GENETIC DIFFERENTIATION BETWEEN ADJOINING POPULATIONS OF *EUCALYPTUS OBLIQUA* L'HERIT.

by

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DECLARATION

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ABSTRACT

Genetic differentiation at the site level was investigated within *Eucalyptus obliqua* L'Herit., by comparing the progeny of phenotypically distinct populations from adjacent sites along ecological gradients. Seed was collected from 12 maternal trees within each of four sites in south-east Tasmania. The Forestier sites comprised a topographic sequence of a wet sclerophyll forest type within a moist gully (Gully population) adjoining an open, dry sclerophyll forest type along an exposed ridge (Ridge population). The Lune sites formed a sequence of a wet sclerophyll forest type from the mid-slope position of a broad ridge system (Mid population) adjoining an open woodland from the lower slopes and plain (Plain population). Studies of the progeny from each of the 48 maternal trees were undertaken in various laboratory experiments and in planting trials established within each of the four sites.

The results provided evidence of genetic differentiation between and within the populations from the Forestier and Lune sites. Within the Lune populations, significant differences between progeny from the Mid and Plain were demonstrated for a number of attributes. The Mid population had higher germination energy, larger cotyledons, faster growth to age five years and lower susceptibility to leaf spotting fungi than the Plain population. Progeny from the Plain had higher frost resistance, higher persistence of coppice and a lower susceptibility of coppice shoots to browsing by native mammals.

Within the Forestier populations, there were no differences between progeny from the Gully and Ridge for factors such as growth to age five years, leaf morphology, frost resistance or coppicing ability. However, significant differences were demonstrated for a range of other attributes. The Gully population had higher germination energy, lower production of lignotubers and lower susceptibility to infection by leaf spotting fungi and browsing by native mammals than the Ridge population.

Many of the differences between adjacent populations could be regarded as evidence of adaptation to environmental factors operating at the local site level. The results indicated that differential selection forces may result in genetic differences between populations of *E. obliqua* over the scale of hundreds of metres, in addition to the tens or hundreds of kilometres normally associated with broad geographic variation at the ecotypic or provenance level. Variation at the site level has implications for the conservation of genetic diversity and for the probability of successful regeneration and long term adaptation and growth across heterogeneous sites within native forests.

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CONTENTS

CHAPTER 1 LITERATURE REVIEW AND OBJECTIVES

1. Introduction

The conservation of genetic diversity is fundamental to the maintenance of the evolutionary potential of a species (Frankel 1972). In natural populations, genetic diversity provides a species with an adaptive response to environmental change. In managed populations, the maintenance of genetic diversity maximises the opportunities for selecting superior genotypes for specific management purposes. Genecological studies have been undertaken in more than 34 species of eucalypt (Eldridge *et al.* 1993, Moran 1992). Genetic variation has been recorded for a number of traits, including: growth rates; resistance to frost, drought, insects and diseases; adaptation to particular soil factors; wood properties; and a range of morphological and other characters. The extent and patterns of genetic diversity within natural populations have also been investigated through the use of isozyme techniques, and more recently, molecular markers. Most studies have focussed on an evaluation of the genetic diversity within species that have commercial importance, rarity or a restricted distribution (House and Bell 1994).

Forest gene pools can be conserved *in situ* as reserves, or *ex situ* in plantings or seed stores (Pederick 1976). The reserve system generally relies upon the formal dedication of land areas such as national parks for specific conservation purposes. However, a useful complement to the formal system of *in situ* conservation can be provided by the implementation of suitable management regimes in multiple-use forests. A general objective common to the forest practices codes of both Tasmania and Victoria is that the regeneration of native forests should maintain species patterns and contribute to the conservation of local gene pools (CFL 1989, Forestry Commission 1993). Forest management activities such as timber harvesting may be compatible with the objectives of gene conservation provided that appropriate regeneration systems are used (Shepherd 1974, Pederick 1976). Inappropriate regeneration systems, such as the use of foreign seed in artificial sowings and plantings, may cause changes in the genetic structure of local populations, often leading to a loss of genetic diversity (Gömöry 1992).

Special seed collection guidelines have been developed for clearfelled stands that are to be regenerated by artificial sowing within Tasmania and Victoria (Forestry Commission 1991,

CNR 1994). These guidelines recommend that the seed to be used for regeneration purposes should be collected from the area to be regenerated, or from similar forest types in the same general locality. The rationale for the guidelines is that seed from a site that closely matches the area to be regenerated is most likely to contain genotypes best adapted to the site, thus minimising the risk of poor quality regeneration. In addition, the guidelines specify that collections should be made from an adequate number of trees in order to minimise the risk of a narrowing of the genetic base.

In practice, the proportion of on-site seed used to regenerate clearfelled areas has been very low. This is generally caused by low seed crops in areas scheduled for logging, a problem exacerbated by the irregularity of good seed crops in most of the major commercial species (Cremer *et al.* 1978). As a result, forest managers collect large quantities of seed during good seed years in order to meet the expected needs for the next five year regeneration programme (Forestry Commission 1991). Subsequent sowing programmes are then organised to conform as closely as possible to the guidelines by matching the seed source to the regeneration area on the basis of altitude, dryness, coldness, height of the mature forest, and geographic proximity. The closeness of matching depends upon the availability and the economic cost of alternative sources of seed and a judgement about the level of similarity that is necessary in order to meet the general objective of using seed that is likely to be adapted to the sowing site. This judgement is based on current knowledge about the patterns of genetic diversity within individual species, and the extent to which this diversity can be captured and maintained by seed collection and sowing practices.

In a review of isozyme studies, Moran (1992) concluded that the majority of genetic variation within eucalypt species occurs within, rather than between populations. For species within a widespread distribution, Moran estimated that the mean proportion of total diversity due to differences between populations was less than 15%. However, the pattern for individual traits may be quite different. For example, Tibbits and Reid (1987a) attributed 75% of the variation in frost resistance of *E. nitens* to differences between provenances. Likewise, Zobel and Talbert (1984) argue that 90% of all variation is due to differences between provenances and to differences between individual trees, and that the relative contribution of either will depend upon the particular trait under study. It is therefore apparent that seed collection programmes for the regeneration of native forests should endeavour to capture genetic diversity at two levels. Initially, programmes should aim to maintain a high level of genetic diversity in order to meet the general objectives of gene conservation. At a finer level of

detail, the programme should identify those traits that are most likely to be of substantial adaptive value and ensure that the appropriate alleles are maintained within the collected gene pool.

2. Sources of genetic variation within *Eucalyptus*

The amount and distribution of genetic diversity within a tree species are determined by the interaction of factors such as its breeding system, geographic distribution, longevity and fecundity with the processes of natural selection and adaptation.

2.1 Breeding system

A number of aspects of the breeding system of the eucalypts are of particular importance to the determination of genetic diversity within the species.

2.1.1 Fertilisation

Eucalypts are predominantly outcrossing, although there is a considerable degree of self-fertility within some species (Pryor 1976). The out-crossing rate has been found to average about 75% in the seed from natural populations (Eldridge *et al.* 1993). Moran and Griffin (1983) found that the out-crossing rate did not vary between four populations of *E. delegatensis,* but Brown *et al.* (1975) estimated that the rate varied between 64% and 84% in four populations of *E. obliqua.* Variation within populations tends to be higher for out-crossing species than for inbreeding species, whilst variation between populations tends to be higher for inbreeding species (Moran and Hopper 1983).

The eucalypts have a number of barriers to self-fertilisation which help to maintain the preferential out-crossing system. In general, out-crossing is favoured by the protandrous behaviour of individual flowers and a spread in the peak of anthesis between different individuals (Pryor 1976). There is also experimental evidence to suggest that outcross pollen has superior penetration of ovules than self pollen (Sedgley and Smith 1989), and that inbred embryos have a lower viability and competitive ability than outcross embryos and are

therefore less likely to survive in the developing fruit (Griffin *et al.* 1987). This latter factor may be regarded as an early manifestation of in-breeding depression, a phenomenon which has been associated with low seed yield, reduced germination and slower growth of seedlings and young trees (Hodgson 1976, Potts *et al.* 1987, Tibbits 1988 and Eldridge and Griffin 1983). These attributes generally mean that in-bred trees are at a competitive disadvantage compared to vigorous out-crossed stems and are therefore more likely to be eliminated by natural selection.

2.1.2 Pollen and seed dispersal

Pollination in eucalypts is primarily by animals (mainly insects and birds), not by wind (Pryor 1976). Animal pollination is generally regarded as a less effective means of gene flow than wind pollination and this has been cited as a possible reason for animal-pollinated species having higher levels of genetic differentiation between populations than wind-pollinated species (Moran 1992). Various studies have estimated that the dispersal distance for eucalypt pollen is generally limited to about 50m but may extend to distances of up to 300m (Barber 1965, Potts and Reid 1983, Eldridge *et al.* 1993). However, the possibility of even longer range dispersal of pollen is indicated by the presence of isolated trees of the intermediate *E. regnans / E. obliqua* form that have been observed to occur in *E. regnans* forest up to 1 km from the nearest *E. obliqua* boundary (Ashton 1981).

Eucalypt seed is wind distributed but the dispersal distance is limited because of the absence of any specialised dispersal mechanisms such as seed coat wings (Boland *et al.* 1980). The majority of seed falls within a distance of about two tree heights (60-90m) and very little appears to be displaced any further downwind (Cremer 1966). Thus, the restricted dispersal distances of both pollen and seed are likely to limit the rate of gene flow between populations of eucalypts.

Limited dispersal distances for seed means that natural regeneration within a stand probably consists of patches of closely related full-sibs, half-sibs and selfs. This pattern would magnify the potential for in-breeding over a period of successive generations, if it was not for the strong selection forces that preferentially favour the heterozygous products of the out-crossing system (Brown *et al.* 1975). These forces ensure that distant matings are more likely

to succeed than closer matings, resulting in a disparity between pollen dispersal and gene flow (Potts *et al.* 1987).

2.1.3 Hybridisation

Hybrids occur between many pairs of eucalypts (Pryor and Johnson 1971). However, despite the ease of many artificial hybrids, natural hybridisation is restricted by geographical, ecological, temporal and systematic barriers (Pryor 1976, Griffin *et al.* 1988). Hybrids may form as individuals within microsites that provide an intermediate niche between relatively uniform populations. These sites may be rare and highly localised as with the hybridisation between *E. obliqua* and *E. pulchella* (Potts and Reid 1983). Alternatively, the hybrid zones may be relatively common and widespread, as with the intermediate form of *E. obliqua* and *E. regnans* (Ashton 1981).

Hybridisation is important in the study of eucalypt genetics because it provides a potential mechanism for gene transfer between species through the process of introgression which occurs when hybridisation is followed by backcrossing over a number of generations. Introgression may explain some of the patterns of intra-specific population differentiation that occur within a continuous distribution of a species, although such patterns may also result from the alternative mechanism of selection forces acting upon a genetic continuum (Barber and Jackson 1957, Davidson *et al.* 1987).

2.2 Geographic distribution

The genus *Eucalyptus* contains species with distribution patterns that range from extensive continuous or discontinuous populations, to very small and isolated populations. Even within the continuous range of most species, the spatial distribution of populations is usually a mosaic determined by relative adaptation to different environments. Eucalypts, like most other tree species, generally show high levels of genetic variability. Although the majority of this variability appears to occur within populations, species differ considerably in the amount of variability between populations (Moran 1992). As a general rule, genetic differentiation is most likely to occur between populations that have been geographically isolated for evolutionary periods (Moran 1992) or between those that have been exposed to differential

selection forces (Barber and Jackson 1957). The effect of distribution patterns on the genetic diversity within the eucalypts is discussed in section 3.

2.3 Longevity and fecundity

Eucalypt species can produce viable seed from about age 2-10 years (Eldridge *et al.* 1993) and individual stems are believed to live for up to about 350-400 years (Gilbert 1959). The annual production of viable seeds has been estimated to be about 5,000 to 100,000 for individual large mature trees (Cremer *et al.* 1978) or about 0.9 million per hectare in pole forests and about 3.5 million per hectare in mature forests (Ashton 1975). In natural forests, generation times will depend upon the ecological processes associated with the regeneration of new stands. The wet eucalypt forests of Tasmania are generally considered to contain evenaged strata resulting from wildfires that are likely to occur at intervals of 80-400 years (Gilbert 1959, Mount 1965, Jackson 1968). In contrast, wildfires in the dry eucalypt forests are generally more frequent but of lower intensity, resulting in unevenaged structures containing a mosaic of different age classes.

Generation times in the eucalypt forests will therefore encompass the range of about 20-400 years, with the average likely to be in the order of hundreds of years. This longevity means that there may be very little genetic differentiation between populations that have been geographically separated for thousands of years since this only equates to a few generations of selection (Coates and Sokolowski 1989). The long generation times for the eucalypts may also provide a buffer against the loss of genetic diversity due to reductions in population size resulting from clearing or selective logging during the last 200 years of European settlement.

2.4 Natural selection and adaptation

Adaptations are defined by Stern and Roche (1974) as "character combinations, metabolic processes, and developmental pathways that enable an organism to survive in a given niche; and to occupy an optimal position in relation to the specific physiological possibilities set by its evolutionary history". Adaptive strategies may be based primarily on either phenotypic plasticity or population differentiation, or on some combination of the two (Rehfeldt 1979).

Phenotypic plasticity is expressed when a species produces variable phenotypes within different environments and yet all phenotypes retain a similar genetic make-up. In such species, there is little selection pressure for genetic differentiation and as a result, the species complement of genetic variability is widely maintained and is available for the development of further phenotypes as environmental conditions change. In a heterogeneous environment, phenotypic plasticity provides a range of phenotypes adapted to different niches, rather than an optimum phenotype for a specific niche (Stern and Roche 1974). Traits associated with plant vigour (such as height growth, lamina size and plant habit) are often demonstrated to be highly plastic in eucalypt species (Potts 1985).

Population differentiation occurs when the forces of natural selection lead to the development of phenotypes that are genetically different from one environment to another. Such differentiation is most likely to occur as a result of intense selection for an attribute of substantial adaptive value, such as the possession of glaucous leaves in high altitude populations of *E. urnigera* (Barber and Jackson 1957). Genetic changes may be slower to develop where the selection forces are less intense or where the population is subjected to intense selection that is 'density-dependent' (Barber 1965) rather than due to differences in adaptation or growth. Barber cites the intense thinning in the stocking of seedlings of *E. regnans* as evidence of density-dependent selection. Natural thinning reduces the stocking of stems per hectare from over 100,000 at age one year to about 700 by age 25 years, and to as few as 12 by age 400 years (Gilbert 1959). Selection in such stands is likely to favour the most vigorous trees that have the ability to out-compete their neighbours. However, selection for vigour is likely to be diluted by the effects of density-dependent mortality and the adaptation of the species is more likely to be expressed as plasticity in growth rather than as population differentiation (Barber 1965). In contrast, on less favourable growing sites, selection may be independent of density and more related to characteristics associated with survival and tolerance of environmental stresses such as frost, drought and low soil fertility (Pederick 1976). Intense selection of this form is most likely to lead to the genetic differentiation of populations (Barber and Jackson 1957).

3. Patterns of genetic variation within *Eucalyptus*

3.1 Levels of genetic variation

Phenotypic variation within natural stands is generally recognised at the following levels within the range of a species (Zobel and Talbert 1984):

1. Provenance (geographic) variation
2. Sites within provenances
3. Stands within sites
4. Individual trees within stands
5. Within trees (where applicable).

3.1.1 Provenance variation

Zobel and Talbert (1984) regard *provenance* as synonymous with terms such as *geographic source* and *geographic race.* A race is defined as a "subdivision of a species consisting of genetically similar individuals, related by common descent, and occupying a particular territory to which it has become adapted through natural selection" (Wakeley, unpub., cited by Zobel and Talbert 1984). By this definition, a provenance (or race) is not simply a "geographic locality" or "seed source", but is a distinctive population that is genetically different to any other within the range of the species. Genetic variation between provenances may correspond to differences in climate, altitude or soil factors. Alternatively, distinctive provenances may arise because of geographical isolation or other barriers to gene flow.

3.1.2 Variation between sites

The broad area occupied by a "provenance" will usually consist of a mosaic made up of smaller units of heterogeneous sites. These sites may be associated with substantial differences in environmental factors such as soil type, temperature, moisture availability and drainage. Differences may occur over relatively short distances, particularly where they are related to changes in physiography. For example, temperature generally decreases by 0.7°C

for every 100m increase in altitude (Australian Bureau of Statistics 1994). However, more extreme temperature differences of up to 9°C per metre of vertical distance have been recorded in localised areas of cold air drainage (Davidson and Reid 1985) and temperature differences between northern and southern aspects can be equivalent to a 300m difference in altitude (Hocker 1979). These changes in local site factors are often far larger than the broad differences that exist between many of the more widely distributed provenances within the range of a species. For example, Eldridge (1972) argues that an altitudinal difference of about 700m in Victoria has probably exerted a greater selection pressure on *E. regnans* than the latitudinal difference of 6° or 660 km that exists between the northern and southern limits of its distribution.

The environmental variation between different sites determines the local pattern of species distribution. In eucalypt forests, the occupation of specific niches is dependent upon the relative competitive ability of a species, rather than on its absolute restriction by some limiting environmental factor (Florence 1969). Within this pattern of inter-specific variation, there may also be substantial variation in the phenotype of an individual species across the range of sites within the provenance. Such phenotypic differences are often environmentally based and may only contribute a small amount to the total genetic variation within a species (Zobel and Talbert 1984). However, where site differences are very large, specific niches may be occupied by discrete populations of adapted individuals. Such populations are normally referred to as *ecotypes* and are regarded as being almost synonymous to races, but are generally at a finer scale. According to Stern and Roche (1974) a fundamental definition of ecotypes is that they should be specifically adapted, genetically distinct units that have boundaries that coincide with the distribution of an environment or environmental factor.

3.1.3 Variation between stands

Natural stands on uniform sites are likely to be subjected to uniform selection pressures, therefore genetic differentiation between stands is likely to be low (Zobel and Talbert 1984). However, modification of the population by human activities such as selective felling and artificial regeneration treatments may result in substantial variation between stands. The potential impacts of management activities on genetic diversity within forests are discussed in section 6 of this chapter.

3.1.4 Variation between trees

Substantial phenotypic variation generally occurs between the individual trees of a natural stand. This variation is due to both environmental and genetic factors. In provenance trials, the genetic component comprises variation due to differences between families (i.e. differences between the progeny from different open-pollinated mother trees) and differences between trees within families (differences between the progeny from the same open-pollinated mother trees). Variation due to differences between trees within families is often a major source of the total variation within a species. For example, between-tree variation for frost resistance in *E. nitens* is greater than the combined variation due to differences between families and between altitudinal populations within provenances (Tibbits and Reid 1987a).

3.1.5 Variation within a tree

Certain characteristics may vary according to the relative position or ontogenetic stage of development within the tree. Positional variation in factors such as foliage characteristics is particularly important in the eucalypts, given the distinctive changes that accompany the transition from juvenile to adult foliage in most species (Pederick 1979, Potts 1985). Other factors such as basic wood density and pulp strength may vary with the age and growth rate of the tree, and many of these properties are highly heritable (Matheson *et al.* 1986, McKimm 1985a,b, McKimm and Ilic 1987).

The levels of variation described above generally correspond to the heterogeneity of sites within the range of a species. Such variation is often discontinuous, resulting in discrete populations of genotypes at one of the hierarchal levels of variation described above. Within the range of many species, site variation may also occur along a continuous gradient, corresponding to changes in factors such as latitude or altitude. Such variation is referred to as *clinal variation* and it may be genetically or environmentally based. By definition, a cline is "a gradient in a measurable characteristic which follows an environmental gradient" (Zobel and Talbert 1984). Clines differ from ecotypes in that they are based on the concept of continuous variation of a single characteristic, whereas ecotypes represent discontinuous

populations of whole genotypes that are specifically adapted to distinct (discontinuous) environments.

The range of most species will contain some environmental factors that have continuous geographic gradients and other factors that are discontinuous. The variation pattern within a species will therefore contain continuous as well as discontinuous components, depending upon the variation of the niches (in time and space) that control selection, the selection coefficients of the alleles concerned and the migration rate (Stern and Roche 1974).

Genetic variation within eucalypts has been demonstrated from work based on isozyme analysis and studies into quantitative traits related to adaptation, survival and growth. More recently, molecular techniques such as Random Amplified Polymorphic DNA (RAPD) markers have been developed for application in advanced selection and breeding programmes.

3.2 Isozyme studies

Isozyme analyses have been undertaken to study genetic variability and breeding systems within a number of eucalypt species. The isozyme technique permits the study of discrete genes by the analysis of enzymatic loci. It is therefore regarded to be a very effective method for measuring genetic variation which is close to the DNA level and relatively free of environmental effects (Brown and Moran 1979).

Many plant genera demonstrate a significant correlation between geographic range and the level of genetic variability, with widespread species having greater variability than their more restricted congeners (Karron 1987). Eucalypts appear to fit this model, with species that occupy similar geographic distributions having comparable allelic distributions (Moran and Hopper 1987). However, Coates and Sokolowski (1989) argue that geographic range is not necessarily an accurate predictor of the *pattern* of genetic variability in congeneric species, since the pattern of variation is also determined by the population structure, especially the level of temporal and spatial separation that may occur between isolated populations. Eucalypt species with widespread distributions have been reported to have relatively high levels of genetic diversity with little genetic differentiation occurring between populations (Coates and Sokolowski 1989, House and Bell 1994) This is possibly due to the lack of

major barriers to gene flow and the presence of similar selection pressures within the range of the species. In contrast, high levels of population differentiation have been reported for species that occur within small and isolated populations (Moran and Hopper 1983). In *E. diversicolor*, a species with a population structure that comprises both a main (continuous) distribution and several (discontinuous) groups, Coates and Sokolowski (1989) found that most of the genetic variation occurred within populations, rather than between. However, certain isolated outlying populations were substantially different to the populations of the main forest and there was an indication that the genetic divergence was related more to time since separation, rather than to geographic distance from the main forest.

In a review of isozyme studies conducted within 17 species of eucalypt, Moran (1992) estimated the total genetic diversity and the distribution of diversity within and between the populations of each species. The results (summarised in Table 1.1) indicate that:

- the majority of genetic diversity appears to occur within, rather than between populations;
- widespread species have the highest levels of total diversity and within-population diversity;
- regional species have the highest proportion of diversity between populations, in particular those with disjunct distributions (38.6%) compared to those with continuous distributions (11.2%).

Table 1.1 Estimates of total genetic diversity and the distribution of diversity within and between the populations of eucalypt species (from Moran 1992).

Distribution type of the species*	Mean no. of alleles per locus	Total genetic diversity	Mean genetic diversity within populations	Mean proportion of total diversity due to differences between populations (%)
Widespread	3.19	0.231	0.198	14.7
Regional	2.99	0.208	0.154	24.9
Localised	2.45	0.199	0.173	14.2

* Widespread species = a range of 600 km in at least one direction
Regional species = a range of 150-600 km
Localised species = a small number of populations, usually of limited size and endemic to a restricted area of <100 km.

Despite the usefulness of isozyme analysis in studies of genetic variation, a number of authors have indicated that the results have given inconsistent associations with environmental factors or with quantitative traits important for adaptation, survival and growth. House and Bell (1994) found a lack of isozyme differentiation between populations of *E. urophylla* despite the high level of differentiation in morphological characters and growth rate. Moran (1992) found that in a number of widespread eucalypt species there was no pattern between population levels of isozyme variation and environmental factors such as latitude, altitude and soil type. However, Moran reported that populations differentiated on the basis of isozyme analysis in both *E. delegatensis* and *E. nitens* corresponded to populations that had been differentiated on the basis of morphological characters. Other studies have demonstrated a correlation between the gene loci selected for isozyme studies and several climatic variables (Guries and Ledig 1979). However, the general conclusion is that there is not necessarily a direct relationship between the genes studied and the morphological and physiological factors related to traits such as adaptation (Brown and Moran 1979). The real value of isozyme techniques is in providing a direct study of the genetic structure of a species, particularly in terms of assessing total genetic diversity, levels of diversity within different populations, and the degree of differentiation between populations (Brown and Moran 1979). Such information provides an important complement to the quantitative studies of genetic diversity.

