate to conditions for hardening in a maritime compared to a continental climate. Thus in two maritime environments, Griffin *et al.* (1982) reported a strong correlation between their ranking of 25 provenances of *E. regnans* at Maydena, Tasmania with those of Rook *et al* (1980) in New Zealand. Unfortunately Griffin (1982) did not present any results for frost hardiness for similar provenance trials in Victoria but in a continental environment. Burgess (1983) reported that *E. grandis* was able to survive frosts as cold as -8.8°C in Canberra. This was perhaps unexpected as this species suffered from severe frost damage following a frost of -8.5°C when planted in Florida (Franklin and Meskimen, 1983). Since it appears from the data from the Tarraleah provenance trial that frost hardiness, in *E. delegatensis*, is a dynamic process in which the plant continually adjusts to changes in temperature, the nature of the temperature environment is important. Whether the autumn and winter temperatures follow a steady decline with continuous low night temperatures (continental) or whether they decrease on average with many small increases and decreases (maritime) would influence the degree to which a plant hardened and its tolerance to a severe frost.

Results from growth chamber experiments (Chapter 4) supported the idea of a dynamic equilibrium between frost hardiness development and temperature in that they demonstrated that colder nights induce greater frost hardiness and that dehardening is a faster process than hardening. Rates of hardening of *E. delegatensis* were slow (0.07°C/day) compared with reported rates for many other species e.g. *E. pauciflora* 0.25°C/day, (see Chapter 4, Table 4.5). However, Tibbits and Reid (1986) also reported that *E. nitens* hardened at 0.07°C/day. Further testing should be done to find out whether nonlethal frosts would increase frost hardiness as reported by Greer and Warrington (1982) for *Pinus radiata*. The results from the field trial at Tarraleah appear to support this, as plants developed greater frost hardiness during the winter of 1983 than in 1984 (see Chapter 3, Fig. 3.6). Although the mean monthly minimum temperatures did not differ greatly in 1983 and 1984, the extreme minimum in 1983 was -13°C compared with -8°C in 1984. The mean lethal temperature for Ben Lomond provenance in August 1983 was -10°C and must have been lower in July to have survived the -13°C experienced that month. In August 1984 the mean lethal temperature for Ben Lomond provenance was -8.6°C.

Since dehardening is a faster process than hardening (see Chapter 4), work should be done to find out if it is completely temperature controlled in eucalypts and what period of dehardening conditions is necessary to stimulate dehardening to begin. Aronsson (1975) reported that for *Pinus silvestris* and *Picea abies*, which frost harden in response to shortening photoperiod, temperature was of more importance than day length for dehardening. Dehardening was a much faster process than hardening for these species. Whether dehardening can be reversed once begun or whether it is seasonally linked is important for plant survival. At Tarraleah (Chapter 3) plants did not appear to fully deharden each summer since after the severe winter of 1983 levels of frost hardiness remained higher than they did following the milder winter of 1984. It appears that some sort of equilibrium is established with the environmental conditions. It has been suggested that frost hardiness and growth rate are negatively linked genetically (Marien, 1983) since in general the most frost hardy plants come from high altitudes and growth is negatively related to altitude of seed source. This hypothesis has not been substantiated in provenance trials of *E. regnans* (Griffin *et al.*, 1982) and this would also appear to be the case for *E. delegatensis*. At the Esperance sites (Chapter 5) the Guildford provenance was more frost hardy and had greater height and diameter growth than the Maydena provenance at all altitudes. Further, although the other species did not have a significant difference in frost hardiness between provenances there were significant differences in height and diameter growth.

The results from the Esperance sites (Chapter 5) also demonstrated that it is possible for temperature conditions at a site to be suitable for good growth rates as well as good frost hardiness development. The 60 m site had both the warmest maximum temperatures and the coldest minimum temperatures and thus produced the greatest growth and frost hardiness for most species. This has not been demonstrated previously because information on frost hardiness has been obtained by assessing damage to provenance trials (e.g. Griffin, 1982; Boland and Dunn, 1985) and data has not been available from more than one site or altitude. Alternatively, frost hardiness has been measured over several sites, but no growth data has been presented (Rook *et al.*, 1980).

Thus a reliable method of testing frost hardiness nondestructively, such as that developed in the present study, has much potential for making comparisons between frost hardiness and growth performance of trees at sites experiencing different climatic conditions. Further, selection for both good growth at high temperatures and the ability to harden would appear essential ingredients for fast growing plantations of eucalypts both in Australia and overseas. Temperatures <5°C have been confirmed as the primary stimulus for hardening of eucalypts, but the role of shortening photoperiod still remains unclear.

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