3.3 Quantitative studies

3.3.1 Continuous (clinal) variation

Clines are common within the eucalypts, accounting for considerable variation within and between species. Clinal variation has been reported in a number of species, including *E. urnigera* (Barber and Jackson 1957), *E. pauciflora* (Pryor 1956, Green 1969, Slatyer and Morrow 1977), *E. regnans* (Ashton 1958), *E. ovata* (Ladiges *et al.* 1981), *E. brookerana* (Ladiges *et al.* 1981), *E. camaldulensis* (Eldridge 1975, Grunwald and Karschon 1983) and *E. cordata* (Potts 1989). In some species, clinal variation occurs as part of a continuum resulting from intergradation between two species e.g. *E. viminalis/E.dalrympleana* (Phillips and Reid 1980), *E. regnans/E. obliqua* (Ashton 1981), *E gunnii/E archeri* (Potts and Reid 1985a,1985b,Potts 1985), *E. nitida/E. coccifera* (Shaw *et al.* 1984), *E. amygdalina/*

E. pulchella (Kirkpatrick and Potts 1987), *E. grandis/E. saligna* (Burgess and Bell 1983)and *E. camaldulensis/E. tereticornis, E. globulus/E. pseudoglobulus* and *E. maculata/ E. citriodora* (Eldridge *et al.* 1993).

The above studies have indicated that clinal variation may be associated with strong environmental gradients in factors such as altitude (as in *E. urnigera, E. pauciflora, E. regnans* and *E. nitida/E. coccifera*) and latitude (as in *E. camaldulensis, E. ovata* and *E. brookerana*). The environmental gradient may occur over a distance of a few hundred metres as with the altitudinal cline for *E. urnigera* on the slopes of Mt Wellington (Barber and Jackson 1957), or over the vast distances of thousands of kilometres associated with the latitudinal and longitudinal clines within *E. camaldulensis* (Eldridge 1975, Grunwald and Karschon 1983). Variation may be controlled by a single environmental factor such as moisture availability or temperature but is often more complex, involving the interaction of several environmental factors. For example, Potts and Reid (1985a,1985b) and Potts(1985) attributed two major genetically based clines within *E gunnii/E archeri* to gradients in both latitude and altitude. Phillips and Reid (1980) found that clinal variation between *E. viminalis* and *E.dalrympleana* was correlated with several environmental variables but the reason for the variation between populations could not be determined.

Quantitative traits associated with clinal variation include certain morphological and physiological characters that can be related to site adaptation. Commonly, altitudinal clines are associated with phenetic differences such as decreasing tree height, decreasing leaf size, increasing lamina thickness, increasing persistence of juvenile or intermediate foliage in mature trees, and increasing glaucousness with increasing altitude (Pryor 1956, Barber and Jackson 1957, Potts and Reid 1985a, 1985b, Potts 1985). Some of these characters are the result of substantial phenotypic plasticity whilst others indicate varying levels of genetic differentiation. Large plastic responses are often reported for characters that are a function of plant vigour (e.g. height, lamina size, internode length) but minimal plasticity tends to occur in other characters such as glaucousness and leaf shape (Potts 1985) which may be under significant genetic control (Barber and Jackson 1957). Clinal variation in glaucousness has been reported for a number of species that occur along altitudinal gradients e.g. *E. coccifera* and *E. delegatensis* (Barber 1955), *E. urnigera* (Barber and Jackson (1957), and *E. viminalis* (Banks and Whitecross 1971). Genetic variation has also been reported for clines in factors such as: frost resistance in *E. regnans* (Ashton 1958) and in *E. pauciflora* (Pryor 1956);

photosynthetic characteristics in *E. pauciflora* (Slatyer and Morrow 1977); leaf oil gland density in *E. ovata* and *E. brookerana* (Ladiges *et al.* 1981); and water availability/drainage in *E. cordata* (Potts 1989).

3.3.2 Discontinuous variation

Discontinuous patterns of variation have been reported in many genecological studies of the eucalypts (Eldridge *et al.* 1993). Variation in morphological characters is commonly used to provide the taxonomic basis for the differentiation of populations into separate species, sub-species or provenances (Boland and Dunn 1985, Boland 1985). Tree breeders have tended to concentrate on factors related to environmental tolerance, productivity and wood quality. These factors include growth rate, stem straightness, wood properties, resistance to frost, drought, insects or fungi, and tolerance of soil conditions such as salinity, alkalinity, acidity and waterlogging.

Discontinuous variation is most likely to occur in species that are distributed on environmentally different or geographically separate sites. Broad scale variation in factors such as seedling growth rate and frost resistance has been reported to occur between provenances of species such as *E. obliqua* (Brown *et al.* 1976), *E delegatensis* (Boland and Dunn 1985), *E. globulus* (Volker and Orme 1988), *E. ovata* (Clucas and Ladiges 1979) and *E. nitens* (Tibbits and Reid 1987a). Often, differences between populations can clearly be seen as an adaptive response to a specific environmental factor. For example, "edaphic ecotypes" have been reported for populations that demonstrate adaptation to specific soil types in *E. viminalis* (Ladiges and Ashton 1974) and *E. obliqua* (Anderson and Ladiges 1978, 1982 and Anderson 1982a, 1982b).

As discussed in the preceding sections, the genetic diversity of most species will contain both continuous and discontinuous components, depending upon factors such as the variability of the environment and the continuity of distribution and gene flow within the species range. Patches of discontinuous variation often occur within a broader pattern of continuous (clinal) variation. These patches may occur as specific niches within a locally modified environment, or as geographically isolated populations within the range of a widely distributed species. For example, Turnbull (1973) identifies the pattern of genetic variation within

E. camaldulensis as patches of discontinuous variation within the general pattern of large scale clinal variation. Turnbull attributes the discontinuous variation to restricted gene flow between the separate drainage basins that comprise the distribution of this species.

In many studies, it is not possible to determine whether patterns of variation are continuous, discontinuous or some combination of the two, due to the restricted number of samples used for experimentation. The following sections briefly review the literature on the patterns and levels of genetic variation for a number of specific traits.

4. Studies of genetic variation in specific traits within *Eucalyptus*

4.1 Growth rate

Growth rate is the most commonly measured trait in studies of genetic variation within eucalypt species as it is generally regarded as the most important attribute for commercial forestry (Matheson *et al.* 1986). Obviously, long term growth (such as biomass production over one or a series of rotations or generations) is difficult to monitor for long-lived species such as the eucalypts. A number of studies have indicated a good correlation between short term growth (e.g. results at age 2 to 5 years) and longer term growth (e.g. results at age 10 to 20 years) (Eldridge 1972, Pederick 1985). However other studies have indicated that substantial changes may occur in the ranking of specific provenances, particularly when the statistical differences in the growth between the provenances is not high (Volker and Orme 1988). In particular, the growth of seedlings in the nursery or during the first growing season may not be a reliable predictor of subsequent growth (Griffin *et al.* 1982, Krishnaswami *et al.* 1986).

Discontinuous variation in growth rate at the provenance level has been recorded in most species that have been studied, including: *E. obliqua* (Brown *et al.* 1976); *E. regnans* (Griffin *et al.* 1982); *E. delegatensis* (Moran *et al.* 1990); *E. globulus* (Volker and Orme 1988); *E. nitens*, (Pederick 1979); *E. grandis/E. saligna* (Burgess 1988); *E. camaldulensis* (Eldridge *et al.* 1993); *E. fastigata* (Wilcox 1982); *E. tereticornis* (Matheson and Mullin 1987); *E. viminalis* (Eldridge *et al.* 1993); *E. ovata* (Clucas and Ladiges 1979); *E. perriniana* (Wiltshire and Reid 1987); *E. occidentalis* (Mughini 1985); *E. urophylla* (Eldridge *et al.* 1993); and *E. deglupta* (Davidson 1983).

Clinal variation in growth rate has also been demonstrated for altitudinal transects in *E. regnans* (Eldridge 1972); *E. gunnii/E archeri* (Potts 1985); and *E. pauciflora* (Pryor 1956). These studies have demonstrated a linear relationship between tree growth and altitude, with the higher altitude seedlings growing more slowly, even at low altitude planting sites. Slower growth at high altitude may reflect a physiological adaptation to the environment, allowing traits such as growth rates and cold resistance to be synchronised with the seasonal climatic pattern (Ashton 1958).

The above studies have indicated major changes in the ranking of provenances on different sites (or large genotype x environment interactions) for species such as *E. delegatensis*, *E. nitens*, and *E. camaldulensis*, indicating significant differentiation in terms of the relative adaptation of individual populations to specific sites. However the interactions are either small or non-significant in species such as *E. obliqua*, *E. deglupta* and *E. regnans* suggesting that there has been relatively equal selection pressures for growth. Vigorous growth is likely to be an important factor in the natural selection of populations on optimal sites, where success is related to the competitive ability of the individual seedling to exploit the growing environment ahead of the very high density of other stems and competing vegetation. The genetic capacity for fast growth may be associated with considerable phenotypic plasticity, with fast-growing populations often producing the best growth even on relatively poor sites (Eldridge *et al.* 1993). In contrast, on harsh sites, natural selection appears to be less dependent upon growth rate and more dependent upon adaptation to environmental stresses such as drought, frost or poor drainage. Namkoong (1969) argues that some species may possess an ecological peak in the interior of their range which is coincident with an optimal combination of environmental and climatic factors, and that genotypes from these optimal ecological zones will outgrow populations from the more marginal areas of the species distribution. Populations from sites with low growth rates are often among the lower rankings for growth in provenance trials. For example, *E. obliqua* progeny from low rainfall sites in South Australia have produced poorer growth than progeny from higher rainfall areas (Brown *et al.* 1976). However, this is not always the rule: Eldridge *et al.* (1993) report that poor phenotypes from a harsh site within the range of *E. camaldulensis* have produced highly vigorous progeny in plantations established in Mediterranean regions.

4.2 Adaptation to cold temperatures and frost

The timing and severity of frost can be a major selective factor in determining the distribution of eucalypt species (Davidson and Reid 1985). Numerous studies have demonstrated continuous and discontinuous patterns of genetic variation in the relative frost resistance of populations within the range of a species. Variation at the population level has been strongly correlated with altitudinal clines in species such as *E. regnans* (Ashton 1958), *E. pauciflora* (Pryor 1956); *E. viminalis* (Paton 1972), *E. fastigata* (Boden 1958), *E. delegatensis* (see review by Eldridge *et al.* 1993) and *E. urnigera* (Thomas and Barber 1974a). However, the pattern of frost resistance is not always continuous or well correlated with the temperature trends associated with altitude. A lack of significant differentiation in frost resistance between altitudinal populations may occur as a result of other physiographic factors that influence the degree of cold air drainage and radiation frost. For example, increased frost resistance in low altitude populations has been observed for progeny originating from sites subject to cold air drainage in *E. pauciflora* (Harwood 1980) and *E. regnans* (Ashton 1958). Other environmental factors such as waterlogging may also be involved in the development of frost resistance (Paton 1972, Potts 1985, Davidson and Reid 1985).

Studies of discontinuous variation in frost resistance has demonstrated substantial population differentiation within many species. Components of variation for differences between provenances were 3 to 4 times larger than for differences between families (within provenances) in *E. fastigata* (Wilcox 1982). Similarly, in *E. nitens*, 75% of the variance was attributed to differences between provenances and of the remaining variance, differences between seedlings within family were greater than the differences between families within provenance (Tibbits and Reid 1987a). However, very high levels of within provenance variation have been noted for other species. Paton (1972) found that for *E. viminalis*, the variation within half-sib families and within provenances was often significant and of the same order of magnitude as the variation between provenances. Paton suggests that the maintenance of high levels of within provenance variation is a function of natural selection being balanced between adaptation for frost resistance and adaptation for some different, but equally severe selection pressure. This form of balancing selection means that in years of unseasonal or catastrophic frost, selection will favour individuals with high frost resistance, whereas in mild years selection will favour individuals with other adaptive traits.

Frost resistance varies with season in response to environmental factors that determine the level and rate of hardening and de-hardening. The frost resistance of seedlings at various levels of hardening may reflect differential survival strategies since damaging frosts can occur to fully hardened seedlings in mid-winter or to partially or totally de-hardened seedlings in spring and autumn. A consistent pattern of frost resistance for hardened, partially hardened and unhardened seedlings has been found for provenances of *E. fastigata* (Wilcox *et al.* 1983) and *E. nitens* (Tibbits and Reid 1987a). However, Tibbits and Reid found highly significant interactions for frost resistance x season at the family (half-sib) level. In contrast, Awe and Shepherd (1975) recorded major differences between southern and northern provenances of *E. camaldulensis*. The northern provenances had the highest levels of frost resistance for unhardened seedlings whilst the southern provenances had superior resistance for hardened seedlings. Awe and Shepherd suggested that the ability of the northern provenances to withstand frost damage in the unhardened state was an adaptation to the sudden occurrence of mild radiation frosts during periods when daytime temperatures are relatively high, conditions that are commonly experienced in the northern range but not in the cooler southern range of *E. camaldulensis*.

Results from clinal studies indicate a trend of reductions in both growth rate and frost sensitivity with increasing altitude of the seed source (e.g. Eldridge 1972, Pryor 1956). Growth retardation and the development of frost hardiness has been linked to environmental factors such as decreasing temperatures and daylength (Scurfield 1961). However, Paton (1981) demonstrated that providing night temperatures were close to freezing, rapid hardening was independent of photoperiod, light source and day/night temperature differentials. Paton found that increased frost resistance was associated with ontogenetic increases in the content of a growth regulator in seedlings of *E. grandis*. It is not clear to what extent growth rate may contribute to frost sensitivity *per se*, and therefore whether the slow growth of high altitude provenances may be of direct adaptive value in reducing the sensitivity of seedlings to frost. Other populations may have a different survival strategy for avoiding frost damage. For example, vigorous height growth may allow seedlings to rapidly position their growing tips beyond the level of a radiation frost layer. This strategy has been observed in a low altitude provenance of *E. fastigata* which, despite greater frost damage in the first growing season, produced 75% greater volume production by age 2.5 years than the more frost resistant but slower growing high altitude provenance (Sherry and Pryor 1967). In contrast, Griffin *et al.* (1982) found no correlation between early height growth and frost hardiness in seedlings of *E. regnans*.

Other adaptive strategies have been proposed to link frost resistance with morphological characters such as leaf glaucousness. A number of studies have indicated a clinal pattern of increasing leaf glaucousness and frost resistance with increasing altitude (Thomas and Barber 1974a, Potts 1985, Tibbits and Reid 1987a). However, it is not clear whether the two factors are linked or are under different, but parallel control mechanisms (see discussion under section 4.6).

Although frost is generally regarded as a major factor on many sites, selection may also be determined by the relative adaptation of individuals to low temperatures *per se*. Temperature influences growth by altering the rates of physiological processes such as photosynthesis and respiration, and species may vary in their response to different temperature regimes (Kramer and Kozlowski 1960). Clinal variation in photosynthetic characteristics has been reported in altitudinal populations of *E. pauciflora* (Slatyer and Morrow 1977). Peak rates of photosynthesis were observed at a lower temperature in the higher altitude populations. Slatyer (1977a, 1977b) also demonstrated that the photosynthetic temperature optimum of each population could be shifted several degrees by acclimation to contrasting growth temperatures. The rate of acclimation was also shown to vary between populations, with the lower altitude population able to respond more quickly to temperature changes than the higher altitude population (Slatyer and Ferrar 1977). Slatyer (1977b) argues that photosynthetic acclimation is of adaptive value for evergreen woody species such as the eucalypts, particularly in environments in which the growing tips are exposed to low temperatures which may limit photosynthesis.

4.3 Adaptation to drought and water stress

Differential adaptation to drought has been demonstrated in populations of *E. camaldulensis* (Awe *et al.* 1976, Grunwald and Karschon 1982) and *E. viminalis* (Ladiges 1974b). The ability of *E. camaldulensis* seedlings to survive a rapidly drying soil profile is due to a capacity to produce a massive root system, and some provenances can do this more rapidly than others (Awe *et al.* 1976). Once established, there are differences in tissue water relations between different provenances. Grunwald and Karschon (1982) found that a Victorian provenance of *E. camaldulensis* was better able to tolerate drought, by having lower water potentials and the ability to maintain higher growth rates at low soil moisture levels, than a West Australian provenance.

In *E. viminalis*, seedlings from two low rainfall populations showed greater drought tolerance than seedlings from two high rainfall populations (Ladiges 1974b, Ladiges and Ashton 1974). Within the two low rainfall populations, there were differences between the drought tolerance of seedlings from a granite soil type and a basalt soil type, suggesting that greater selection pressures have occurred on the more drought-prone soil type. This work demonstrated that drought resistance in *E. viminalis* was not simply due to differences in stomatal closure or water potential, but appeared to be associated with some physiological resistance of the protoplasm to desiccation.

Provenance variation has also been reported for the germination of seeds under different conditions of water stress (Gibson and Bachelard 1987). Differences in the germination of seeds from two provenances each of *E. camaldulensis, E. nitens* and *E. obliqua* were consistent with differences in seed coat characteristics and the environment of the parent trees, providing support for the view that the provenances were specifically adapted to their natural environments. Ladiges (1974a) also demonstrated that germination rate and total germination percentage were higher for populations of *E. viminalis* from high rainfall sites than from low rainfall sites. Ladiges suggested that rapid establishment was a selective advantage on moist sites, whereas irregular or delayed germination may be an advantage on dry sites where suitable conditions for establishment may be more sporadic.

4.4 Adaptation to edaphic factors

Genetic differentiation on the basis of tolerance to edaphic factors such as fertility, acidity, alkalinity and salinity has been reported in *E. viminalis* (Ladiges and Ashton 1977), *E. obliqua* (Anderson and Ladiges 1978, Anderson 1982a, 1982b, Anderson and Ladiges 1982) and *E. camaldulensis* (Sands 1981). Ladiges (1974a) found that seedlings of *E. viminalis* from a low rainfall population maintained slow growth rates even when given increased nutrient supply. Ladiges and Ashton (1974) argue that slow growth may be a selective advantage on infertile soils in low rainfall areas, since fast growth rates (in response to higher nutrient levels) may render the seedlings more susceptible to drought. Further studies of *E. viminalis* have also shown that some populations are adapted to calcareous soils and differ in their susceptibility to lime chlorosis (Ladiges and Ashton 1977). However, these authors noted that soil factors could be quite variable at any one site, and that all of their seedling populations showed considerable variation in both yield and degree of lime

chlorosis, suggesting that differences in chlorosis-susceptibility may involve more than one gene.

Similar differences in adaptation to calcareous and acid soils have been reported for *E. obliqua* (Anderson and Ladiges 1978, Anderson 1982a, 1982b, Anderson and Ladiges 1982). These workers demonstrated the existence of edaphic ecotypes, with populations from acidic loam, neutral sand and alkaline sand showing different growth rates when grown under glasshouse conditions. Adaptations were expressed in a number of forms, including differential germination response at high levels of Ca (Anderson 1982b), and reduced susceptibility to lime chlorosis due to different mechanisms for the uptake of Fe under conditions of high pH and high P (Anderson 1982a).

Differential tolerance of salinity has been noted in *E. camaldulensis* by Sands (1981) who found that a salt concentration of 400mM resulted in the death of all seedlings of one provenance, no ill effect on the health of the seedlings of another provenance, and an intermediate effect on the seedlings of the remaining provenance. Sands also noted that germination under saline conditions was least affected in populations from dry, high salinity sites and most affected in populations from wet, low salinity sites. Eldridge *et al.* (1993) indicate that highly tolerant populations of *E. camaldulensis* have been found on highly saline sites but that high levels of tolerance also appear to exist in some populations growing in soils of low salt content. This suggests that adaptation to salinity may be determined by variable selection forces.

4.5 Tolerance of disease and insect damage

Fungal and insect parasites play an important role in the maintenance of distribution patterns within eucalypt forests (Burdon and Chilvers 1974). Host specificity or host preference is commonly observed towards trees of a particular species or provenance. Substantial differences have been reported in the susceptibility of provenances of *E. viminalis* and *E. dalrympleana* to damage by the eucalyptus snout beetle (*Gonipterus scutellatus*) in South African provenance trials (Richardson and Meakins 1986). In Australia, the variable growth performance of *E. globulus* in provenance trials has been linked to provenance variation in the resistance of the juvenile foliage to defoliation by two insect species (Farrow *et al.* 1994). Inter- and intra-provenance variation has been demonstrated for damage by a range of insects

to the foliage of *E. camaldulensis* and *E. blakelyi* (Floyd *et al.* 1994). Similarly, Potts(1985) found preferential leaf grazing by phytophagous insects at the population level within *E. gunnii/E. archeri* and he suggested that damage by insects may be a significant selective force contributing to population differentiation within this cline.

Provenance variation in susceptibility to disease has also been recorded in species such as: *E. grandis* for canker and rust diseases (Eldridge *et al.* 1993); *E. nitens* for leaf spotting disease (Purnell and Lundquist 1986); and *E. urnigera* for canker diseases (Eldridge *et al.* 1993). The mortality of seedlings of *E. regnans* due to *Phytophthora cinnamomi* has been shown to be highly variable at both the family and provenance level (Harris *et al.* 1985).

4.6 Variation in morphological characters

Substantial phenotypic variation is often noted in many morphological characters over the distribution range of many species. For example, tree form in *E. viminalis* can vary from stunted, open-grown trees in the open woodland on low rainfall sites to the tall, straight bole trees in the tall, dense understorey forests on high rainfall sites (Ladiges and Ashton 1974). Characters such as leaf shape may also vary in response to environmental factors. As noted under the discussion on clinal variation (section 3.3.1), environmental conditions may account for a large proportion of the phenotypic variation associated with characters that are a function of plant vigour (e.g. height, lamina size, internode length). However, other characters that have been under strong selection forces may be associated with substantial genetic differentiation.

A pioneering study of natural selection and genetic differentiation of morphological characters has been described for leaf glaucousness in *E. urnigera* along an altitudinal cline in Tasmania (Barber and Jackson 1957). In this work, the authors demonstrated a strong pattern of clinal variation, with both the glaucous and green leaf forms present at mid-altitudinal sites, but the glaucous and green form only present at the higher and lower altitudinal sites respectively. Progeny raised from seed collected along the altitudinal transect indicated that within the intermediate altitudinal zone, both leaf forms were produced from green and from glaucous parents. However, at either end of the transect green parents only produced green leaf form seedlings, and glaucous parents only produced glaucous seedlings. These results indicate that the glaucous condition is directly, or

indirectly, associated with superior survival at the higher altitude, but appears to have been selected against at the lower altitude sites. Similar tends for clinal variation in leaf glaucousness have been reported in *E. coccifera*, *E. gunnii*, and *E delegatensis* (Barber 1955). Differences in the structure and quantity of leaf surface waxes have also been reported between ecotypes of *E. viminalis* (Banks and Whitecross 1971). Various hypotheses to explain the relative advantages and disadvantages of glaucousness were proposed by Barber and Jackson (1957). Thomas and Barber (1974a) suggested that the greater water repellency of glaucous leaves allowed the dry leaves to supercool and hence tolerate lower temperatures than the wettable green leaves. However, Paton (1981) found no relationship between the level of frost resistance and intensity of leaf glaucousness in either segregating progeny of *E. urnigera* or F_2 and backcross progenies between *E. pulverulenta* and *E. grandis*. Cameron (1970) provided evidence that leaf glaucousness increased the reflectance of light and therefore reduced the photosynthetic ability of leaves at low light intensities. On this basis, it has been speculated that glaucous leaves will be a disadvantage in closed forests where the dense regeneration of seedlings and understorey plants may lead to acute competition for light. In contrast, glaucous leaves may be an advantage in exposed, high altitude areas where light intensities may greatly exceed those of lower altitudes (Cameron 1970, Thomas and Barber 1974b). Since high light intensities and frost-prone locations frequently coincide (Potts 1985), it is difficult to determine the major selective force that may favour leaf glaucousness along altitudinal clines. In addition, leaf glaucousness may be associated with other adaptive features. For example, ecotypic variation in the glaucousness of leaves has been linked to superior insect resistance in *E. camaldulensis* (Floyd *et al.* 1994).

Differences in characters such as leaf shape have been reported in other studies of variation within and between species. Variation in leaf shape is often continuous, with intermediate characteristics generally demonstrated by populations within the middle range of the species or hybrid complex. For example, a trend of broad leaf lamina to narrow lamina has been observed in clines of *E. viminalis* (Ladiges and Ashton 1974), and in clines or in zones of hybridisation between *E. dalrympleana/E. viminalis* (Phillips and Reid 1980), *E. obliqua/ E. pulchella* (Potts and Reid 1983), *E. coccifera/E. nitida* (Shaw *et al.* 1984), *E. gunnii/ E. archeri* (Potts 1985), and *E. risdonii/E. amygdalina* (Potts and Reid 1985c). Discontinuous variation in factors such as leaf shape has been recorded in species such as *E. ovata* (Clucas and Ladiges 1979) and *E. camaldulensis* (Grunwald and Karschon 1983). Ladiges (1974a) suggested that the trend for *E. viminalis* to have narrower leaves on drier sites may be related to heat stress. Narrow leaves may help to reduce the overheating of leaf

tissue on dry sites, whereas on wet sites where moisture stress is less likely, large leaves may give a competitive advantage by providing a greater photosynthetic area.

Distinctive patterns of ontogenetic development are noted in many species, with the transition from juvenile to adult foliage often under strong genetic and environmental control. Davidson *et al.* (1981) have argued that heteroblastic development in eucalypts is of substantial adaptive value since there is greater variability upon which selective forces can act. The retention of juvenile or intermediate foliage for longer periods has been observed on very harsh sites at extremes of altitude (e.g. *E. gunnii, E. vernicosa, E. urnigera* and *E. coccifera*) and drought (e.g. *E. viminalis* and *E. tenuiramis*) (Davidson *et al.* 1981, Potts 1985). Genetic variation in ontogenetic development has been demonstrated in *E. gunnii* by Potts (1985) who noted that the genetic component of the variation matched the plastic response, i.e. the populations from the most extreme sites retained the juvenile foliage type longer than seedlings from other populations. Substantial genetic differentiation on the basis of ontogenetic development has also been recorded in *E. nitens,* where the persistence of juvenile foliage was very clearly associated with faster growth rates (Pederick 1979). According to Pederick, the superior growth of the juvenile form may be due to a greater leaf area, and possibly because their horizontal orientation allows for the interception of more solar radiation than the vertical orientation of the adult leaf form.

Comprehensive studies of morphological variation have been undertaken in populations of *E. camaldulensis* (Grunwald and Karschon 1983). Discontinuous variation was noted for leaf size and shape, and clinal variation with latitude and longitude was found for other characters. Increasing latitude was associated with decreases in lignotuber percent, oil gland density and index of leaf fading, and with increases in sclerophylly, epicuticular waxes and frost resistance. Lignotuber percent also showed an increase with longitude. Although the data clearly indicated that the *E. camaldulensis* ecotypes were closely correlated with major drainage systems, Grunwald and Karschon did not attempt to define these morphological differences in terms of adaptations to specific environmental factors. In fact, they showed that the between-family variation was high, suggesting that relatively equal selection pressures may operate within the range of the species.

Population differences in the development of lignotubers has been cited as evidence of adaptation in a number of eucalypt species. The pattern of variation is distinctive between provenances of *E. camaldulensis*, with all seedlings developing lignotubers in the northern

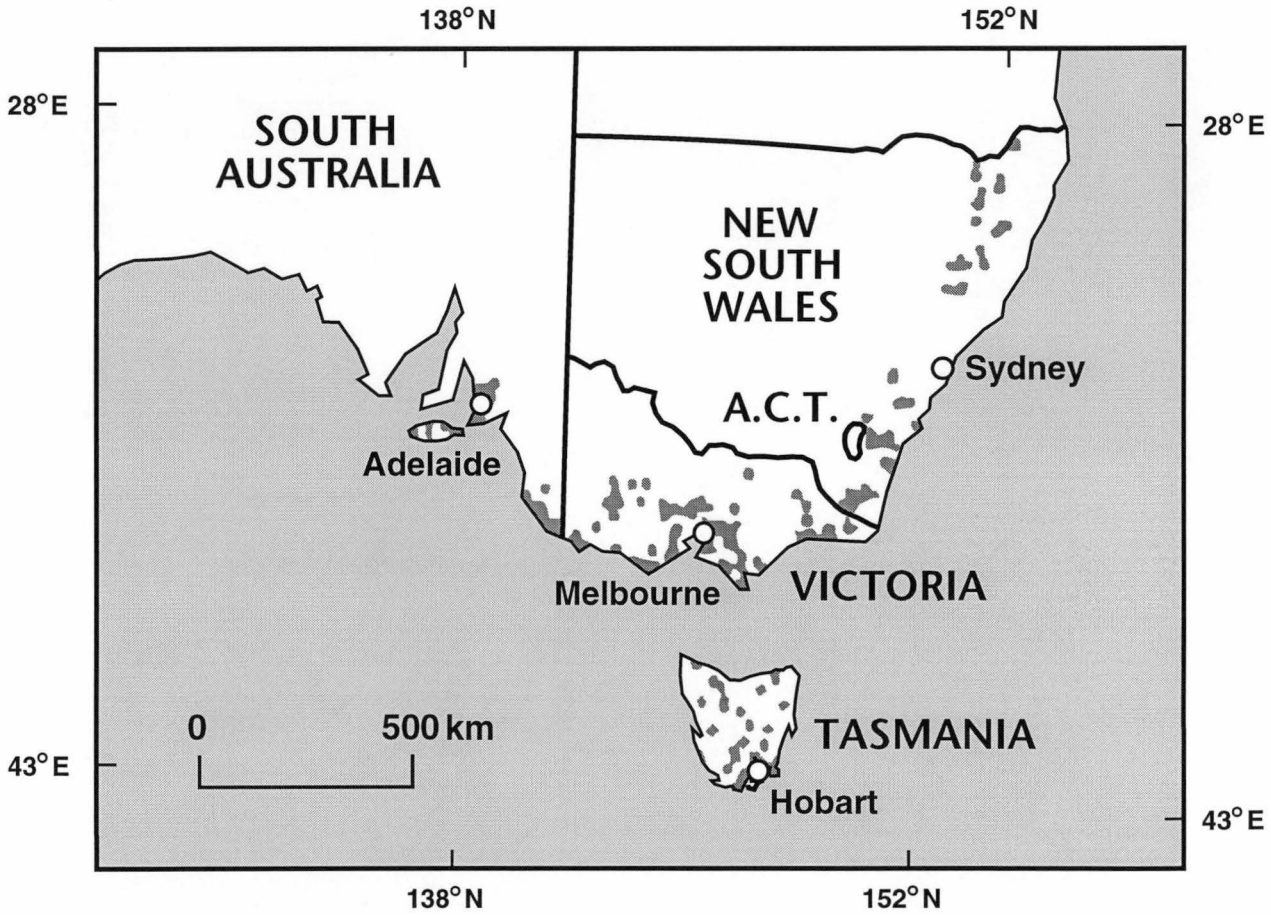
populations and very few or none in the southern populations (Eldridge *et al.* 1993). Ecotypic variation has been observed in other species, including *E. viminalis* (Ladiges and Ashton 1974), *E. ovata* (Clucas and Ladiges 1979), *E. obliqua* (Green 1971), *E. camaldulensis* (Eldridge *et al.* 1993) and *E. gunnii/E. archeri* (Potts 1985). In these species, an increase in lignotuber development has been associated with populations from more open forest types that are prone to frequent drought or wildfires.

Variation in wood properties has also been noted between provenances of several eucalypts. Phenotypic variation in pulp yield was reported for different populations of *E. globulus* in Tasmania but the genetic component of variation was not determined (Turner *et al.* 1983). Provenance trials in *E. obliqua* indicated that both pulp yield and strength were positively correlated with the growth rate of the populations, but that there was no geographic pattern to the variability (Matheson *et al.* 1986). Significant provenance variation has been found in *E. nitens* with respect to some wood properties, such as longitudinal strain, fibre dimensions, basic density, compressive strength, moduli of elasticity and rupture, crushing strength and hardness (McKimm 1985a,b, McKimm and Ilic 1987). However, the variations were generally of relatively low magnitude, in contrast to the very substantial differences that have been noted for growth rate and other morphological characters (Pederick 1979). Provenance variation in wood density has also been recorded in *E. urophylla* by Ngulube (1989) and *E. camaldulensis* (Eldridge *et al.* 1993). In a review of the *E. camaldulensis* studies, Eldridge *et al.* (1993) noted that the southern provenances had faster growth, lower wood density and higher shrinkage than the northern provenances.

5. Genetic variation in *E. obliqua*

Eucalyptus obliqua is a widespread and economically important species of south-eastern Australia. Its natural range extends from northern New South Wales to southern Tasmania and as far west as Adelaide, South Australia (Figure1.1 after Boland *et al.* 1985). The altitudinal range varies from sea level to 750m in Tasmania and from near sea level to 1000m in Victoria and to 1200m in northern NSW (Boland *et al.* 1985). It occurs on a wide range of soil types and within a rainfall range of 750 to 1250 mm (Green 1971). The summers are mainly mild and the winters cool to cold with 10-100 moderate to severe frosts per year and snowfalls at the higher altitudes (Boland *et al.* 1985).

Figure 1.1 **Natural distribution of *E. obliqua* (after Boland *et al.* 1985)**



Eucalyptus obliqua occupies a broad ecological range and populations show large phenotypic variation. On high quality sites, the stands occur as wet eucalypt forests 45 to 90m in height (Boland *et al.* 1985). On sites with lower growth potential, the stands consist of short, poorly formed trees in open forest or woodland communities. Within the main range of its distribution, *E. obliqua* occurs as pure stands or as a dominant or co-dominant species in mixtures with other species. In Victoria and NSW, associated species include *E. fastigata, E. nitens, E. cypellocarpa* and *E. viminalis* (Boland *et al.* 1985). In Tasmania, mixtures occur with *E. regnans, E. globulus, E. viminalis, E. amygdalina* and *E. delegatensis*. In this State, the species is replaced by *E. regnans* on the more fertile and moister sites, by *E. delegatensis* on the colder sites above 300m in altitude and by peppermint species (*E. amygdalina, E. pulchella* and *E. nitida*) on the drier or infertile sites. The boundaries between these species may be sharp, in response to distinctive changes in environmental factors, or more diffuse, leading to the formation of ecotones within a mosaic pattern corresponding to the ecological preferences of each species. Within these ecotones, the formation of hybrid zones may occur with other ash species; *E. regnans* (Ashton 1958) and *E. delegatensis* (Duncan 1989) and in rare situations, with the peppermint species *E. pulchella* (Potts and Reid 1983) and *E. nitida* (Duncan 1989). Intermediate populations of *E. obliqua / E. regnans* may also have arisen due to long term introgressions; from gene mutations and selection; or as a relic of a common ancestor (Ashton 1981). The extent of these intermediate populations may vary, depending upon the relative selection pressures operating on the gene pools within each site. Intermediate populations have traits such as bark thickness and lignotuber development which are intermediate between the parents, thus conferring higher fire resistance than for pure *E. regnans,* but lower resistance than for pure *E. obliqua* (Griffin and Eldridge 1980). Growth of the intermediate populations may be equal to the most vigorous populations of *E. regnans* (Griffin and Eldridge 1980) and much faster than other sources of *E. obliqua* (Pederick 1974). These combinations of traits may give the intermediate populations a selective advantage over the pure form of either *E. regnans* and *E. obliqua* on sites where the environmental transition between stands of the two species is gradual. Ashton (1981) notes that in Victoria, intermediate populations form large stands on maritime mountain sites, but are much more limited in extent or absent on sites in the Central Highlands where there is a steeper environmental gradient. Such inter-specific variation may be an important contribution to the total level of intra-specific variation within *E. obliqua* on various sites. For example, Griffin and Eldridge (1980) found that there was as much variation in growth traits among intermediate populations as between the pure forms of the two species.

Eucalyptus obliqua is included in the Series *Obliquae*, Section *Renantheria* of the Subgenus *Monocalyptus* under the informal taxonomic classification described by Pryor and Johnson (1971). This series also includes the widely distributed species: *E. regnans, E. delegatensis, E. fastigata, E. sieberi* and *E. pauciflora.* Intraspecific variation within *E. obliqua* was recognised in five formal varieties by Blakely (1934). However, Green (1971) subsequently found that only one variety (*E. obliqua* var. *megacarpa)* from Furner (South Australia) was worthy of separate taxonomic consideration.

Evidence for genetic differentiation within *E. obliqua* was provided by the isozyme studies of Brown *et al.* (1975) who demonstrated clear differences in the allelic frequencies of four provenances from NSW, South Australia, Victoria and Tasmania.

Genecological studies into the species were initiated by Green (1971) who collected open-pollinated seed from 22 localities throughout Australia. Green's localities were selected systematically from a grid superimposed over the natural range of the species. At each site, ten trees were randomly sampled within an area of about 2.5 km^2. Progeny from each seedlot were subsequently planted in three separate planting trials, at two sites in Victoria and at one site in Tasmania (Brown *et al.* 1976). The Tasmanian trial included additional seedlots that were collected to evaluate the performance of progeny from four different sites within 2km of each other at Strathblane in the Southern Forests of Tasmania. The four sites had site indices (i.e. estimation of mean height of predominant trees at age 50 years) of 18m, 29m, 34m and 50m. Further details are provided in chapter 3. These collections have been used as the basis for a number of studies (Green 1971, Brown *et al.* 1976, Nicholls and Matheson 1980 and Matheson *et al.* 1986). The results from these studies have indicated significant genetic variation between provenances and between families within provenances, as summarised below.

Phenotypic variation in parental populations - morphological characters such as tree height and diameter varied significantly between provenances with the tallest trees in the wet forests at Maydena (Tasmania) and the shortest in the South Australian populations. Leaf and capsule dimensions also demonstrated highly significant variation between and within provenances (Green 1971).

Seed germination - the difference in the rate of germination between stratified and unstratified seed was significant for only one provenance and there appeared to be no relationship between provenance and the response to stratification (Green 1971).

Seedling characters - controlled environment studies indicated genetic variation in seedling characters such as height growth, leaf dimensions and lignotubers. Furthermore, there were significant parent-progeny correlations for plant height and leaf length/breadth ratio (Green 1971). In the planting trials established by Brown *et al.* (1976) there were significant differences to age 43 months for seedling survival, height, diameter and volume growth. The greatest source of variation was between provenances; variation between families was also significant but amounted to less than 5% of the variation due to provenances. There were also significant interactions between planting sites and provenances. The Tasmanian provenances did not appear to be highly variable, with similar results being achieved on all planting sites. In particular, there were few differences between the Strathblane populations, thus providing no indication of differentiation at the fine geographic scale, despite substantial differences in site quality.

Wood characteristics - Nicholls and Matheson (1980) found that there were highly significant differences between provenances but not between families for the traits of bark thickness, heartwood proportion and incidence of kino veins. Significant differences between provenances and families were found for latewood ratio and for within-ring density range. No differences were recorded for wood density. In subsequent studies, Matheson *et al.* (1986) recorded significant differences between provenances for pulp yield and strength but not for basic density. In contrast, variation between families was significant for basic density and pulp strength but not for pulp yield. Matheson *et al.* concluded that provenances which had the fastest growth produced the strongest pulp and the highest yield of pulp.

The studies of Brown *et al.* (1976) indicated that whilst there were statistically significant genotype-environment interactions in growth and survival over the three planting sites, the magnitude of the interactions was small. This is not surprising in view of the relative similarity of these favourable sites (altitude 150-370m, annual rainfall 1000-1300mm). Superior vigour of specific families has been observed to occur across a more diverse range of planting sites and often the non-local seed sources grow much faster than the local sources. For example, Pederick (1974) found that populations from the highest site qualities grew better than the local populations, even on lower quality sites. However, Brown *et al.* (1976)

argue that superior growth may not confer an advantage on sites outside the normal range of the species. These authors report that the families used in their main study were also planted on a cold, high altitude site near Canberra. Here, the ranking of populations for survival and good growth was different to the other three (mild) sites, with the most successful populations from northern NSW.

The differentiation of populations on highly contrasting sites was investigated by Anderson and Ladiges (1978, 1982) and Anderson (1982a, 1982b) who clearly established the existence of populations that were adapted to particular soil types ("edaphic ecotypes"). This work compared the growth of three different populations of *E. obliqua*: one population from a tall forest, high rainfall site with acidic soil; and two populations from open forests on low rainfall sites, one with calcareous (neutral) and the other with calcareous (alkaline) soils. The two calcareous populations were located within about 4km of each other, and both were about 30km away from the acidic population. The results of these studies provided evidence of genetic differentiation between the acidic population and the calcareous populations. However, differences between the two calcareous populations were very minor. Glasshouse trials indicated that the acidic population was most susceptible to lime chlorosis and possible induced P toxicity, resulting in reduced growth when grown on calcareous soils of intermediate pH (Anderson and Ladiges 1978). Planting trials confirmed these differences, with the acidic population growing significantly faster than the calcareous populations on the acidic soil site, and showing poorer growth on the calcareous site (Anderson and Ladiges 1982). These trials also demonstrated that the seedlings from the open forest (calcareous) sites developed lignotubers much faster than the seedlings from the tall forest (acidic) site. Further investigations indicated that the calcareous populations are adapted to the calcareous sites because they have a more efficient mechanism for absorbing and retaining Fe in the presence of high concentrations of external P, thus reducing the potential risk of P toxicity (Anderson 1982a). The adaptation also appears to extend to processes such as germination, since the calcareous populations were able to maintain a high germination capacity at a wide range of Ca concentrations, whereas the germination capacity of the acidic population was reduced at high Ca levels (Anderson 1982b).

Overall, the various studies of *E. obliqua* have been able to demonstrate significant genetic variation at both the provenance and family levels. Both continuous and discontinuous forms of variation have been noted. The distribution pattern of *E. obliqua* (Figure 1.1) is similar to

that of *E. delegatensis* and could therefore be described as 'widespread' under the classification system of Moran (1992). According to the isozyme studies reviewed by Moran, the widespread species of eucalypts tend to have high levels of both total genetic diversity and variation within populations, but low levels of variation between populations (see Table 1.1). However, high levels of variation between-populations can be found in widespread species that have a disjunct distribution, such as *E. nitens* in which 30% of the total diversity is due to variation between populations, compared to the average figure of about 15% for other widespread species (Moran 1992). Moran believes that much of the variation between populations can be attributed to distinctive geographic boundaries. For example, in *E. delegatensis* 12.5% of the total variation is due to differences between populations. Moran apportions about two-thirds of this figure (8%) to differences between regions and the remaining one-third (4%) to differences between populations within regions.

In summary, it could be expected that genetic variation within *E. obliqua* would conform to a pattern of high levels of total variation, with large variation within populations, and relatively low levels of variation between populations. The exception to this pattern may occur between populations that are geographically isolated, or between populations that occupy very distinctive environments.

6. Potential impacts on the maintenance of genetic diversity in forest trees

The genetic diversity of forest trees can be modified by a number of impacts, including forest clearing, forestry and other management activities, and environmental change.

6.1 Forest clearing

Forests occupy 3.317 million ha or 49% of the total land area in Tasmania (Forestry Commission 1994a). This area is estimated to represent about 82% of the total forest cover that existed at the time of European settlement (Brown 1992). Eucalypt forests cover about 2.5 million ha of which about 50% is available for wood production (Forestry Commission 1994a). Forest clearing, primarily for conversion to agricultural crops or tree plantations

continues at a rate of about 6,000ha per year, representing a major threat to the conservation of some plant communities (Kirkpatrick 1991). Land clearing also increases the fragmentation of some forest types, resulting in restricted gene flow and higher rates of inbreeding (Ledig 1988).

6.2 Forest management

The impact of forest operations on genetic diversity will depend upon the felling and/or regeneration practices that have been implemented over time. Intensive selection through a regime of early and repeated thinnings could lead to a narrowing of diversity in favour of individuals with desired attributes such as superior vigour and form. In contrast, negative or dysgenic selection could lead to a narrowing in favour of individuals with less vigour or poorer form. However, the potential impacts of either regime depend upon the intensity of tree selection, and on the alternative sources of regeneration for the next generation of forest. For example, selective fellings that are applied to the mature component of a stand may exert little selective pressure, for two reasons. Firstly, natural selection forces in a eucalypt forest may typically reduce initial stocking levels from as high as 2.5 million seedlings per ha (Ashton 1976) to about 100-200 stems per ha in a mature forest (Jackson 1968), indicating that substantial selection for adaptation to the site has already occurred. Secondly, the presence of mature trees of poor phenotypic form may reflect site heterogeneity or damage by factors such as fire and natural windfall. However, more intensive regimes may exert a greater selection pressure, particularly if a low number of retained trees are relied upon to provide the sole source of propagules for regeneration. The greatest impacts are likely to result from seedtree systems or from clearfell systems that use regeneration methods such as artificial sowing and planting.

In a seedtree system, the seed source for regeneration may be provided by as few as seven trees per ha (Forestry Commission 1994b). This may equate to even fewer trees per species in a mixed species stand. Stands regenerated by this system are likely to be made up of patches of related individuals (full-sibs, half-sibs and selfs) that are even more extensive than the patches that have been observed in naturally regenerated forests (Richmond 1971). Thus, seedtree regeneration systems may result in a shift in genetic make-up if the retained trees comprise a low number of individuals with common attributes that may not necessarily be representative of the heterogeneity within the stand. In contrast, genetic diversity may be

maintained or even increased by the retention of a higher number of trees as seed sources. For example, the shelterwood system has been shown to increase genetic diversity in stands of Douglas Fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco), probably because of the positive effects of a high out-crossing rate among a large effective population of retained trees (Neale 1985).

Clearfelling systems that rely upon regeneration methods such as artificial sowing or planting provide the greatest opportunities for modifying gene pools. In the extreme case, sowing or planting with non-local species can result in the loss or reduction of local species. At the individual species level, the maintenance of local genetic diversity will depend upon the source of the seed used in regeneration operations. The effect of using off-site seed will depend upon whether seed sources are genetically equivalent or not. Differences between populations will result in some non-local seed sources growing more vigorously than the local seed, whilst others may be less adapted and grow very poorly (Pederick 1976). Where seed is collected from a large number of widely spaced individuals, artificial sowing can theoretically increase the genetic diversity within a site. This is because the mixing of seed from individual trees prior to sowing tends to minimise the degree of relatedness that would otherwise occur within naturally regenerated patches. In contrast, a substantial reduction in genetic diversity may result from the use of seed collected from a small number of individuals, particularly if the trees are isolated or closely related and therefore likely to produce a high proportion of seed that is selfed, full-sib or half-sib (Pederick 1987). Isozyme studies of Norway spruce (*Picea abies* Karst.) reported by Gömöry (1992) have confirmed that the genetic structure of artificially regenerated stands may be substantially different to those of virgin forests. In the artificially regenerated stands the genetic diversity is generally decreased, probably as a result of the restricted number of maternal trees that contribute to the sowing mix (Gömöry 1992). In plantations, there is a greater likelihood of lower genetic diversity than for artificially sown areas because much less seed is initially required (Pederick 1976).

The use of foreign species and provenances may also have effects on the genetic diversity of stands in proximity to the regeneration area by providing a pollen source for potential inter- and intra-specific hybridisation.

6.3 Other management activities

Eucalypt forests are ecologically adapted to periodic fire and any alteration to natural fire regimes may impact on the structure and composition of the forests. In the absence of fire, mixed eucalypt forests are replaced by rainforest (Gilbert 1959), leading to the loss of the local eucalypt genotypes. In the drier forest, repeated burning may prevent the establishment of eucalypt regeneration by killing the sensitive seedlings and encouraging the development of a fire-adapted understorey such as grass or bracken. This process may be exacerbated on sites subjected to continued grazing by domestic animals such as sheep. Fragmented forests in agricultural locations are most susceptible to potential changes in genetic structure for two reasons. Firstly, in the absence of regeneration, the progressive loss of trees due to selective fellings and natural mortality, may deplete the final stocking of trees to very low levels, resulting in an open woodland structure, or isolated clumps or individuals within an open landscape (Orr 1991). Thus genetic diversity may be decreased as a result of a smaller population sizes and lower rates of gene flow between remaining individuals or populations. Secondly, the natural environment may be highly modified as a result of the cumulative effects of factors such as increased exposure, altered fire regime, grazing, increased predation by mammals and insects, and soil changes associated with the long term modification of the understorey or fertiliser application. These changes may impose severe selection pressures, resulting in the loss of genotypes that are not adapted to the new environment. Where the environmental changes are also accompanied by a lack of recruitment, the opportunities for evolutionary adaptation to the new environment are greatly diminished (Kile 1981). The widespread decline of trees such as *E. viminalis* from dryland areas of Tasmania (McMurray 1983) exemplifies the potential loss of genetic diversity associated with such changes.

6.4 Environmental change

Forest trees depend upon the maintenance of high levels of genetic diversity and heterozygosity in order to retain an adaptability to changing environments (Gregorius 1989). Genetic diversity is greater in large populations that have remained intact over long periods of time, compared to populations that have colonised areas in more recent times (Ledig 1988). Ledig found that the southern forests of America that had survived during glacial periods had twice the genetic diversity of the more northern forests that were re-established by colonisation following the last glaciation.

Substantial climatic fluctuations have occurred in Australia since the evolution of the eucalypts over 10 million years ago (Kemp 1981). The present distribution and patterns of genetic diversity within eucalypt species in Tasmania probably reflects the environmental changes that accompanied the major climatic warming following the last glaciation 10,000 years ago (Macphail 1980). Palynological evidence indicates that, prior to European settlement, an equilibrium between vegetation, climate and fire existed in Tasmania for about 3,600 to 6,000 years (Macphail 1980). Current vegetation patterns are now regarded to result from the interactions, in time and space, of climate, fire, vegetation, soil fertility and human practices (Gilbert 1959, Jackson 1968, Macphail 1984, Ellis and Thomas 1988, Podger et al 1988).

Recent climatic changes present a major threat to forest tree gene pools. Major impacts are likely to result from large increases in atmospheric carbon dioxide and other green-house gasses, increased ultra-violet radiation due to the depletion of the stratospheric ozone layer, and the cumulative effects of local air pollution, including acid rain and smog (Pittock 1987). These changes are forecast to continue at a very rapid rate, which may exceed the rate at which long generation plants such as forest trees can adapt (Gregorius 1989). The selection of individuals that are able to tolerate the environmental changes may lead to the loss of species, or to within -species population changes. Karnosky *et al.* (1989) suggest that the widespread loss of genotypes sensitive to air pollution in Europe and North America may lead to decreased genetic diversity within the surviving populations, thus reducing the future adaptability of the species.

7. Objectives of the current study

The current study sought to further investigate the pattern of genetic differentiation within *E. obliqua*, one of the most widespread and commercially important tree species in Tasmania. Previous research has provided evidence of differentiation within *E. obliqua* at the broad provenance level with significant variation between populations from Victoria, South Australia, New South Wales and Tasmania (Green 1971, Brown *et al.* 1976). There is also evidence to indicate that differentiation may occur on highly contrasting sites that are separated by distances of about 30 km (Anderson and Ladiges 1978, 1982, Anderson 1982a, 1982b).

Within Tasmania, *E. obliqua* has a widespread and relatively continuous distribution pattern. According to Moran (1992), such species are likely to have high levels of variation within populations, but relatively low levels of variation between populations. This view was supported by the results from provenance trials, which indicated little difference in growth between the five Tasmanian populations (Brown *et al.* 1976, Matheson *et al.* 1986). These populations were taken from across the geographic range within Tasmania. However, they were primarily wet forest types, and did not cover the more open forest types, with the exception of one series of four collections across a range of forest types within the Strathblane area of the Southern forests. Brown *et al.* (1976) and Matheson *et al.* (1986) found no evidence of differentiation between the Strathblane populations and concluded that the selection of superior genotypes at such a fine geographic scale would not be worthwhile even though populations may appear to be very diverse.

Eucalyptus obliqua exhibits marked phenotypic variation across its natural range within Tasmania. Often this variation is associated with ecological gradients from tall, wet forest types on productive sites, to open forest types on dry or harsh sites. The transition between forest types at the local scale is often related to differences in physiographic location and may be gradual or abrupt, depending upon the magnitude of changes in environmental factors such as water availability, drainage, soil fertility and climate.

Forest managers seek to ensure that the seed used for reforestation purposes is genetically adapted to the area (Forestry Commission 1991). Many forest coupes are heterogeneous, containing a mosaic of phenotypically different forest types. Much of the variation in phenotype can be attributed to differences in site quality. However, information on potential genetic differentiation between populations of *E. obliqua* at the local site level was limited, and there were continuing concerns about the potential loss of genetic diversity resulting from inappropriate seed collection and sowing programmes (Duncan 1985).

The objective of the current study was therefore to further evaluate the potential genetic variation between populations at the local geographic scale, corresponding to the 'site' level of variation (*sensu* Zobel and Talbert 1984). The study was designed to compare a number of attributes that may have adaptive importance for natural populations on specific sites. The study was not designed to provide an evaluation of differences in terms of selection for tree-breeding purposes.

CHAPTER 2 SAMPLING OF POPULATIONS AND ESTABLISHMENT OF PLANTING TRIALS

This chapter provides general information on the selection and description of the populations and sites used in the studies. Details on the methodologies used in the individual experiments are presented in the following chapters.

1. Location and description of seed sources

The seed sources used in these studies were selected from two geographical areas within Tasmania, at Forestier and Lune (Figure 2.1). These areas are regarded as comparable seed zones on the basis of the close matching of the environmental attributes of altitude, dryness and coldness (Forestry Commission 1991). However, they are identified as separate geographic sources because they are separated by a direct distance of 100km (largely across a water surface) or an indirect distance of about 130km via a continuous land surface. Both areas contain distinctive ecological gradients that are commonly found within the extensive range of *E. obliqua*. The Forestier area comprises a series of ecological gradients related to changes in topographic position and aspect. Typically, wet sclerophyll forests are found in sheltered gullies and on the lower slopes of southern aspects. These forests grade into more open dry sclerophyll forests on the ridge-tops and on the higher slopes of northern aspects. In the Lune area, major ecological changes occur along the gradient from well-drained slopes to poorly drained plains. The slope positions are occupied by tall wet eucalypt forests whilst the plains are characterised by open woodland with a sedgy understorey.

Two distinctive forest types were selected from the range of an ecological gradient within each provenance (Figures 2.2 to 2.5) as follows:

• Forestier provenance:

Gully population - wet sclerophyll forest within moist gully;

Ridge population - open dry sclerophyll forest along exposed ridge;

•Lune provenance:

Mid population - wet sclerophyll forest from well-drained mid-slope position;

Plain population - open woodland on lower slopes adjoining poorly drained plain.

Figure 2.1 Location of Forestier and Lune provenances

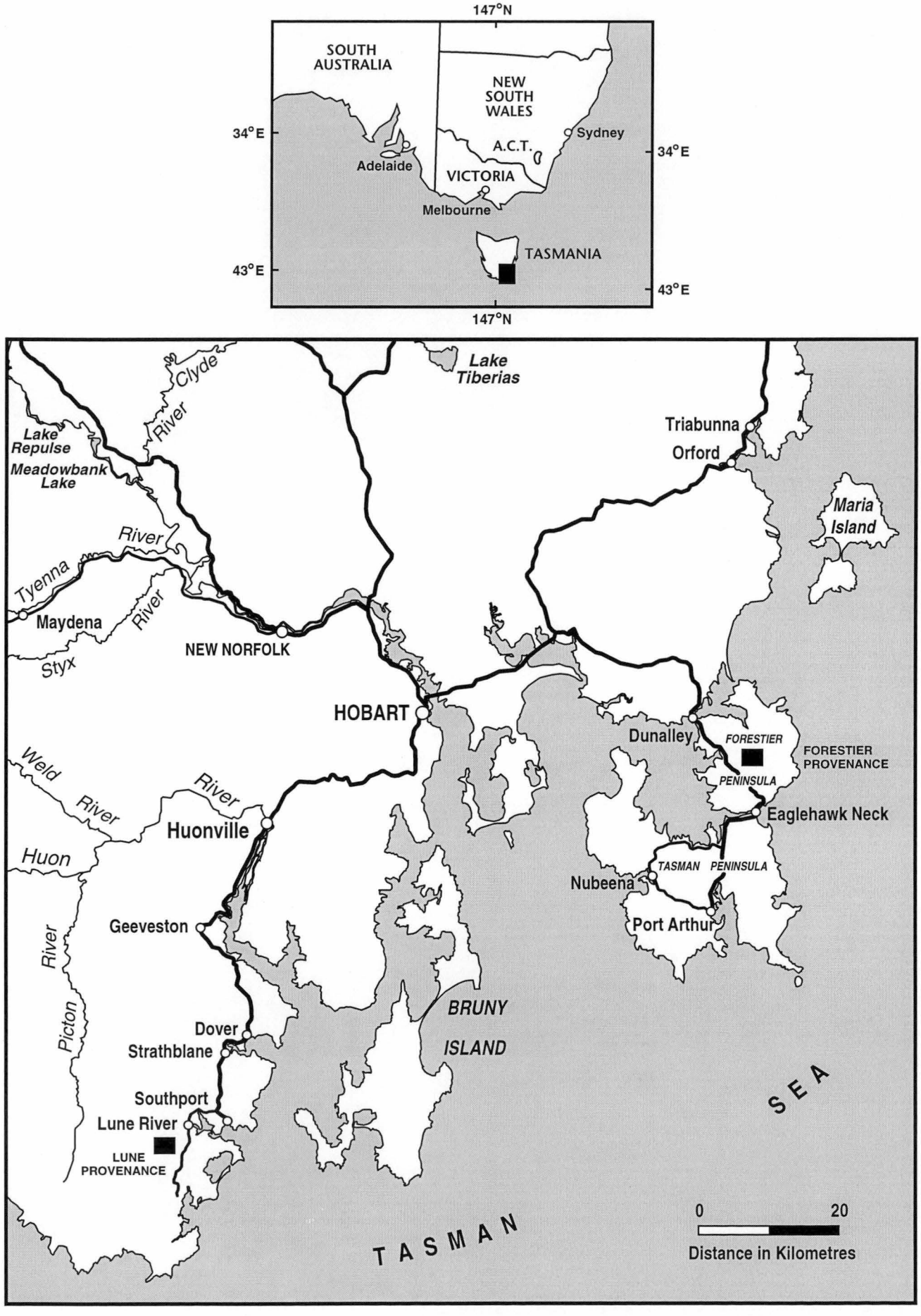


Figure 2.2 Location of Forestier Gully and Ridge populations

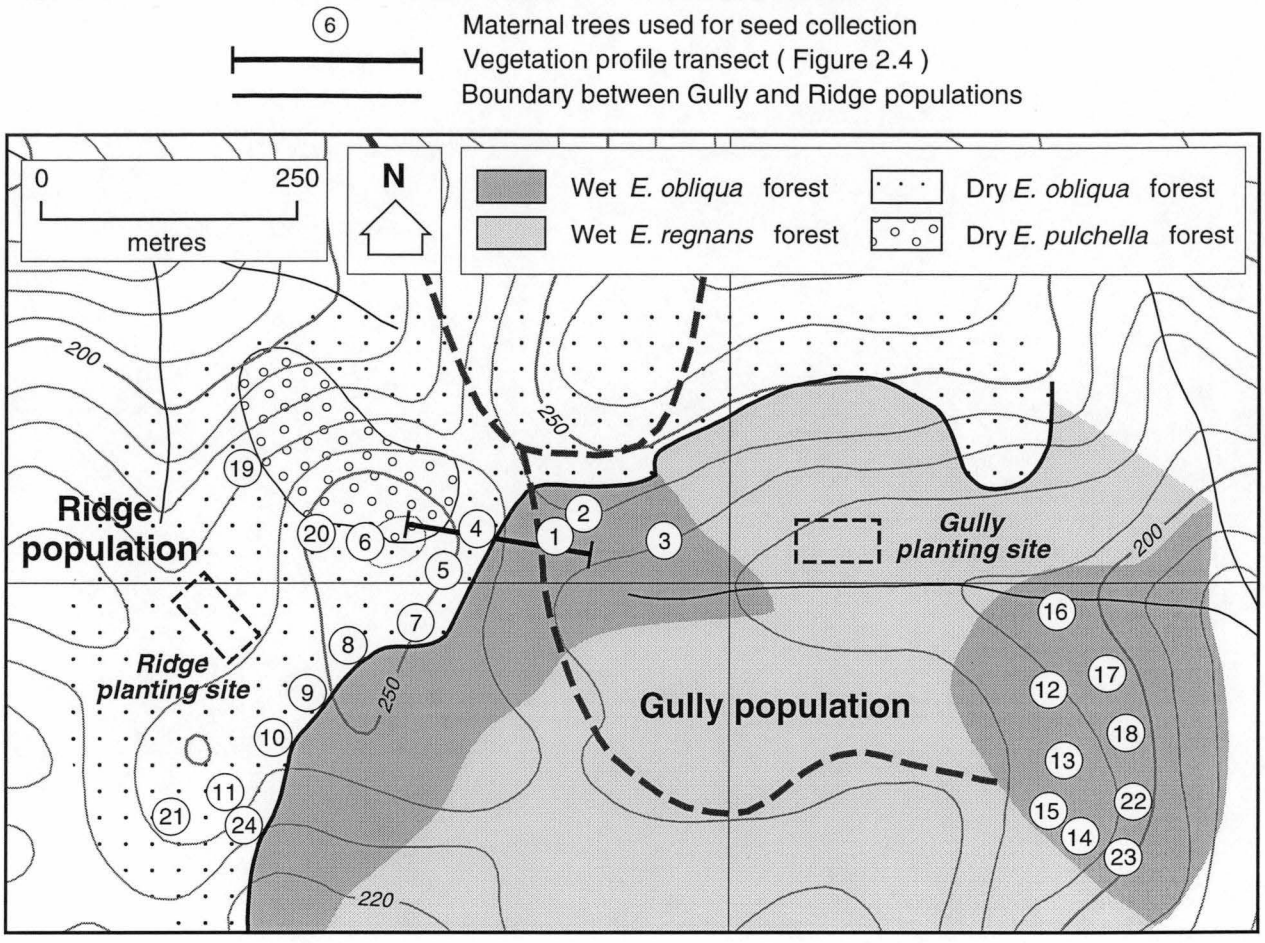


Figure 2.3 Location of Lune Mid and Plain populations

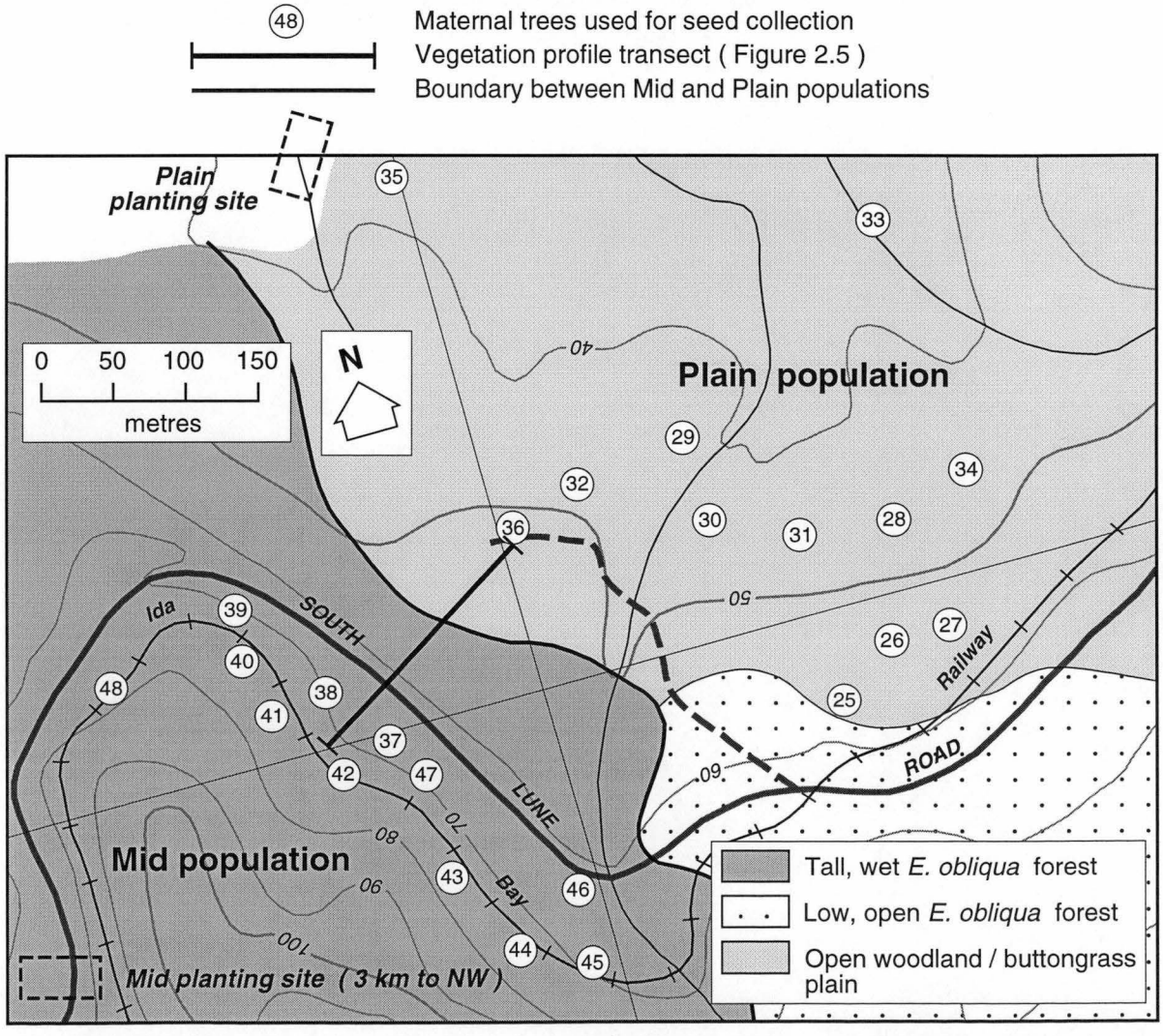
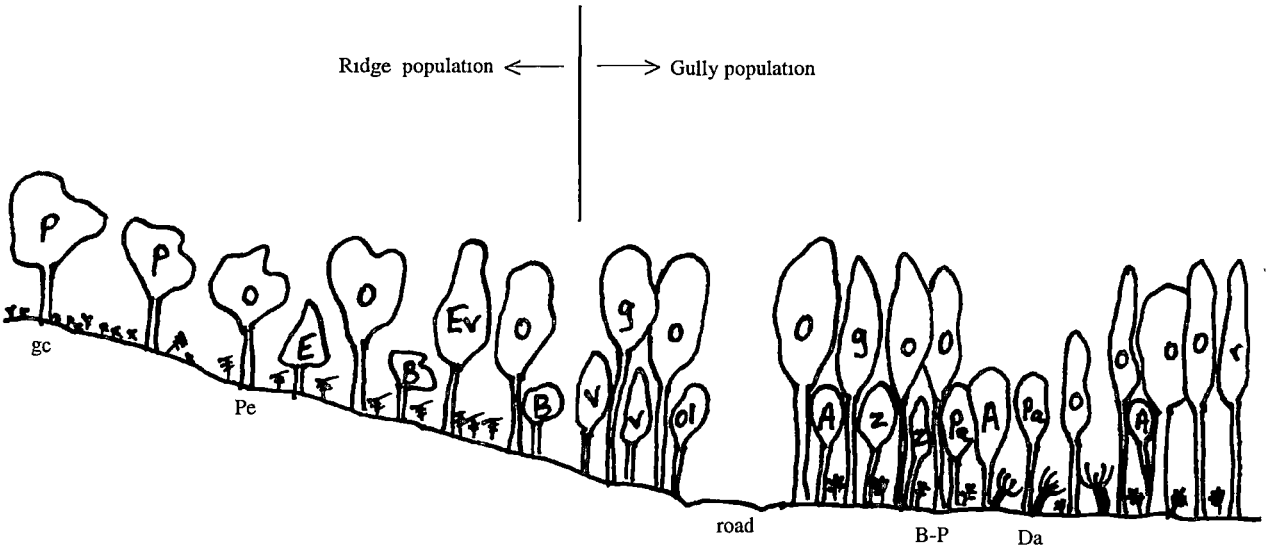
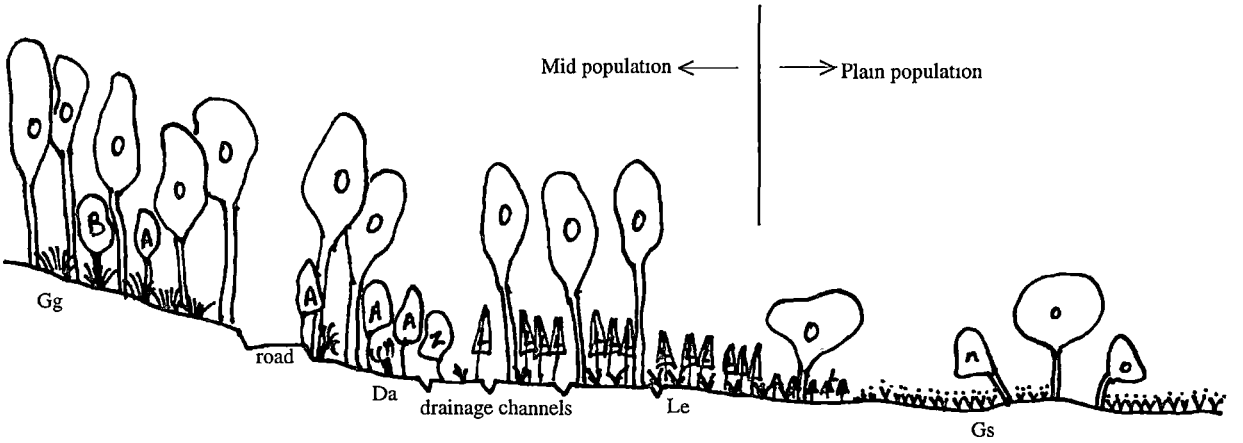


Figure 2.4 Vegetation profile along transect between Gully and Ridge populations at Forestier.



Species code: O = *E obliqua;* P = *E. pulchella;* Ev = *E. viminalis;* r = *E. regnans;* g = *E. globulus,*
A = *Acacia melanoxylon;* z = *Zieria arborescens;* Pa = *Pomaderris apetala,*
v = *Acacia verticillata;* ol = *Olearia argophylla;* E = *Exocarpos cuppressiformis;*
B = *Banksia marginata;* gc = ground cover of *Lomandra, Goodenia, Lomatia, Pimelea* spp
Pe = *Pteridium esculentum,* B-P = *Blechnum nudum* and *Polystichum proliferum,*
Da = *Dicksonia antarctica.*

Figure 2.5 Vegetation profile along transect between Mid and Plain populations at Lune.



Species code: O = *E .obliqua;* n = *E. nitida;* B = *Banksia marginata;* A = *Acacia verticillata;*
Z = *Zieria arborescens;* L = *Leptospermum scoparium;* Gg = *Gahnia grandis;*
Da = *Dicksonia antarctica;* Le = *Lepidosperma elatius;* Gs = *Gymnoshoenus sphaerocephalus.*

For the purposes of this study, the Forestier and Lune areas are regarded as *provenances*. The two forest types selected within each provenance correspond to variation at the *site* level (Zobel and Talbert 1984). The four seed sources will be referred to as *populations* in the current study in order to avoid confusion with the terminology used to study levels of variation due to the (planting) site in subsequent experiments (section 4).

Site descriptions for the populations are provided below. Soil profiles were sampled and descriptions were derived from pits established at representative profiles within each population (Table 2.1). The presence of plant species (Table 2.2) was recorded by surveys conducted at 12 sites within each population as described in section 2. Climatic data are summarised in Tables 2.3 and 2.4.

1.1 Forestier Gully

The Gully population is located in compartment FT2d of the Forestier forest block (Figures 2.1 and 2.2). The selected trees occupy a sheltered easterly aspect along a moist gully. The altitude ranges from 180-240m and the slopes average 5-10%. Soils are derived from Jurassic dolerite and are defined as xanthozems under the Great Soil Group classification (Stace *et al.* 1968) or brown ferrosols under the classification proposed by Isbell (1992). The A1 horizons comprise a dark grey-brown clay-loam about 9cm deep with an abrupt boundary to a light medium clay with prominent yellowish-brown mottles and dolerite fragments. Soil drainage is moderate, with slow permeability. The forest type is predominantly *E. obliqua* wet sclerophyll forest (community OBO 110 *sensu* Kirkpatrick *et al.* 1988, Photograph 2.1), grading into patches of mixed *E. obliqua / E. regnans* wet sclerophyll forest (community REG 1000) and pure *E. regnans* wet sclerophyll forest (community REG 1001). Selective logging operations were conducted throughout the forest about 60 to 80 years ago. This logging was closely followed by wildfires, which have resulted in the current mosaic of forest structures containing patches of scattered individuals of old growth trees 150 to 300 years old and regrowth trees 60 to 80 years old. There appears to have been no further fires (and hence no further age classes of regeneration) since the fires that gave rise to the 60 year old regrowth. Photo-interpretation (PI) maps indicate that the old growth trees provide 5 to 70% crown cover with an average height of 27 to 34m; the regrowth trees account for 10 to 50% crown cover with an average height of 37 to 44m (Forestry Commission 1988). The overstorey trees comprise *E. obliqua, E. regnans* and *E. globulus*, with *Acacia melanoxylon* as a scattered sub-dominant 15 to 27m in height. A shrub layer 8 to 15m in height dominates the understorey, containing species such as *Pomaderris apetala, Acacia verticillata, Zieria*

arborescens, Cyathodes glauca and *Acacia melanoxylon.* The ground layer is predominantly covered with litter and mosses. Rainforest species such as *Atherosperma moschatum* and *Hymenophyllum* spp. are present in undisturbed, sheltered patches. Annual rainfall is about 890mm and all months receive greater than 50mm. The distribution shows a winter bias, with the wettest months of June-August receiving about 50% more rainfall than the driest months of January-March. Annual pan evaporation is about 20% higher than the annual rainfall, with deficits occurring in all months except April to August. Temperatures are mild, with maxima averaging about 21°C in summer and 12 °C in winter. Minimum temperatures are about 12 °C in summer and 5°C in winter. Frosts are relatively common in the months of May to October.

1.2 Forestier Ridge

The Ridge population adjoins the Forestier Gully population within compartment FT2d (Figures 2.1 and 2.2). The selected trees occur along the top and upper north-western slopes of the ridge system that forms the boundary with the Gully population to the south-east. Slopes average about 5 to 20% within an altitude range of 240 to 260m. Soils are derived from Jurassic dolerite and up to 60% of the ground surface is covered by rock fragments. The soil type is a krasnozem (red ferrosol) which consists of a shallow hemic peat organic layer over a light clay loam A1 horizon. Lower horizons are yellowish-red clays that are well drained with moderate permeability. The forest type is dry sclerophyll, dominated by shrubby (doleritic) *E. obliqua* forest (Photograph 2.2) which grades into open grassy *E. pulchella* forest (*sensu* Duncan and Brown 1985) on the driest north-western slope. There is a rapid transition from the wet sclerophyll communities of the Gully population to the dry sclerophyll community of the Ridge population (Photograph 2.5, Figure 2.4). Logging operations do not appear to have been carried out within the Ridge populations, probably because their low sawlog volume precluded them from the selective operations that occurred in the adjoining higher commercial quality wet sclerophyll forests 60 to 80 years ago. A high proportion of deep fire scars in the mature trees indicates a recent history of frequent fires, with the natural fire boundary closely matching and probably contributing to the sharpness of the ecological boundary between the wet and dry sclerophyll communities. The resulting forest structure is unevenaged, with old growth trees up to about 200 years old and 27 to 34m in height providing an overstorey of about 40 to 70% crown cover (Forestry Commission 1988). *Eucalyptus obliqua* is the dominant overstorey species, forming mixtures with *E. amygdalina, E. pulchella, E. globulus* and *E. viminalis.* The forest has an open shrub stratum, 5 to 8m in height, containing species such as *Exocarpos cupressiformis, Banksia*

marginata and *Bedfordia salicina*. The ground cover is dominated by rocks, litter, grasses and a high diversity of herbs and forbs such as *Gonocarpus humilis*, *Pimelea nivea*, *Goodenia ovata* and *Viola hederaceae*. The climatic conditions are equivalent to those described above for the Forestier Gully populations, although differences in aspect and topographic position are likely to result in the Ridge populations being exposed to higher levels of solar radiation, wind and evaporation.

1.3 Lune Mid

The Mid population occurs within the Lune forest block (Figures 2.1 and 2.3). The selected trees occur within a contour range that corresponds to the mid-slope position of a broad north-easterly slope. The altitude range is from 70 to 90m and slopes average 20%. The forest type abruptly changes to open forest and buttongrass plain at an altitude below 50m (Photograph 2.6). Soils are xanthozems (brown ferrosols) derived from Jurassic dolerite and Triassic mudstone. They are characterised by a dark greyish-brown clay loam A1 horizon over deep medium clay horizons. Soil drainage is imperfect, with very slow permeability. The forest type is *E. obliqua* wet sclerophyll (community OB0111 *sensu* Kirkpatrick *et al.* 1988, Photograph 2.3). Selective logging operations and periodic wildfires have occurred within the Lune forest area since the 1880's (Kostoglou 1994). As a result, the Mid population consists of a series of age classes, including scattered old growth trees 150 to 200 years old, and regrowth trees up to 100 years old. PI maps indicate that the old growth trees currently provide less than 5% crown cover, with an average height of 55 to 76m (Forestry Commission 1985). Regrowth stems provide about 70 to 90% crown cover, with an average height of 27 to 37m (Forestry Commission 1985). The overstorey contains a single species, *E. obliqua*, which forms extensive areas of pure stands throughout the Lune and adjoining forests. A dense shrub layer forms an understorey to a height of about 5 to 8m and includes species such as *Acacia verticillata*, *Olearia stellulata*, *Zieria arborescens*, *Bedfordia salicina*, *Melaleuca squarrosa* and *Leptospermum scoparium*. The ground layer is covered with litter, *Pteridium esculentum*, *Goodenia ovata*, *Gahnia grandis*, mosses and ferns. The rainforest species *Atherosperma moschatum* occurs in areas protected from recent disturbance. Annual rainfall is about 1400mm p.a. and all months receive greater than about 70mm. There is a marked winter bias with the months of June-August receiving almost double the rainfall of January-March. Pan evaporation totals about 690mm per year and monthly evaporation only exceeds rainfall during January and February. Temperatures are mild, with maxima of about 20°C in summer and 12 °C in winter. Minima temperatures average about 9 °C in summer and 3 °C in winter. Frosts can occur in any month but are frequent from May to October.

1.4 Lune Plain

The Plain population is distributed on foothill slopes and on slightly elevated islands within a broad, poorly drained buttongrass plain (Figure 2.3). Altitude ranges from 30 to 50m with slopes of 3 to 8%. The soil is derived from alluvial deposits and is defined as a peaty podzol or oxyaquic hydrosol. It consists of a sapric peat layer to 25cm in depth over silty clay loam and medium clay horizons. Soil drainage is very poor and permeability is very slow. The vegetation type comprises a mosaic of sedgy *E. nitida* woodland (sensu Duncan and Johnson 1995) within a buttongrass plain (Photograph 2.4). Frequent burns have occurred as wildfires or as deliberate burns intended to protect the nearby settlements established since the 1880's (Kostoglou 1994). The study area was most recently burnt by wildfire in 1993 and 1989. These fires have probably resulted in a slightly accelerated rate of tree mortality due to butt damage and tree collapse. Previous fires have also given rise to regeneration which has either developed to the sapling and young tree stage or has been held at the lignotuberous seedling stage, depending upon the pattern in the extent and intensity of subsequent fires. The resulting forest structure is predominantly an open woodland of 100 to 200 year old trees, with small and isolated patches of regeneration. There is a sharp boundary between the tall wet forests of the Mid population and the open forests of the Plain (Photograph 2.6). Overall, the forest boundaries have probably receded since settlement as a consequence of the higher fire frequency which would have favoured the expansion of the moorland vegetation (Jarman *et al.* 1988). PI maps indicate that the old growth trees have an average height of 15 to 27m and provide less than 5% crown cover (Forestry Commission 1985). *Eucalyptus obliqua* is the dominant overstorey tree on the higher ground and is replaced by *E. nitida* and *E. ovata* on the lower woodland sites. The understorey consists of a low sedge or shrub layer 0.3 to 2m in height. Species include *Gleichenia dicarpa, Melaleuca squarrosa, Gahnia grandis, Leptospermum scoparium, Gymnoshoenus sphaerocephalus* and Poaceae spp. The general climate for the Plain population is as described for the Lune Mid population with some important microsite variations. The major difference is due to the effect of topography on air drainage and cold temperatures. The plain sites are subjected to cold air drainage which results in extreme vertical temperature gradients. This means that frosts may occur in any season and severe frosts of -3 °C to -7 °C are common from May to November.

Photograph 2.1 Forest type at the Forestier Gully site - wet sclerophyll forest with dense understorey

Photograph 2.2 Forest type at the Forestier Ridge site - dry sclerophyll forest with open understorey

Photograph 2.3 Forest type at the Lune Mid site - wet sclerophyll forest with dense understorey ←

Photograph 2.4 Forest type at the Lune Plain site - open woodland with sedgey understorey ↓

Photograph 2.5 Boundary between the Forestier Gully and Ridge sites (abrupt change in the understorey from tall, dense shrubs in LHS of photograph to low open understorey in RHS)

Photograph 2.6 Boundary between the Lune Mid and Plain sites (abrupt transition from dense eucalypt regrowth in the background of the photograph to *Leptospermum* and then sedges in the foreground).

Table 2.1. Description of soils in the planting sites

Site: **Forestier Gully**
Drainage: Moderately well drained *Permeability*: Slow
Lithology: Dolerite *Surface fragments*: none
Great soil group: Xanthozem *Principal profile form*: Gn3.21
New Australian classification (Isbell): FEABAGDQAEMOW3 - Brown Ferrosol

Horizon	*Depth (cm)*	*Description*
A1	0-9	Very dark greyish brown (10YR3/2) clay loam; moderately developed 10-20mm subangular blocky structure; weak strength, moderately moist, firm; 10-20% subrounded 200-600mm dolerite fragments; many medium roots, field pH 5.6; abrupt boundary,
B21	9-55	Olive brown (2.5Y4/3) light medium clay; 20-50% <5mm prominent yellowish brown (10YR5/8) mottles; strongly developed 20-50mm angular blocky breaking to moderately developed 10-20mm angular blocky structure; smooth-ped; 10-50% distinct clay skins; very firm strength, moderately moist, stiff; 20-50% subrounded 200-600mm dolerite fragments; common medium roots, field pH 5.6; gradual boundary,
B22	55-85	dark yellowish brown (10YR4/6) medium heavy clay; 10-20% <5mm distinct mottles; strongly developed 20-50mm angular blocky breaking to moderately developed 10-20mm angular blocky structure; smooth-ped; 10-50% distinct clay skins; very firm strength, moderately moist stiff; 10-20% subrounded 200-600mm dolerite fragments; common medium roots, field pH 5.7; gradual boundary,
B3g	85-100+	olive grey (5Y5/2) medium heavy clay; 2-10% <5mm prominent strong brown (7.5YR5/8) mottles; moderately developed 20-50mm angular blocky structure; firm strength, moist, firm; few coarse roots, field pH 5.4.

Site: **Forestier Ridge**
Drainage: Well drained *Permeability*: Moderate
Lithology: Dolerite *Surface fragments*: 20-50% stones, 2-10% boulders
Great soil group: Krasnozem *Principal profile form*: Gn3.11
New Australian classification (Isbell): FEAAGCDBEMOV3 - Red Ferrosol

Horizon	*Depth (cm)*	*Description*
O2	1-0	Hemic peat, sharp boundary,
A1	0-12	light clay loam; moderately developed 2-5mm granular structure; very weak strength, moist, soft; 20-50% subrounded 60-200mm dolerite fragments; common medium roots, field pH 5.7; clear boundary,
B1	12-26	light clay; weakly developed 20-50mm subangular blocky breaking to strongly developed 2-5mm granular structure; <10% faint clay skins; weak strength, moist, soft; 20-50% subrounded 60-200mm dolerite fragments; common medium roots, field pH 5.9; clear boundary,
B2	26-70	yellowish red (5YR4/6) light medium clay; <2% <5mm faint dark red (2.5YR3/6) mottles; weakly developed 20-50mm subangular blocky breaking to strongly developed 2-5mm granular structure; weak strength, moist, firm; 20-50% subrounded 200-600mm dolerite fragments; few fine roots, field pH 5.9; gradual boundary,
BC	70-90+	yellowish red (5YR4/6) light medium clay; 20-50% <5mm prominent light olive brown (2.5Y5/4) mottles; massive; firm strength, moist, firm; 20-50% subrounded 200-600mm dolerite fragments.

Site: **Lune Mid**
Drainage: Imperfectly drained*Permeability*: Very slow
Lithology: Dolerite/mudstone*Surface fragments*: 10-20% dolerite stones
Great soil group: Xanthozem*Principal profile form*: Gn3.71
New Australian classification (Isbell): FAABAGDQBEJOX3 - Brown Ferrosol

Horizon	*Depth (cm)*	*Description*
A1	0-12	Dark greyish brown (2.5Y4/2) clay loam; moderately developed 5-10mm angular blocky structure; firm strength, moist, soft; many medium roots, field pH 5.4; gradual boundary,
B1	12-35	greenish grey (5GY6/1) light medium clay; 20-50% <5mm prominent strong brown (7.5YR5/8) and <2% 5-15mm prominent grey (10YR5/1) mottles; moderately developed 10-20mm angular blocky structure; firm strength, moist, soft; common medium roots, field pH 5.3; gradual boundary,
B21	35-90	strong brown (7.5YR5/8) medium clay; 20-50% 5-15mm prominent greenish grey (5GY6/1) mottles; moderately developed 10-20mm angular blocky structure; smooth-ped; 10-50% distinct clay skins; firm strength, moist, firm; few fine roots, field pH 5.3; gradual boundary,
B22	90-140+	light grey (5Y7/2) medium clay; 20-50% 5-15mm prominent yellowish brown (10YR5/6) mottles; massive; smooth-ped, >50% prominent humus coatings; firm strength, wet, firm; <2% subrounded 60-200mm dolerite fragments; few fine roots, field pH 5.2,

Site: **Lune Plain**
Drainage: Very poorly drained*Permeability*: Very slow
Lithology: Alluvial *Surface fragments*: none
Great soil group: Peaty podzol *Principal profile form*: Gn3.91
New Australian classification (Isbell): HYDTFRDWBEMOV4 - Oxyaquic hydrosol

Horizon	*Depth (cm)*	*Description*
P2	25-0	Very dark grey (10YR3/1) sapric peat; wet; abundant medium roots, field pH 4.5,
A1g	0-15	dark grey (10YR4/1) silty clay loam; 2-10% <5mm prominent strong brown (7.5YR4/6) mottles; wet; many medium roots,
B21g	15-55	light medium clay; 2-10% <5mm prominent yellowish red (5YR4/6) mottles; wet; few fine roots,
B22g	55-80+	greenish grey (5GY6/1); <2% <5mm prominent strong brown (7.5YR4/6) mottles; wet; 10-20% angular 6-20mm fragments; few very fine roots,

Table 2.2 Predominant plant species associated with the four populations *

Population	Stratum		
	Tree	**Shrub**	**Ground**
Forestier Gully	*Eucalyptus obliqua*	*Pomaderris apetala* *Acacia verticillata* *Zieria arborescens* *Cyathodes glauca*	*Viola hederaceae* *Beyeria viscosa* bryophyte spp. *Lepidosperma elatius* *Monotoca glauca* *Chiloglottis gunnii*
Forestier Ridge	*E. obliqua* *E. viminalis* *E. pulchella*	*Exocarpos cupressiformis* *Banksia marginata* *Bedfordia salicina* *Olearia lirata*	*Gonocarpus humilis* *Pimelea nivea* Poaceae spp. *Goodenia ovata* *Viola hederaceae* *Cyathodes juniperina* *Pultenaea juniperina* bryophyte spp. *Coprosma quadrifida* *Lomandra longifolia* *Pteridium esculentum* *Chiloglottis gunnii* *Lomatia tinctoria* *Correa reflexa*
Lune Mid	*E. obliqua*	*Acacia verticillata* *Olearia stellulata* *Zieria arborescens* *Bedfordia salicina* *Melaleuca squarrosa* *Leptospermum scoparium* *Coprosma quadrifida*	*Pteridium esculentum* *Goodenia ovata* bryophyte spp. *Gahnia grandis* *Pultenaea juniperina* *Lepidosperma elatius* *Gonocarpus humilis* *Blechnum wattsii* *Daviesia ulicifolia*
Lune Plain	*E. obliqua*		Poaceae spp. *Gleichenia dicarpa* *Melaleuca squarrosa* *Spengelia incarnata* *Gahnia grandis* *Leptospermum scoparium* *Sphaerolobium minus* *Leptospermum lanigerum* *Lepidosperma filiforme* *Hakea epiglottis* *Gymnoshoenus sphaerocephalus*

* recorded as species present on >50% of plots established at each of the 12 maternal trees within the four populations

Table 2.3 Climatic data for the Forestier planting sites

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Mean annual rainfall (mm) [1]	60	54	63	73	80	93	92	83	61	82	66	80	887
Pan evaporation (mm) [2]	163	132	106	69	38	22	26	47	75	113	128	159	1079
Rainfall 1990-93 (% of mean annual) [1]	110	124	87	84	80	63	114	120	123	74	127	105	98
Mean maximum temperature (°C) [2]	21	22	20	17	14	12	11	13	15	17	19	20	
Mean minimum temperature (°C) [2]	12	12	11	9	7	5	4	5	6	8	9	11	
Average number of frosts [2]	0	0	<1	<1	2	5	7	5	2	<1	<1	0	
Highest recorded temp. (°C) 1990-94 [3]:													
Forestier Gully	40	34.5	36	20	24	22.5	14.5	14	19	22	30	31	
Forestier Ridge	39	33	36	23	24	19.5	14	17	21	24	31.5	38	
Lowest recorded temp. (°C) 1990-94 [3]:													
Forestier Gully	0	6.5	0	3	1	-1.5	-1.5	-1	0	-1	0	4	
Forestier Ridge	0	2	1	-4	-3.5	-3.5	-5	-4.5	-4.5	-3	-1.5	2	

Sources:

[1] Data from the nearest comparable rainfall station at Eaglehawk Neck (8km south of the Forestier sites at an elevation of 10m). Rainfall data collected from gauges at the Forestier planting sites were found to be within 9% of the Eaglehawk Neck data during a monitoring period of Aug 1990-Dec 1993.

[2] Data for nearest comparable temperature recording station at Hobart Meteorological Bureau (50km west of the Forestier sites at an elevation of 55m).

[3] Data from max/min thermometers installed at each planting site.

Table 2.4 Climatic data for the Lune planting sites

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Mean annual rainfall (mm) [1]	69	73	88	119	139	150	140	142	129	137	124	106	1416
Pan evaporation (mm) [1]	102	87	59	42	31	21	22	34	48	66	87	90	689
Rainfall 1990-93 (% of mean annual) [1]	112	84	70	58	72	85	129	121	71	72	76	135	89
Mean maximum temperature (°C) [1]	21	21	19	17	13	12	11	12	14	16	17	19	
Mean minimum temperature (°C) [1]	9	9	8	7	5	3	3	3	4	5	7	8	
Average number of frosts [1]	0	0	<1	1	4	9	12	9	6	3	1	0	
Highest recorded temp. (°C) 1990-94 [2]:													
Lune Mid	35	34	33.5	31	17	16	15	15	19	27	30	*	
Lune Plain	42	39.5	39	24	24	23.5	17	18	26	30	37	*	
Lowest recorded temp. (°C) 1990-94 [2]:													
Lune Mid	0.5	5	4.5	4	3	0	-1	0	1	1	5	*	
Lune Plain	*	0	*	-1	-3	*	-7	-5	-5.5	-4	-3	*	

Sources:

[1] Data from the nearest comparable meteorological station at Hastings Chalet (4km north of the Lune Mid site at an elevation of 40m). Rainfall data collected from gauges at the Lune planting sites were found to be within 1.5% of the Hastings Chalet data for samples taken during a monitoring period of Aug 1990-July 1991.

[2] Data from max/min thermometers installed at each planting site.

*: Data not available

2. Location and Description of Maternal Trees

Seed was collected from 12 trees within each population. The criteria for selection of maternal trees were as follows.

- dominant or co-dominant trees within the main overstorey stratum;
- selected trees were spatially separated by a distance equivalent to no less than twice the tree height, to reduce the possibility of in-bred seed due to half-sib parent trees;
- sufficient seed was available for experimental purposes (minimum of 10g).

The locations of selected trees within each population are presented in Figures 2.2 and 2.3. Individual dimensions of selected trees are provided in Table 2.5. Botanical surveys were undertaken using TASFORHAB (Peters 1983) plots at each selected tree. The radius of the circular plots around each tree was approximately 10m (or as varied to ensure that the plot area was uniform and representative). Lists were compiled of all species within the tree, shrub and ground strata. These lists were entered into the ECOPAK programme (Minchin 1986) and subjected to an ordination analysis using the detrended correspondence analysis (DCA) technique of Hill and Gauch (1980). Results of this analysis indicated that the four populations were clearly delineated on the basis of species composition (Figure 2.6). Full species lists compiled for each plot are detailed in Appendix 1 and a summary of the data is presented in Table 2.2.

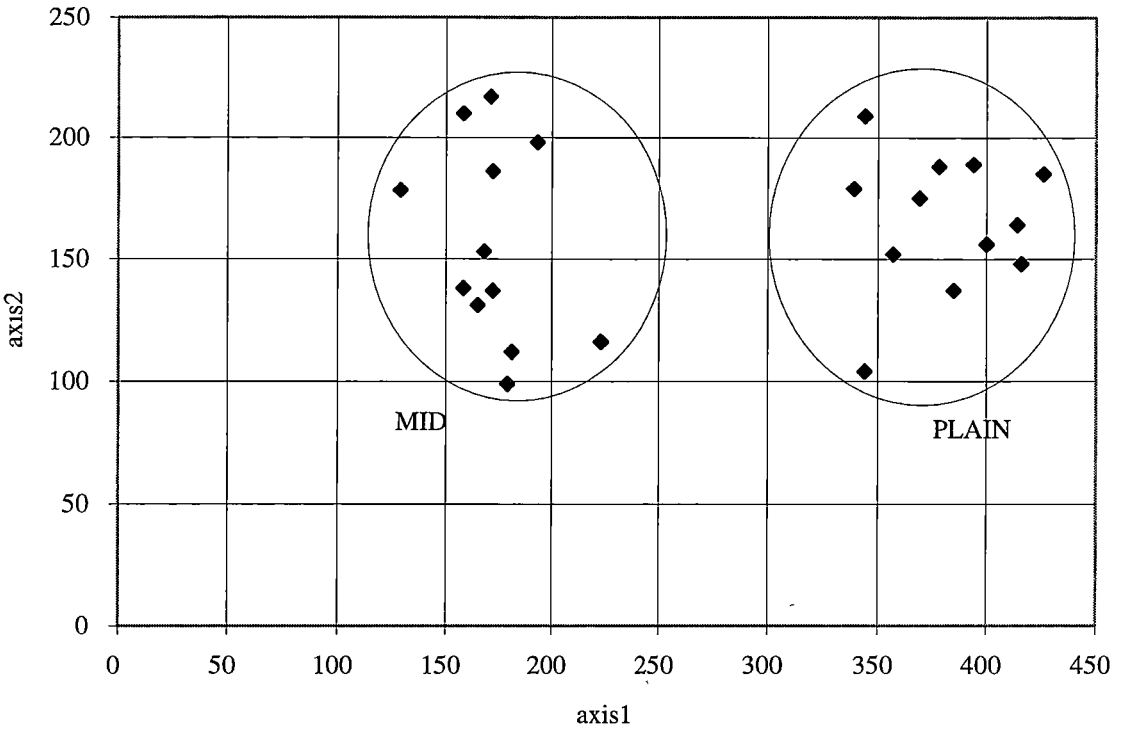
3. Collection of Samples and Seed

Samples of adult leaves and mature capsules (containing seed) were collected from the selected maternal trees at Forestier in March 1989 and at Lune in April 1989. The collection of samples was achieved by the removal of one or more secondary branches from an open, sunny part of the upper canopy. Branches were removed on most trees by either climbing and hand-sawing or by the use of a commando saw (a length of chainsaw chain attached at either end to ropes which are operated in a sawing action from the ground by two operators). Seed was also collected from trees that were either intentionally felled (trees 3 and 23) or recently windblown (tree 17). Capsules were dried in open trays for two weeks at 20°C. During this period the capsules were subjected to regular agitation and sieving to extract the seed material. The seed and chaff material was then stored in closed paper bags and sealed within larger plastic bags. Storage was carried out for 12 months within the laboratory under conditions of darkness and ambient temperature (< 25°C maximum) until the germination tests were concluded. The seed was then transferred to a cold storage room (2°C) for longer term storage.

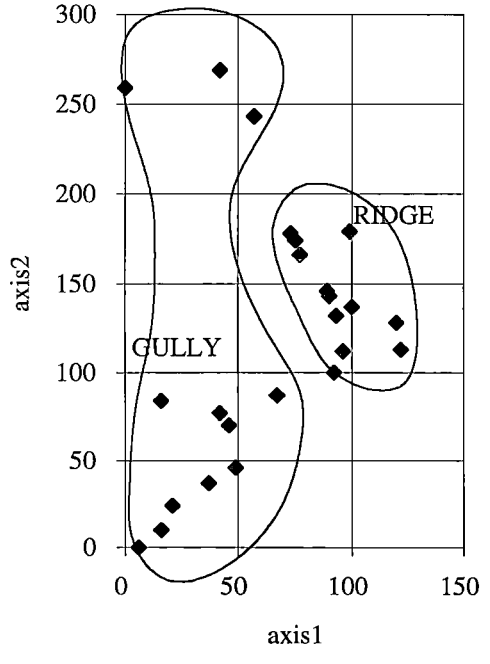
Table 2.5 Height and DBHOB measurements for maternal trees within each population

Population	Maternal tree	Height (m)	DBHOB (cm)
Forestier Gully	1	35	91
	2	31	82
	3	28	81
	12	30	82
	13	31	74
	14	40	115
	15	19	62
	16	30	65
	17	28	71
	18	28	47
	22	20	45
	23	41	140
	mean ± SE	30.1 ± 1.9	79.6 ± 7.8
Forestier Ridge	4	26	85
	5	31	102
	6	16	65
	7	26	76
	8	27	72
	9	35	66
	10	24	67
	11	18	36
	19	25	58
	20	25	84
	21	24	66
	24	24	54
	mean ± SE	25.1 ± 1.4	69.3 ± 4.8
Lune Mid	37	30	73
	38	27	72
	39	28	70
	40	23	102
	41	26	102
	42	34	100
	43	33	64
	44	26	56
	45	31	96
	46	26	71
	47	41	112
	48	18	69
	mean ± SE	28.6 ± 1.7	82.3 ± 5.4
Lune Plain	25	21	54
	26	25	75
	27	18	72
	28	6	12
	29	18	100
	30	25	79
	31	25	82
	32	16	96
	33	24	100
	34	11	33
	35	19	102
	36	12	29
	mean ± SE	18.3 ± 1.8	69.5 ± 8.9

Figure 2.6 Ordination of vegetation plots on the basis of floristic similarity
1. Lune Mid and Plain populations



2. Forestier Gully and Ridge populations



4. Establishment of Planting Trials

4.1 Location of Sites

At each site, the initial selection of maternal trees was carried out within a locality containing a recently clearfelled (<2 years) patch of forest so that a planting site could be located within the area occupied by each population or on a nearby site that had similar environmental attributes. Planting sites were located within the boundaries of the populations at Forestier Ridge, Forestier Gully and Lune Plain, whilst a nearby coupe was selected as the planting site to represent the Lune Mid population (see Figures 2.2 and 2.3).

4.2 Description of Sites

4.2.1 Forestier Gully

This planting site was previously occupied by wet sclerophyll forest within the Forestier Gully population described in section 1.1 (Map sheet Murdunna 25/5624, grid reference 741 452, Photograph 2.7). The site had a south-easterly aspect and was located at an altitude of 220m in a lower slope position, 20m from an intermittent stream. The centre of the planting site contained a natural spring which flowed after periods of heavy rainfall (the spring and drainage area were excluded from the experimental areas - see Figure 2.7). Surface drainage over the remainder of the site was very good. The soil was an xanthozem with moderate sub-soil drainage and prominent mottles in the B2 and B3 horizons (Table 2.1). Climatic data are presented in Table 2.3.

4.2.2 Forestier Ridge

This planting site was previously occupied by dry sclerophyll forest within the Forestier Ridge population described in section 1.2 (Map sheet Murdunna 25/5624, grid reference 734 448). The trial area was located on a north-westerly aspect in the upper slope position, 60m to the north-west of the ridge-top that formed the boundary between the dry sclerophyll forest (to the north-west) and the wet sclerophyll forest (to the south-east). Altitude was about 240m. The soil was a krasnozem with a high proportion of rock fragments on the ground surface and within the soil profile (Table 2.1, Photograph 2.8). Drainage over the trial area was generally very good, although the lower slope positions were less well-drained than the upper portions of the site (this was taken into account in the blocking of the experimental design). Climatic data indicated that there were small differences in the temperature range

Photograph 2.7 Forestier Gully planting site, one year after planting (note the dense regeneration of understorey)

Photograph 2.8 Forestier Ridge planting site, three years after planting (note the low, open understorey)

between this site and the nearby Forestier Gully site. Temperature minima at the Ridge site were consistently about 3°C lower whilst maxima were more variable but averaged about 1 °C higher than the Gully site (Table 2.3).

4.2.3 Lune Mid

The trial area was located within a coupe approximately 3km from the Lune Mid maternal trees (at Map sheet Hastings 25/4819, grid reference 881 906, Photograph 2.9). This site was representative of the wet sclerophyll forest described in section 1.3. The soil type was described as an xanthozem (Table 2.1). The ground surface was very free-draining, although the soil profile indicated imperfect vertical drainage. The planting site occupied an easterly aspect on the mid slope position, about 1km from the upper ridge-line and about 200m from the boundary between the lower slope and buttongrass plain. The site had an altitude of about 100m and a moderate slope of about 20%. Climatic data for the site are presented in Table 2.4.

4.2.4 Lune Plain

The trial site was located on one of the slightly elevated islands within the Lune Plain forest type described in section 1.4 (Map sheet Hastings 25/4818, grid reference 908 894, Photograph 2.10). Mature stems of *E. obliqua* and *E. nitida* were scattered over the general area but no advance growth or younger trees were present on the planting site. The understorey vegetation was representative of that described for the maternal trees (Table 2.2), although the planting site was largely covered by *Gymnoshoenus sphaerocephalus*. The soil was a peaty podzol with a distinctive peat layer 25cm in depth (Table 2.1). Soil drainage was very poor, with a saturated water table occurring very close to the surface. As a result, there was a very restricted rooting depth, with the majority of roots concentrated within the upper 15 cm of the mineral horizons. The site was located about 90m away from the sharp boundary between the forests of the lower slope and the open woodland of the buttongrass plain. The altitude was 40m and the topography was flat. Climatic data presented in Table 2.4 indicate major temperature differences between this site and the Mid planting site due to the strong vertical gradients caused by cold air drainage. As a result, the minima for the Plain site were an average of 5.5°C, and up to 8°C, lower than the Mid site, despite an altitudinal difference of only 60m.

Photograph 2.9 Lune Mid planting site, two years after planting (note the dense regeneration of *Gahnia* understorey)

Photograph 2.10 Lune Plain planting site, at planting (note the low cover of sedges)

4.3 Design of the planting trials

The trials were planted in a randomised complete block (RCB) design. All 48 families (12 families x 4 populations) were randomised separately within each block and within each planting site. Each family was represented by a plot of 6 trees within each block. There were 4 blocks at each site, giving a total of 1152 plants (12 families x 4 populations x 4 blocks x 6 trees) for each trial. Blocks were designed to take account of slight variations in site factors such as topographic position and drainage. Within each block, site variation was minimised by excluding atypical sites such as high intensity ashbed, poorly drained soaks and soil areas compacted or otherwise disturbed by logging activity. Plots were marked in the field using wire pegs and aluminium labels. The location of families within each trial is presented in Figures 2.7 to 2.10.

4.4 Site Preparation

Site preparation operations are normally carried out in plantations in order to modify relevant site factors so that growth rates are maximised. In these studies, the intention was to minimise the extent of site modification so that the inherent characteristics of the individual planting sites were maintained as closely as possible to the natural state. Site preparation at each site is detailed in the following sections.

4.4.1 Forestier Gully

The trial area occurred at the edge of a coupe that was clearfelled in 1988/89 and the heavy residual fuels were subjected to a high intensity slash burn in April, 1989. The coupe was sown to a mixture of *E. obliqua* and *E. regnans* seed in autumn 1989. A planting site (approximately 4500m^2 in area for all planting sites) was fenced with a standard (floppy-top chicken-wire) fence during summer 1989/90 to exclude animal browsing. Weed control was carried out to remove eucalypt seedlings and understorey species such as *Acacia verticillata, Pomaderris apetala and Olearia* spp. The treatment consisted of slashing with a brush-cutter in February 1990, followed by a spray application of amitrole (2 kg a.i. ha^{-1}) and atrazine (4kg a.i. ha^{-1}) in July 1990. The pre-planting application of these herbicides has no detrimental effects on planted eucalypt seedlings (Wilkinson and Neilsen 1990). Prior to planting any surviving eucalypt seedlings were manually pulled out of the ground.

4.4.2 Forestier Ridge

This trial site was located a the edge of a coupe that was clearfelled in 1987/88 and the light residual fuels were subjected to burn of moderate to low intensity in April 1988. The area was sown to eucalypts in autumn 1988 and by the summer of 1989/90 the ground surface was occupied by a mixture of eucalypt seedlings (*E. obliqua, E. amygdalina and E. pulchella)* and short understorey species such as *Goodenia ovata, Lomandra longifolia, Lomatia tinctoria and Pteridium esculentum.* This vegetation was cleared by a crawler tractor and the planting area was fenced with the standard fence during summer 1989/90. No herbicide regime was considered to be necessary since operational experience on these dry sites indicates that conditions of low weed competition are maintained for at least the first year after clearing.

4.4.3 Lune Mid

This coupe was clearfelled in 1988/89 and a high intensity burn was applied to the heavy residual fuels in March 1989. The coupe was sown with *E. obliqua* seed in autumn 1989. The trial area was fenced with the standard fence in autumn 1990. Herbicide was applied in July 1990, as described in 4.4.1, in order to control the undergrowth which was largely dominated by *Gahnia grandis.* Prior to planting any surviving eucalypt seedlings were manually pulled out of the ground.

4.4.4 Lune Plain

This trial site was located within a buttongrass plain that had been burnt by wildfire in March 1989. The few scattered eucalypt trees (*E. obliqua* and *E. nitida*) on the site had been felled as part of the fire control activities. The planting site was fenced with the standard fence in autumn 1990. The foliar herbicide glyphosate (at 0.72 kg ha^{-1}) was applied twice during the autumn to control the regrowth of *Gymnoshoenus sphaerocephalus.* This herbicide has no residual effect on the growth of eucalypt seedlings (Wilkinson and Neilsen 1990).

4.5 Production of Seedlings

Seed from the 48 families was sown into individual germination trays in February 1990 and subjected to a cool, moist stratification for 24 days within a constant temperature cool-room (2°C). The trays were then transferred to a germination room at 20°C. Following germination (3-7 days) the trays were moved to a glasshouse for hardening-off. The cotyledon seedlings were pricked out in April 1990 into peat moss "jiffy-pots" (5cm x 5cm square pots with a 5cm tapered depth) containing a standard nursery potting mix of composted pine-bark, peat moss, coarse river sand and slow release "Osmocote" fertiliser. The seedlings were regularly watered and were sprayed with fungicide, as required, to prevent fungal diseases. The seedlings were removed from the glasshouse after 15 weeks (July 1990) and moved outside to an outside location for hardening off. The seedlings remained outside until planting commenced, 4-5 weeks later. At the time of planting, the average height of the seedlings was 8.9cm.

4.6 Planting and Establishment

Planting was carried out on 22-24 August 1990 (Lune Mid and Plain planting sites) and 28-29 August 1990 (Forestier Gully and Ridge sites). The "jiffy-pots" were planted intact into holes of the exact dimensions. The planting holes were prepared by a special planting tool that was designed to ensure that the amount of site disturbance and any cultivation effect was minimal and uniform for all seedlings. Variation due to differences in planting quality were minimised by only using two planters, with each planter completing two blocks at each trial. Planting conditions at all sites were favourable with moist soils and good follow-up rain.

Rainfall gauges and maximum/minimum thermometers were installed at all planting sites in order to monitor climatic conditions during the establishment stage of the plantings. The measurements were used to select the nearest comparable long term meteorological station. The rainfall data indicate that during the post-planting establishment period of 1990-93, rainfall was 98% of the long term average for the Forestier sites, and slightly drier at 89% for the Lune sites (Tables 2.3 and 2.4). All other climatic factors were within normal ranges for each site, and no unusual events occurred during the course of the trials.

All sites were regularly inspected to check on the fences and the general health of the seedlings. The fences provided complete protection from browsing damage on all sites except for the Forestier Gully site where it was impossible to totally exclude brush-tail possums (see Chapter 6).

The initial site preparation within all the planting trials resulted in minimal growth of understorey species during the first growing season. However, vigorous regeneration occurred on all sites in the subsequent seasons (Table 2.6). A dense stratum of woody shrubs provided competition to the planted seedlings, overtopping many of the weaker plants at the Forestier Gully site. In contrast, the understorey at the Forestier Ridge site remained relatively low and open (Photograph 2.8). The large sedge, *Gahnia grandis*, provided severe competition at the Lune Mid site and a low, dense sward of buttongrass (*Gymnosphoenus sphaerocephalus*) overtopped and caused the suppression of many seedlings at the Lune Plain site (see chapter 3, Photographs 3.1 and 3.2).

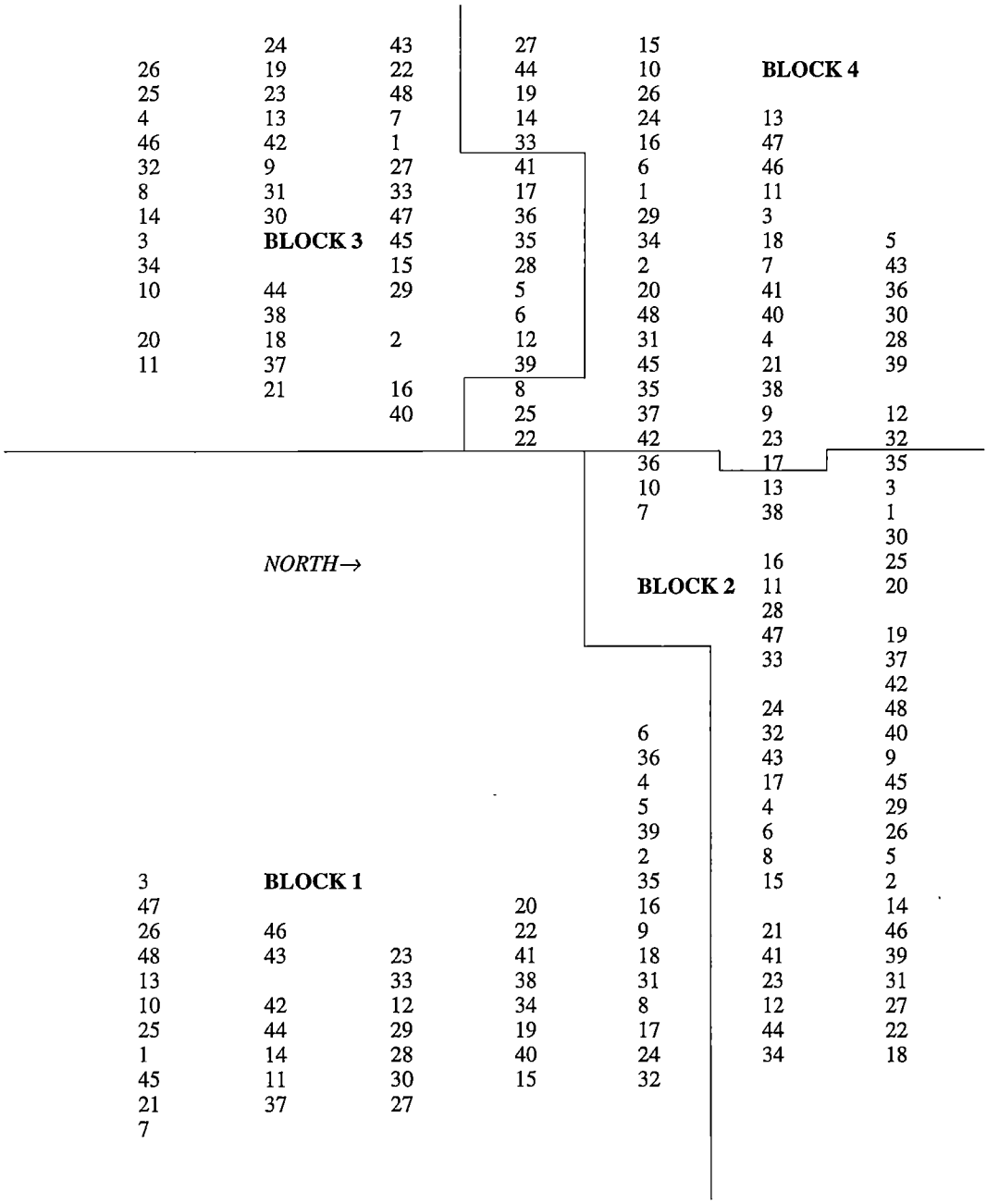
Table 2.6 Dominant regeneration species present on the planting sites 4 years after establishment

Forestier Gully	**Forestier Ridge**
Pomaderris apetala	*Exocarpos cupressiformis*
Acacia verticillata	*Goodenia ovata*
Goodenia ovata	*Lomandra longifolia*
Lepidosperma elatius	Poaceae spp.
	Lomatia tinctoria
	Pteridium esculentum
Lune Mid	**Lune Plain**
Gahnia grandis	*Gymnoshoenus sphaerocephalus*
Acacia verticillata	*Gleichenia dicarpa*
Melaleuca squarrosa	*Melaleuca squarrosa*
Leptospermum scoparium	Poaceae spp.

4.7 Measurements and analysis

Measurements of various attributes were carried out at various times over a five year period following planting, as detailed in the following chapters. Analyses of data were undertaken using various techniques. In all analyses, populations were regarded as fixed terms and families as random terms. Blocks were either regarded as fixed terms or were used to determine mean values for families, depending upon the method of analysis (see methodology within each chapter). Analyses were designed to compare differences between the four populations. The trials were not designed for the purpose of allocating variation between the various strata of provenance, population, family and tree. Similarly, the trials were not intended to provide estimates of genetic correlations or heritabilites used for tree selection and breeding purposes.

Figure 2.7 Experimental design for the planting trial at the Forestier Gully site



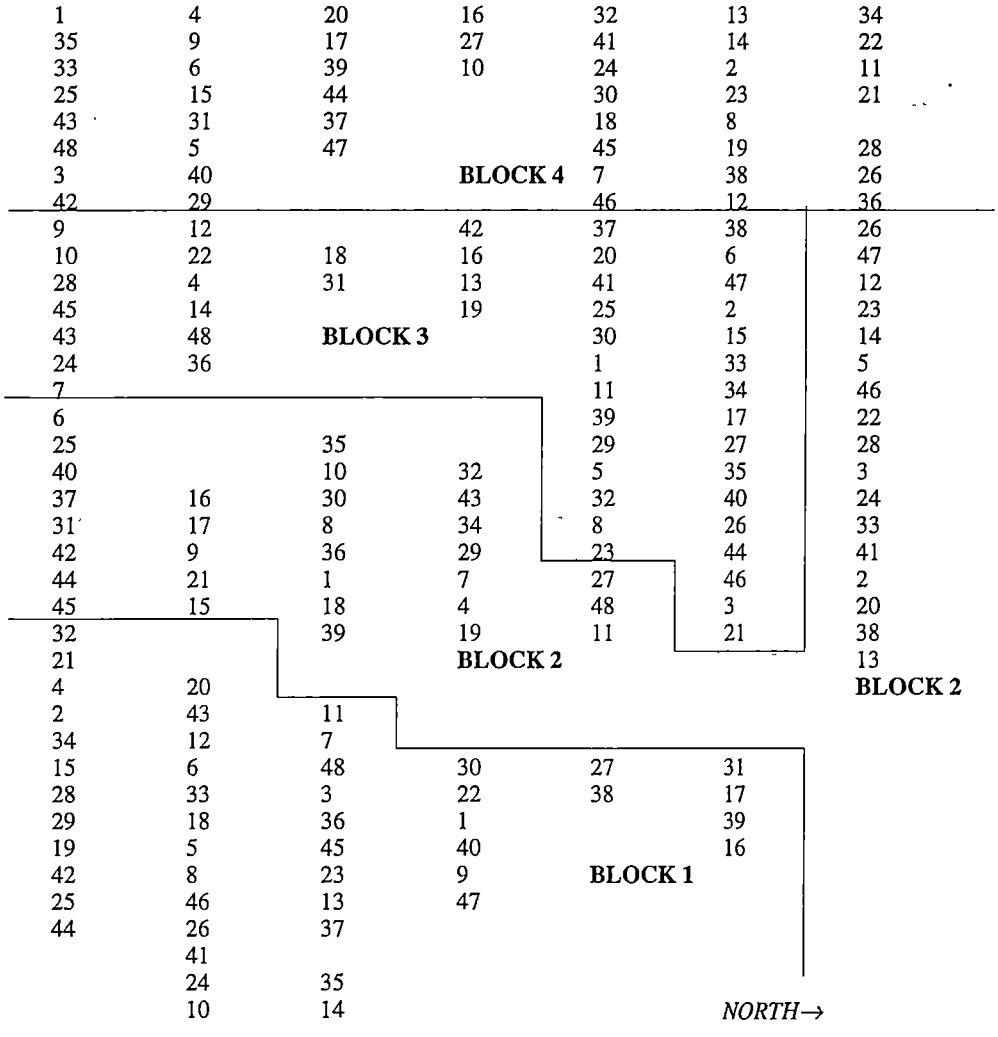
Gully population = Family numbers 1-3, 12-18 and 22-23

Ridge population = Family numbers 4-11, 19-21 and 24

Mid population = Family numbers 37-48

Plain population = Family numbers 25-36

Figure 2.8 Experimental design for the planting trial at the Forestier Ridge site



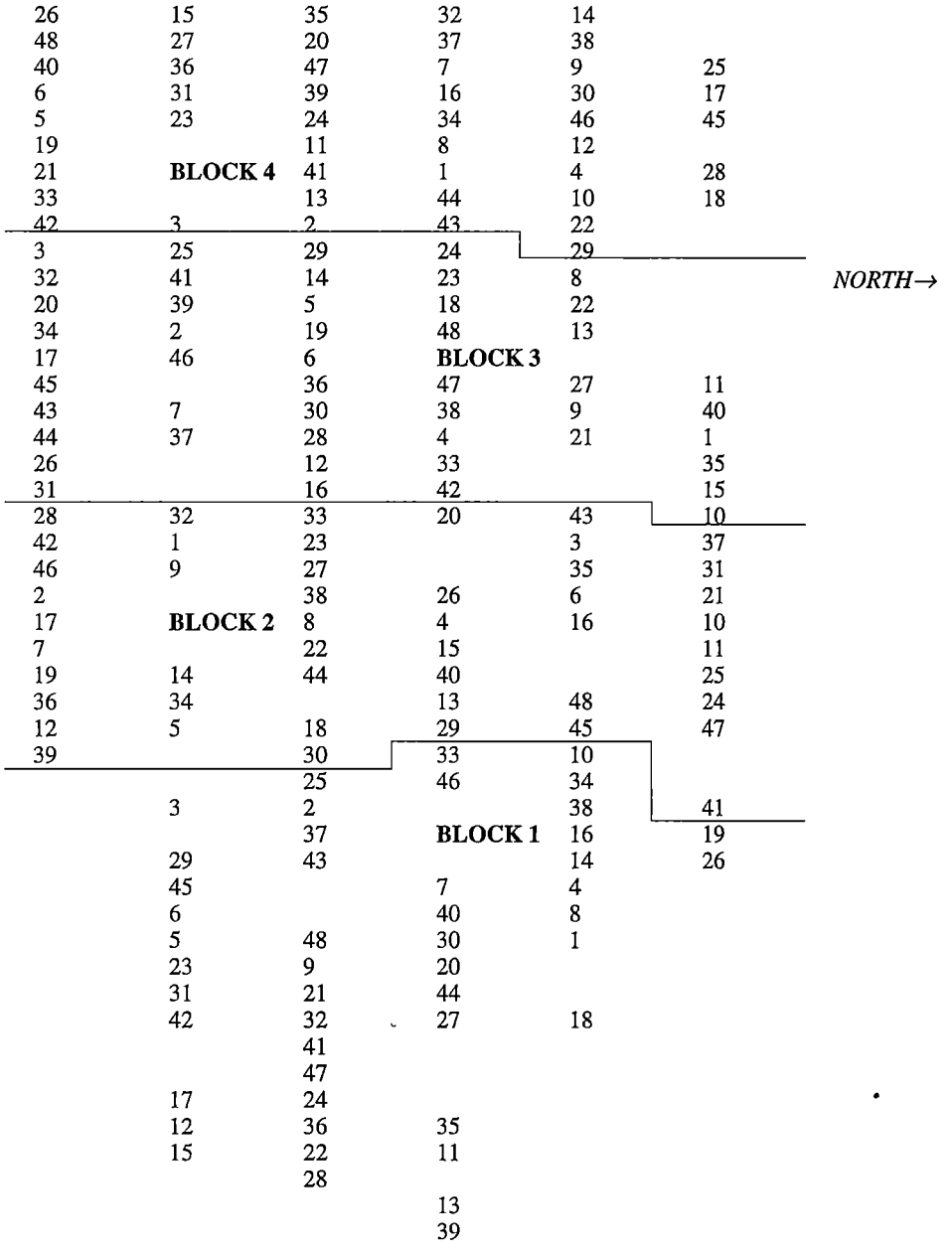
Gully population = Family numbers 1-3, 12-18 and 22-23

Ridge population = Family numbers 4-11, 19-21 and 24

Mid population = Family numbers 37-48

Plain population = Family numbers 25-36

Figure 2.9 Experimental design for the planting trial at the Lune Mid site



Gully population = Family numbers 1-3, 12-18 and 22-23

Ridge population = Family numbers 4-11, 19-21 and 24

Mid population = Family numbers 37-48

Plain population = Family numbers 25-36

Figure 2.10 Experimental design for the planting trial at the Lune Plain site

41	18	38						
28	46	39						
7	4	8						
21	45	43						
35	24	40	**BLOCK 2**					
3	37	20				*NORTH→*		
31	16	19						
1	2	27						
44	26	6						
42	17	47	15	14				
34	10	11	13	12				
25	9	23	36	22				
33	48	29	32	44	48	37	6	
30	5	13	9	41	18	45	35	17
31	30	38	4	33	5	23	38	34
25	44		7	25	43	12	47	24
40	37	42	29	14	11	31	20	46
	48		10	15	22	19	13	
41	**BLOCK 1**	11	2	16	3	36	28	**BLOCK 4**
34	32	35	1	21	39	26	40	
4	33	20	8	30	32	27	42	
9	36	3	24	33	35	30	9	
29	7	19	1	29	42	25	5	
47	28	10	6	36	7		17	
22	39	15	47	48	40	23	2	
24	6	18	12	14	26	11		43
5	1	12	10	4	39	21		27
26	23	43	46	41	38	19		13
46	8	27	22	37	28	3	**BLOCK 3**	
21	2	17	18	44	20	8		31
45	16	14	15	16	45	32		34

Gully population = Family numbers 1-3, 12-18 and 22-23

Ridge population = Family numbers 4-11, 19-21 and 24

Mid population = Family numbers 37-48

Plain population = Family numbers 25-36

CHAPTER 3 SURVIVAL AND GROWTH

1. Introduction

Significant variation in survival and growth has been demonstrated between progeny of *E. obliqua* from 22 provenances across its natural range within Australia (Green 1971, Brown *et al.* 1976). Green (1971) found that differences in height growth were clearly expressed by age 37 weeks but there was no clear correlation between the environmental conditions of the seed source and the subsequent growth of progeny. Brown *et al.* (1976) planted progeny from the same provenances in three large trials, at Narbethong and Silver Creek (both in Victoria) and Lisle (Tasmania), and they demonstrated significant differences in growth at age 43 months. A subsequent measurement undertaken at the Lisle site indicated that significant growth differences persisted to at least age 13 years (Matheson *et al.* 1986).

These planting trials indicated that there was sufficient genetic variation across the range of *E. obliqua* to enable selection of vigorous provenances for tree improvement work (Brown *et al.* 1976). However, there was no reliable site or locality indicator for predicting the growth of progeny and consequently, Brown *et al.* (1976) suggested that future species trials should evaluate an even larger number of provenances. Furthermore, the similar growth of four populations from a range of site indices at Strathblane (Tasmania) led them to conclude that selection at a local site level would not be worthwhile.

A small trial to replicate the evaluation of the Strathblane populations was established at Taranna in south-east Tasmania in the same year as the major planting trials (1970). However, no results from this trial have been published.

The current study was designed to further investigate the survival and growth of progeny originating from diverse sites. Initially, information from the original Tasmanian planting trials was updated by undertaking measurements at each site, followed by the analysis of these and other (previously unpublished) data. Secondly, new planting trials were established to include additional populations as described in chapter 2.

2. Methods

2.1 Measurement and analysis of previously established planting trials

The establishment of the *E. obliqua* provenance trials in 1970 was described by Brown *et al.* (1976). The trial contained 22 provenances collected by Green (1971), including five populations from Tasmania. In addition to these provenances, the trial at Lisle in north-eastern Tasmania included a set of four populations from a range of sites at Strathblane, in the Southern forests region of Tasmania. Of the remaining Tasmanian populations, the Forester provenance is regarded as the closest match to the Lisle site and has been designated as the 'local source' for the purpose of the current analyses.

The Taranna planting site was established at the same time as the Lisle trial by the Forestry Commission. This is a smaller trial and contains only the four Strathblane populations and a local seed source from Taranna (not included in the Lisle trial). Details of the Strathblane collections have not previously been published and are therefore summarised below from internal Forestry Tasmania file records.

Strathblane collections - four sites were identified by K. Felton (Forestry Tasmania) within an area of approximately 50 ha near Strathblane in the Southern forests (Chapter 2). The sites were selected on the basis of potential productivity class as defined by site index (site index is the mean dominant height (MDH) of a stand at age 50 years; MDH is the mean height of the tallest dominant trees at the rate of the tallest tree per 1/30th of a hectare (Forestry Commission 1964)). Seed was collected from 10 mother trees within each of the four sites. Descriptions of the four sites are as follows.

- *Site index 18* - altitude 80m; mixed stand of *E. obliqua* and *E. nitida* regrowth 48 years old, occurring in two patches approximately 2 km apart along the edge of a buttongrass plain.
- *Site index 29* - altitude 87m; stand of *E. obliqua* regrowth, 56 years old.
- *Site index 34* -altitude 93m; stand of *E. obliqua* regrowth, 72 years old.
- *Site index 50* -altitude 160-190m; mixed stand of *E. obliqua*, *E. regnans* and *E. globulus* containing regrowth 72 years old and scattered old growth stems.

Various sources of published and unpublished data on the Lisle and Taranna trials were collated for the purpose of the current review. Published results are available for the Lisle trial at age four years (Brown *et al.* 1976) and 13 years (Matheson *et al.* 1986). Unpublished data for the measurement of the Lisle trial at age 7 years and for all measurements of the Taranna trial (ages 1, 2, 5 and 13) were obtained from Forestry Tasmania records. Measurement of diameter, height and bark thickness were undertaken by the author at both sites at age 19 years (1989) as part of the preliminary investigations for the current studies. Stem volume under bark diameter was calculated for the age 19 years measurement using a standard Tasmanian volume equation for *E. obliqua* regrowth (S. Candy, Forestry Tasmania, pers. comm.):

$$V = \alpha + \beta D^2 H$$

where: V = volume under bark (m^3)

$\alpha = 0.009287$

$\beta = 0.3197$

D = Diameter breast height under-bark (m)

H = Height (m).

The presence of putative '*E. regnans*' types was recorded at the Lisle site as the number of trees with smooth bark persisting along the stem to within one metre of ground level.

The Taranna trial was designed as a randomised complete block design and the data were analysed for differences between populations using ANOVA. The Lisle trial was established as a replicated cubic lattice of 216 ($=6^3$) families and the statistical analyses for the measurements at age four years are described by Brown *et al.* (1976). Data for the age 19 years measurement at Lisle were analysed by Wilkinson and Kube (unpubl.) using residual maximum likelihood (REML) variance components analysis procedure on GENSTAT 5.2 (Genstat 5 Committee 1987). For the purposes of the current review, results from the analysis are presented for the four Strathblane populations and for the Forester provenance (regarded as the 'local seed source' for the Lisle trial; note that the Taranna provenance is regarded as the 'local seed source' for the Taranna trial).

2.2 New trials - Pre-planting growth of seedlings

The initial height of seedlings was measured within the seedling trays (chapter 2) one week prior to planting in the field. This was done because it is much more accurate to measure small and uniform seedlings in the nursery pots, rather than in the field where variation due to differences in planting depth and ground conditions can exceed the variation due to inherent growth rates (W.E. Neilsen, Forestry Tasmania, pers. comm.). Seedlings were raised in four trays per family, with each tray holding up to 30 jiffy pots to ensure a minimum of 24 good quality seedlings for planting at each study site. Two trays from each family were selected at random and the heights of the best 24 surviving seedlings per tray were measured (i.e. a total of 48 seedlings per family or 50% of the total number of planted seedlings). The mean heights of the 48 seedlings were subjected to ANOVA using the families as random terms.

2.3 New trials - Post-planting survival and growth

The establishment of planting trials at the Forestier Gully, Forestier Ridge, Lune Mid and Lune Plain sites is described in chapter 2. Measurements of survival and growth were measured at all sites (except for the Lune Plain site) at the times listed below.

Date		*Time after planting (months)*	*Completed growing seasons (years)*
January	1991	5	0
May	1991	9	0
November	1991	15	1
June	1992	21	2
June	1993	33	3
June	1994	45	4
August	1995	60	5

The timing of measurements at the Lune Plain site was altered because of low survival and negligible growth. Survival was recorded at 5, 9, 15, 26, 31 41 and 60 months and height measured from age 26 months.

The height growth of seedlings was measured using height sticks to 3m and telescopic height poles to 9m. Diameter growth was measured at breast height (1.3m) over bark (DBHOB) using vernier callipers, with the diameter recorded as the mean of two measurements taken at right angles around the stem. Diameter measurements were recorded when the mean tree

height exceeded approximately 1.8m, at ages 3 years and 4 years on the Lune Mid and Forestier Gully sites, and at age 4 years on the Forestier Ridge site.

Total stem volume under bark was determined for each tree using the Opie equation (Opie 1976):

$$\log_{10}(D^2H/V) = 4.762 - 5613/(D + 127)^2$$

where: V = volume under bark (m^3)

D = DBHOB (cm)

H = Height (m).

Measurements of survival and growth to the age of 45 months are based on the total number of surviving trees within each plot. Survival data were recorded as the percentage survival of seedlings within each plot. The data were subjected to the arcsine transformation prior to analysis to ensure that the residuals were normally distributed. Plot data for height and diameter growth were calculated as the mean value for surviving trees within the plot. Volume data were expressed as the total volume of all trees within the plot. Trials at the Forestier Gully, Forestier Ridge and Lune Mid sites were thinned to the largest tree in each plot after the age 45 month measurement (refer to chapter 7) and measurements at age 60 months are therefore based on a single tree per plot.

Data analyses were initially undertaken separately for each planting site. Survival data were analysed by ANOVA, using the mean across the four blocks and treating the families as random terms, since the families within each population are not constant. Height and volume data at the Forestier Gully, Forestier Ridge and Lune Mid planting sites were subjected to REML variance components analysis. The limited height growth recorded at the Lune Plain site was analysed using ANOVA techniques as for survival (above). REML analysis of the plot mean data at the other three sites was undertaken using the following models:

Random model:	population.family + block.population.family
Fixed model:	constant + block + population + block.population

The Wald test was used to determine the significance of fixed effects. The *t*-test was used to determine the significance of the random term population.family. Differences between the means of the populations were compared with the standard error of the difference using the *t*-test.

Mean and standard error (SE) were calculated for survival across all populations within each planting site. Analysis of height and volume data across the planting sites was undertaken using REML variance components analysis. The analysis was restricted to three sites (Forestier Gully, Forestier Ridge and Lune Mid), with the Lune Plain site omitted because of very low survival and growth rates. Data from the age 45 month measurement were used in the REML analysis, since these data provide a more accurate assessment of total growth than the post-thinning data at age 60 months. The following model was used to analyse the plot mean data across the sites:

Random model:	site.block + population.family + site.population.family
Fixed model:	constant + site + population + site.population
Sub-model:	constant + site + population.

The analysis was used to determine the magnitude of the site x population interaction by calculating the deviance between the sub-model and full fixed model. The significance of the interaction was determined by comparing the calculated deviance with the corresponding value of χ^2. The variance components for the random terms were estimated from a REML analysis carried out on the individual (tree) values using the following model:

Random model:	site.block + population.family + site.population.family + site.block.population.family
Fixed model:	constant + site + population + site.population

3. Results

3.1 Previously established planting trials

Survival of progeny at age 19 years at the Lisle site was very high and still averaged 89.6% for the Strathblane populations and the local seed source (Forester provenance). In contrast, survival at Taranna was much lower, due to an average of 50% mortality within the first six months after planting (Figure 3.1), probably as a result of waterlogged soil conditions during the first spring (Forestry Tasmania, unpubl.). Subsequent mortality after the first six months was very low. An apparent increase in survival between the ages of two and 13 years is due to the emergence of seedlings initially hidden by the dense understorey *of Gahnia grandis* (L. Edwards, Forestry Tasmania pers. comm.). Progeny of the site index 18 population had the highest survival at Taranna after the first year of growth, although there was large variability

(e.g. at age 13 years, survival mean= 56.25% SE=9.44) and the differences between this population and the others were not significant ($p>0.05$).

Growth across the Taranna site was also highly variable and differences between the populations were not significant at age 19 years (Figures 3.2 and 3.4). In contrast, there were highly significant differences in growth between populations planted at the Lisle site (Figures 3.3 and 3.4.). By age 19 years, the populations from Forester and site indices 29 and 34 had significantly larger mean diameters and had produced significantly more total volume than the populations from both the lowest (18) and highest (50) site indices.

The presence of the '*E. regnans*' type was recorded in seven out of the 25 different populations planted within the trial. The proportions of putative *E. regnans* types within the five populations under study are given in Table 3.1.

Table 3.1 Proportion of putative *E. regnans* progeny within populations planted at Lisle

Population	**Proportion of *E. regnans* progeny (%)**
Site index 18	4
Site index 29	26
Site index 34	25
Site index 50	35
Local source (Forester)	0

Family heritability for volume across all provenances at Lisle was 0.48 (Table 3.2) and variation between provenances accounted for 39% of the total variation. In contrast, within the Strathblane provenance, population differences generally accounted for less than 3% of the total variation.

Table 3.2 Components of variance, standard error and narrow sense heritability at age 19 years for the volume of *E. obliqua* families and provenances planted at the Lisle site (percentage contribution of components to total variation shown in brackets)

Source of variation	**Tree volume (m^3 $tree^{-1}$)**					
	All 26 provenances/families			Four Strathblane families only		
replication	0.0003	±0.0003	(<1%)	0.0016	±0.0016	(2%)
provenance	0.0297	±0.0091	(39%)	0.0026	±0.0028	(3%)
family.prov.	0.0030	±0.0007	(4%)	0.0028	±0.0021	(3%)
rep x fam.prov. (plot)	0.0034	±0.0011	(5%)	0.0041	±0.0040	(4%)
error (within plot)	0.0392	±0.0014	(52%)	0.0810	±0.0057	(88%)
individual tree heritability	0.17 ± 0.04			0.08 ± 0.06		
family heritability	0.48 ± 0.07			0.32 ± 0.18		

Figure 3.1 Survival of various populations at the Taranna planting site. Identical letters indicate non-significant subsets for each age class (p>0.05) (blank entries indicate data not available)

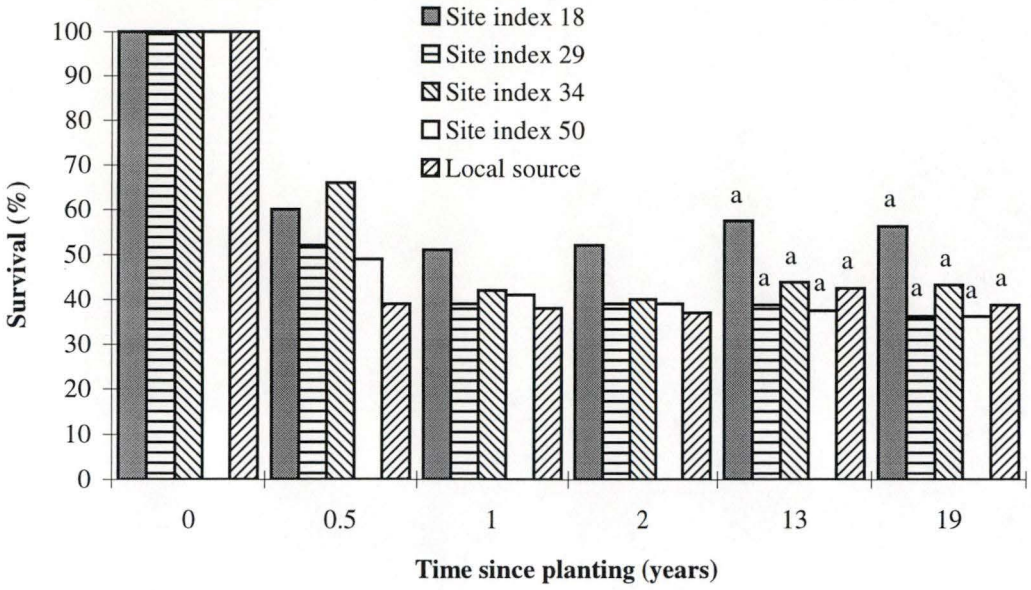


Figure 3.2 Growth (measured as height at ages 1 and 2 years; and DBHOB at ages 3, 13 and 19 years) of various populations at the Taranna planting site. Identical letters indicate non-significant subsets for each age class (p>0.05) (blank entries indicate data not available)

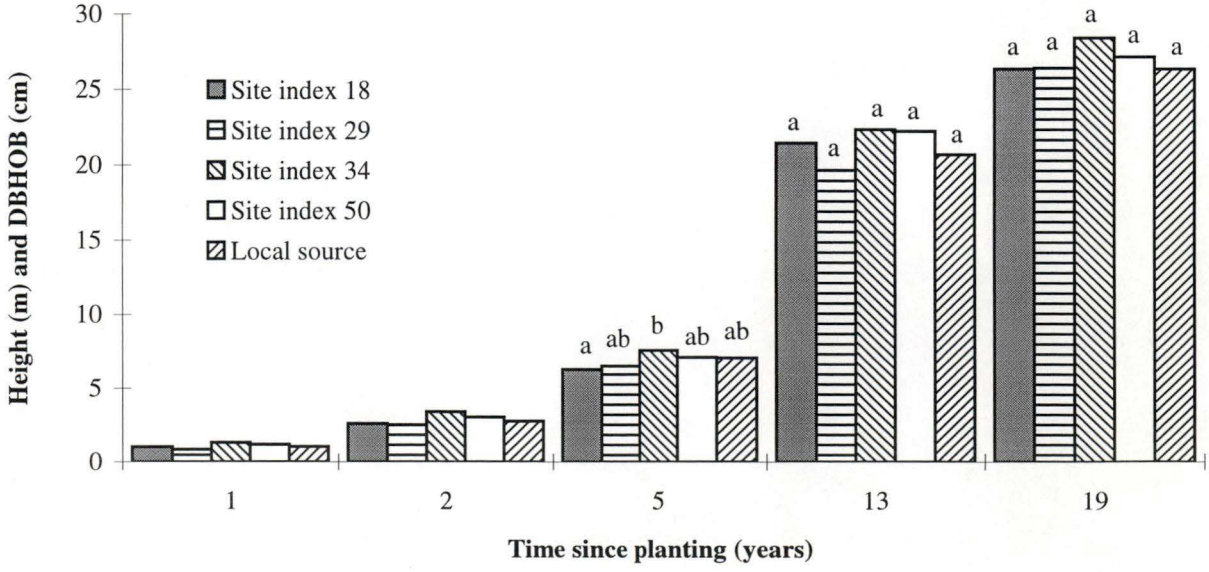


Figure 3.3 Diameter growth of various populations at the Lisle planting site. Identical letters indicate non-significant subsets for each age class (p>0.05) (blank entries indicate data not available)

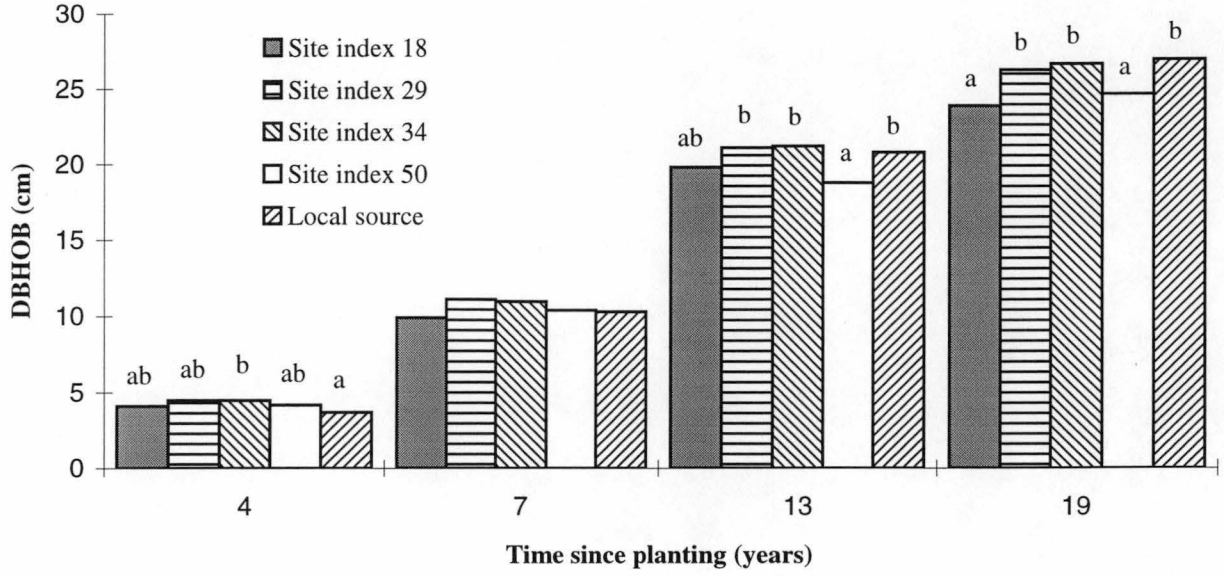
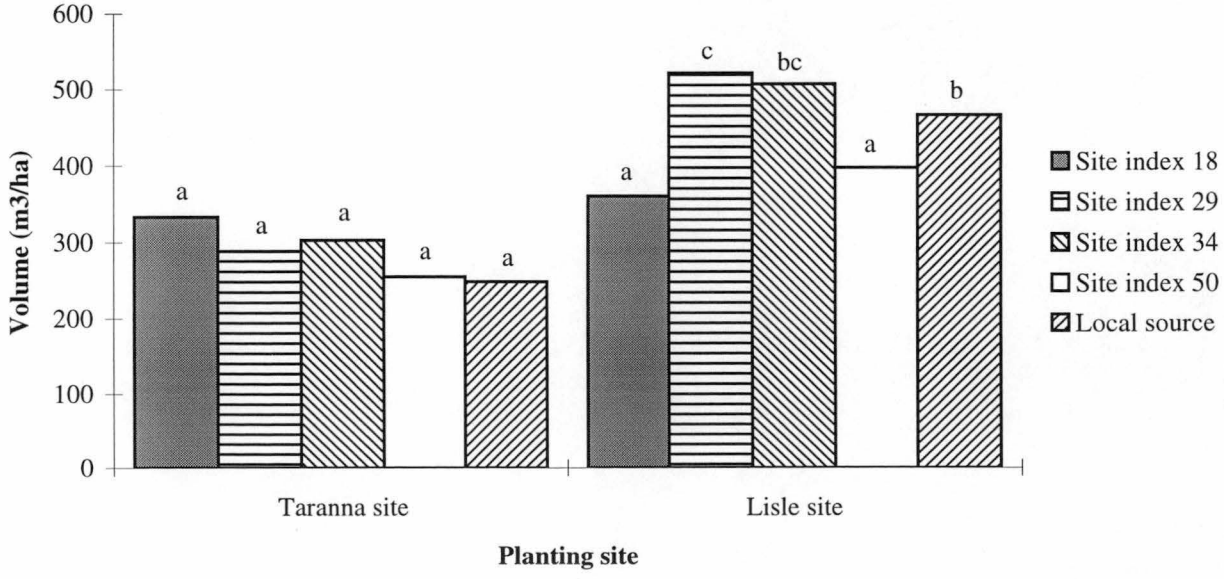


Figure 3.4 Volume production at age 19 years for various populations at the Taranna and Lisle planting sites. Identical letters indicate non-significant differences within each planting site (p>0.05)



3.2 New trials - Pre-planting growth of seedlings

There were no significant differences between populations for the height growth of seedlings in jiffy pots after 20 weeks (immediately prior to planting). (Tables 3.3 and 3.4).

Table 3.3. ANOVA for seedling heights prior to planting

Source	d.f.	M.S.	F-ratio	Significance level
population	3	4.27	1.76	ns
error	44	2.42		

Table 3.4. Mean seedling height and standard errors for populations prior to planting

Population	Mean height (cm) $\pm$ SE
Gully	8.4 $\pm$ 2.59
Ridge	8.7 $\pm$ 2.95
Mid	9.8 $\pm$ 2.83
Plain	8.7 $\pm$ 3.31

3.3 New trials - Post-planting survival and growth

Survival

High survival rates were maintained for all populations at the Forestier Gully, Forestier Ridge and Lune Mid planting sites, although survival at the Ridge site was significantly higher than at the Mid site (Figure 3.5). However, there were no significant differences between the four populations over the first 45 months (Table 3.5, Figures 3.6 to 3.9). In addition, there was no significant site x population interaction for survival across the three sites (Table 3.6). In contrast, survival of all populations at the Lune Plain site was significantly lower than at the other sites, with high mortality occurring within the first five months after planting, and survival continuing to decline to age five years (Figure 3.10). At age 41 months, survival at the Plain site was only 20%, compared to the substantially higher mean survival of 86% at the other three sites (Figure 3.5). By age 60 months, mean survival had fallen to less than 10%, and survival was higher for the Lune Plain population (15%) than the Lune Mid population ((10%) and the two Forestier populations (7%), although this difference was not significant ($p>0.05$).

Table 3.5 Summary of results from ANOVA of transformed values for the survival of seedlings at various times after planting on four sites (F-ratio based on 3 d.f. for populations and 44 d.f. for the error term)

Time since planting (months)	Planting site	F-ratio	Significance level
15	Forestier Gully	1.13	ns
15	Forestier Ridge	0.58	ns
15	Lune Mid	0.95	ns
15	Lune Plain	2.03	ns
21	Forestier Gully	1.76	ns
21	Forestier Ridge	0.52	ns
21	Lune Mid	0.54	ns
26	Lune Plain	1.42	ns
33	Forestier Gully	2.55	ns
33	Forestier Ridge	0.45	ns
33	Lune Mid	0.87	ns
31	Lune Plain	2.11	ns
45	Forestier Gully	2.25	ns
45	Forestier Ridge	0.44	ns
45	Lune Mid	0.92	ns
41	Lune Plain	2.06	ns
60	Lune Plain	2.17	ns

Table 3.6 Site x Population interaction for the survival, height and volume of four populations at age 45 months on three planting sites (Forestier Gully, Forestier Ridge and Lune Mid)

Character	Deviance	d.f.	Significance level
survival	7.276	6	ns
height	11.48	6	ns
volume	17.95	6	0.01

Figure 3.5 Mean survival of all populations at age 45 months on four sites (vertical bars are SE of the mean)

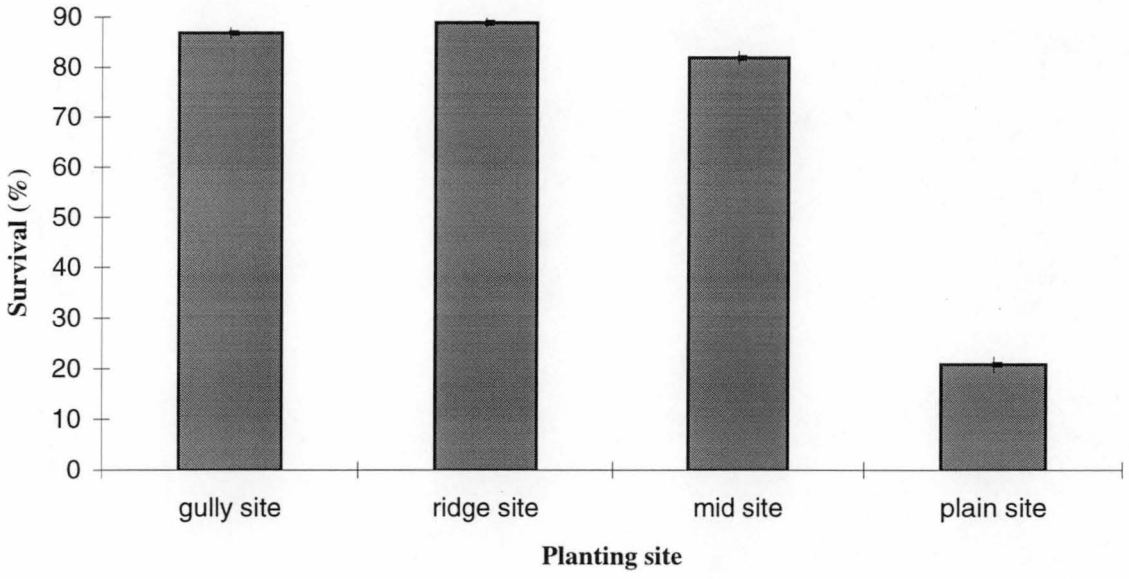


Figure 3.6 Mean survival of four populations at age 45 months across three planting sites at Forestier Gully, Forestier Ridge and Lune Mid. Identical letters indicate non-significant subsets (p>0.05)

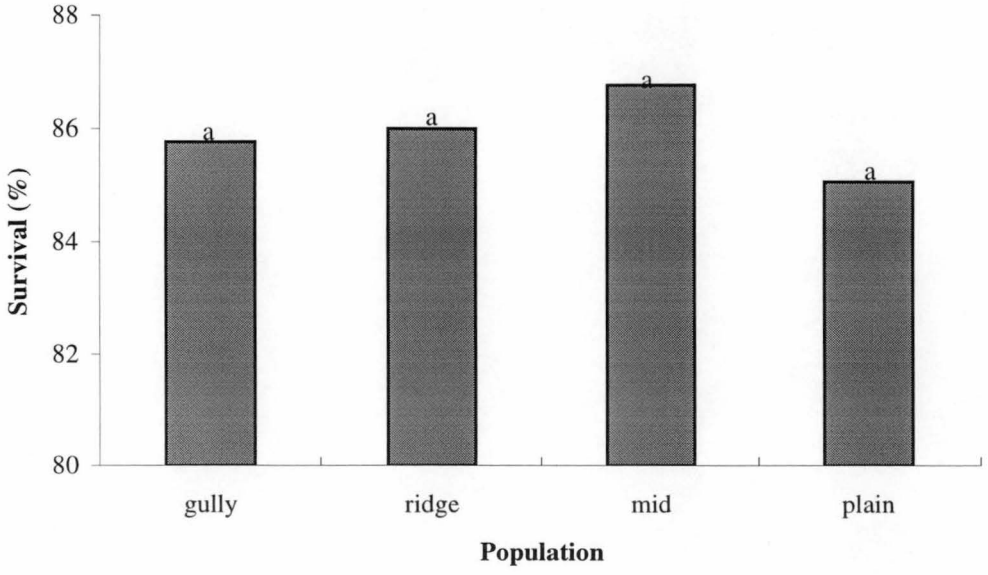


Figure 3.7 Survival of seedlings from four populations at the Forestier Gully planting site

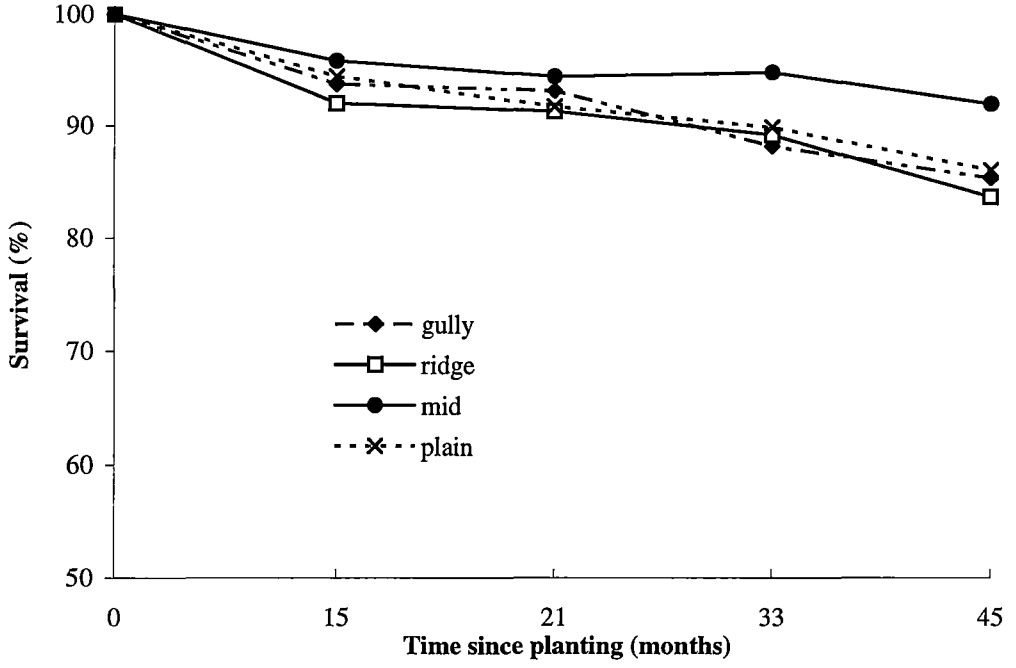


Figure 3.8 Survival of seedlings from four populations at the Forestier Ridge planting site

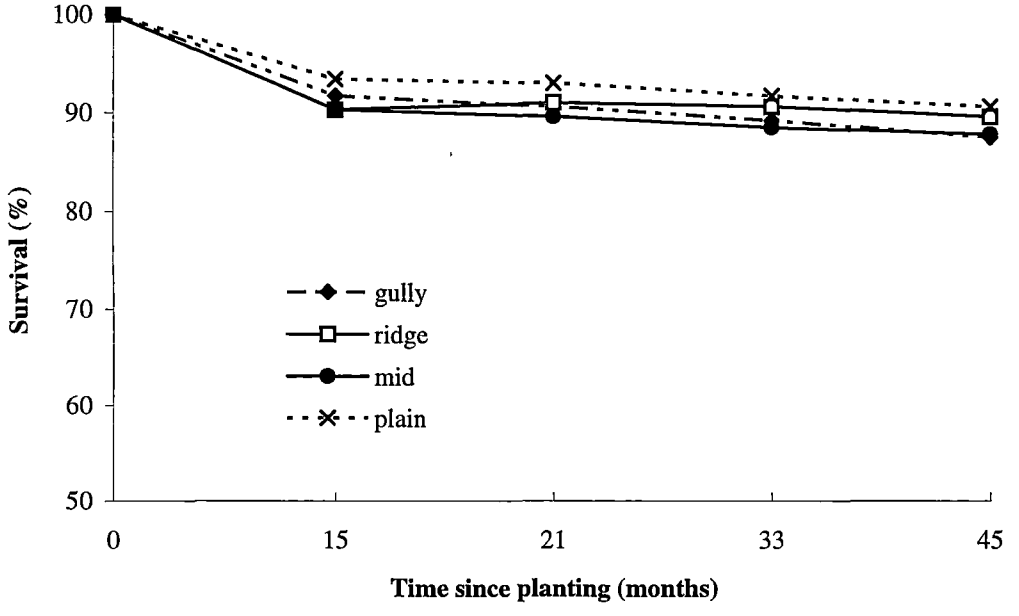


Figure 3.9 Survival of seedlings from four populations at the Lune Mid planting site

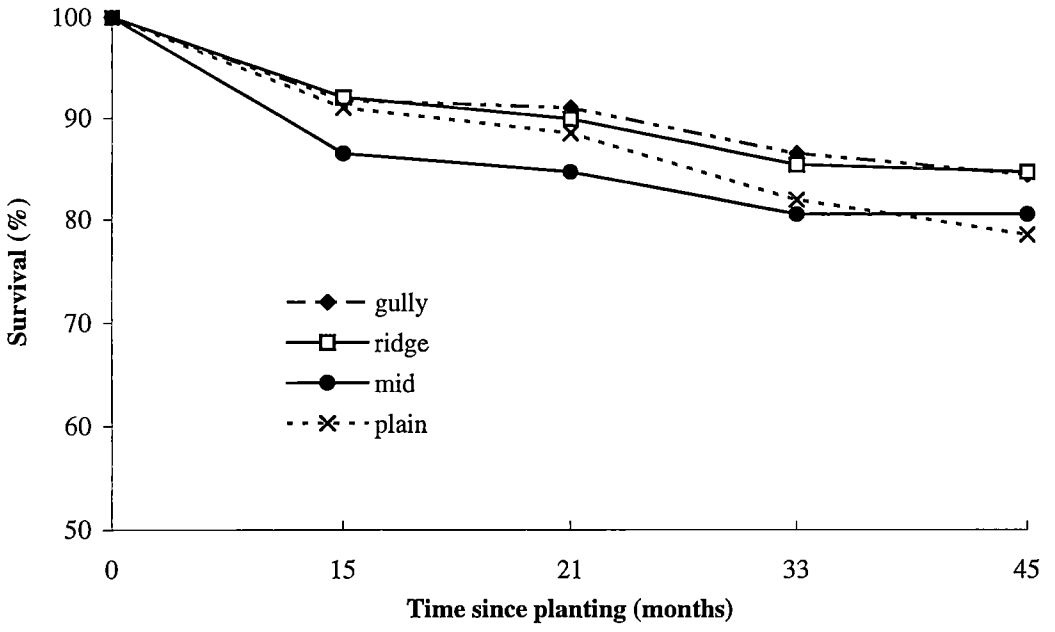
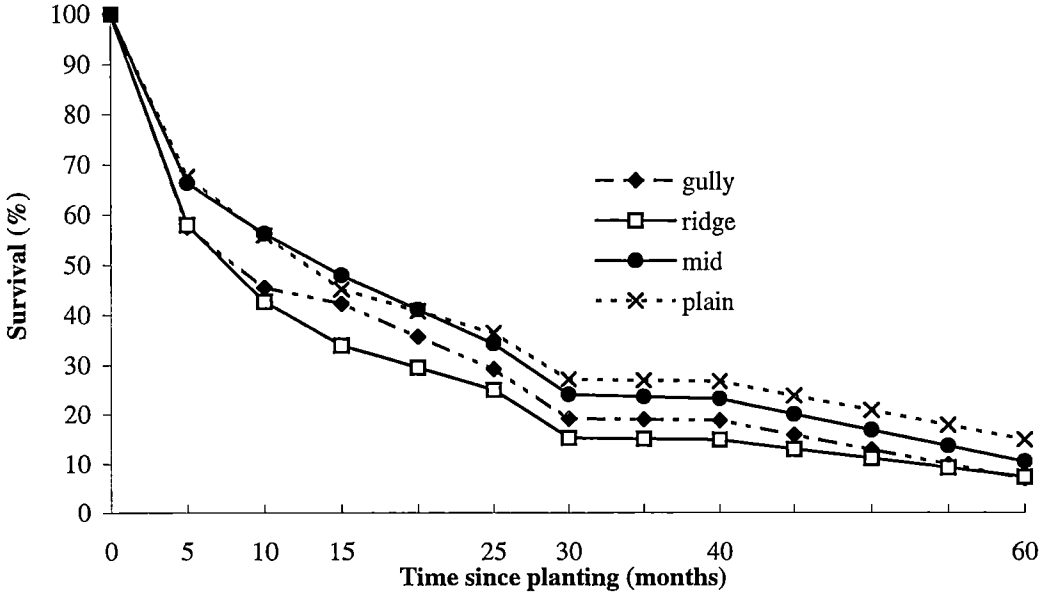


Figure 3.10 Survival of seedlings from four populations at the Lune Plain planting site



Mean height growth

The mean height of seedlings varied significantly on all four planting sites (Figure 3.11). The tallest height growth was recorded at the Forestier Gully site and there was negligible growth at the Lune Plain site. At age 45 months, there were significant differences between populations across the three sites at Forestier Gully, Forestier Ridge and Lune Mid (Figure 3.12). The Mid population was significantly taller than all other populations, and the Plain population significantly shorter than the Mid and Gully populations. The site x population interaction was not significant (Table 3.6) and the population rankings were maintained across each of the three sites.

Differences in the height growth of populations at the Forestier Gully site were expressed by age 9 months when the Mid population was significantly taller than the other three populations (Table 3.7, Figure 3.13). This trend continued to at least age 60 months.

Differentiation of populations occurred slightly later (15 months) at the Forestier Ridge site with the Mid population recording better height growth than the Plain and Gully populations (Table 3.7, Figure 3.14). By age 45 months, the Mid, Gully and Ridge populations had similar heights and were all significantly different to the Plain population. These rankings changed slightly after the subsequent thinning (chapter 7) and by age 60 months the differences in height growth were not significant.

Early differences between the Mid and Plain populations were evident at the Lune Mid site at age 9 months (Table 3.7, Figure 3.15). By age 21 months, the Mid population was significantly taller than the other populations and the Plain population was significantly shorter. This pattern was maintained to at least age 60 months.

Analysis of the variance components for height growth across the planting trials indicated that the majority of variation due to random terms occurred between trees (74%) and plots (16%) (Table 3.9). The Gully site was the only planting site to record significant variation between families within populations, with up to 26% of the total random variation attributed to this source (Table 3.7).

Height increment at the Lune Plain site averaged less than 4 cm yr^{-1} to age 60 months (Figure 3.16). At this age, the mean height of the Mid and Plain populations was 23 cm, compared to 12 cm for the Gully and Ridge populations, but this difference was not significant (Table 3.8). Segregation of the surviving seedlings into height classes showed that the Mid and

Plain populations represented an increasingly larger proportion of the seedlings in the taller height classes (Figure 3.17). The future survival of seedlings <90 cm in height was assessed to be unlikely because of severe competition from the dense understorey vegetation. The Ridge and Gully populations were not represented in the >90 cm and >120 cm height classes respectively.

Table 3.7 Results from REML variance components analysis of data for height of surviving trees at various ages on the Gully, Ridge and Mid planting sites

Age (mths)	Planting site	Fixed term	d.f.	Wald statistic [#1]		Random term	Variance components ± SE [#2 #1]				
5	Forestier	block	3	55.6	**	pop.fam	9.62	±	3.59	(26%)	*
	Gully	pop	3	5.7	ns	block.pop.fam	26.95	±	3.32		
		block x pop	9	4.3	ns						
	Forestier	block	3	27.8	**	pop.fam	6.65	±	4.10	(18%)	ns
	Ridge	pop	3	7.6	ns	block.pop.fam	36.94	±	5.11		
		block x pop	9	10.7	ns						
	Lune Mid	block	3	3.4	ns	pop.fam	3.35	±	2.37	(11%)	ns
		pop	3	4.9	ns	block.pop.fam	27.97	±	3.44		
		block x pop	9	4.3	ns						
9	Forestier	block	3	25.8	**	pop.fam	67.6	±	36.1	(15%)	ns
	Gully	pop	3	12.9	**	block.pop.fam	371.3	±	45.1		
		block x pop	9	9.7	ns						
	Forestier	block	3	47.3	**	pop.fam	5.27	±	3.28	(13%)	ns
	Ridge	pop	3	7.4	ns	block.pop.fam	36.66	±	4.51		
		block x pop	9	12.4	ns						
	Lune Mid	block	3	8.0	*	pop.fam	2.2	±	13.7	(1%)	ns
		pop	3	11.2	*	block.pop.fam	216.4	±	26.6		
		block x pop	9	6.6	ns						
15	Forestier	block	3	31.0	**	pop.fam	184.3	±	79.9	(21%)	*
	Gully	pop	3	19.2	**	block.pop.fam	706.4	±	87.0		
		block x pop	9	11.0	ns						
	Forestier	block	3	90.9	**	pop.fam	7.18	±	6.94	(7%)	ns
	Ridge	pop	3	9.4	*	block.pop.fam	90.55	±	11.15		
		block x pop	9	12.6	ns						
	Lune Mid	block	3	10.5	*	pop.fam	12.7	±	36.6	(2%)	ns
		pop	3	20.6	**	block.pop.fam	556.0	±	68.4		
		block x pop	9	8.0	ns						
21	Forestier	block	3	38.5	**	pop.fam	581	±	271	(19%)	*
	Gully	pop	3	16.4	**	block.pop.fam	2548	±	314		
		block x pop	9	10.8	ns						
	Forestier	block	3	74.0	**	pop.fam	19.8	±	20.8	(7%)	ns
	Ridge	pop	3	12.2	**	block.pop.fam	276.8	±	34.1		
		block x pop	9	11.1	ns						
	Lune Mid	block	3	4.8	ns	pop.fam	27	±	106	(2%)	ns
		pop	3	29.4	**	block.pop.fam	1635	±	201		
		block x pop	9	8.4	ns						

Table 3.7 (cont.) Results from REML variance components analysis of data for height of surviving trees at various ages on the Gully, Ridge and Mid planting sites

Age (mths)	Planting site	Fixed term	d.f.	Wald statistic [#1]		Random term	Variance components ± SE [#2 #1]				
33	Forestier	block	3	21.3	**	pop.fam	1692	±	928	(15%)	ns
	Gully	pop	3	12.4	**	block.pop.fam	9709	±	1195		
		block x pop	9	10.2	ns						
	Forestier	block	3	78.0	**	pop.fam	47	±	91	(3%)	ns
	Ridge	pop	3	12.6	**	block.pop.fam	1327	±	163		
		block x pop	9	12.2	ns						
	Lune Mid	block	3	3.7	ns	pop.fam	325	±	362	(6%)	ns
		pop	3	34.9	**	block.pop.fam	4884	±	601		
		block x pop	9	11.2	ns						
45	Forestier	block	3	31.8	**	pop.fam	3273	±	1348	(22%)	*
	Gully	pop	3	13.0	**	block.pop.fam	11338	±	1396		
		block x pop	9	10.5	ns						
	Forestier	block	3	68.3	**	pop.fam	780	±	657	(9%)	ns
	Ridge	pop	3	12.8	**	block.pop.fam	8255	±	1016		
		block x pop	9	9.3	ns						
	Lune Mid	block	3	4.3	ns	pop.fam	118	±	222	(4%)	ns
		pop	3	29.1	**	block.pop.fam	3256	±	401		
		block x pop	9	9.4	ns						
60	Forestier	block	3		**	pop.fam	234	±	1567	(1%)	ns
	Gully	pop	3		**	block.pop.fam	24655	±	3046		
		block x pop	9		ns						
	Forestier	block	3		**	pop.fam	268	±	767	(2%)	ns
	Ridge	pop	3		*	block.pop.fam	13251	±	1631		
		block x pop	9		ns						
	Lune Mid	block	3		*	pop.fam	1459	±	1356	(8%)	ns
		pop	3		**	block.pop.fam	17409	±	2151		
		block x pop	9		ns						

[#1] * = significant at p<0.05, ** = p<0.01, *** = p<0.001, ns = not significant (p>0.05)
[#2] percentage of total random variation shown in brackets

Table 3.8 Results from ANOVA for mean height of seedlings from four populations at various ages at the Plain planting site

Age (months)	Source	d.f.	M.S.	F-ratio	Significance level
25	population	3	25.8	1.96	ns
	residual	44	13.1		
30	population	3	108.1	2.04	ns
	residual	44	53.1		
40	population	3	156.4	2.56	ns
	residual	44			
60	population	3	573.4	2.65	ns
	residual	44	216.1		

Figure 3.11 Mean height of all populations at age 45 months on four sites. Identical letters indicate non-significant subsets p>0.05 (plain site not included in analysis)

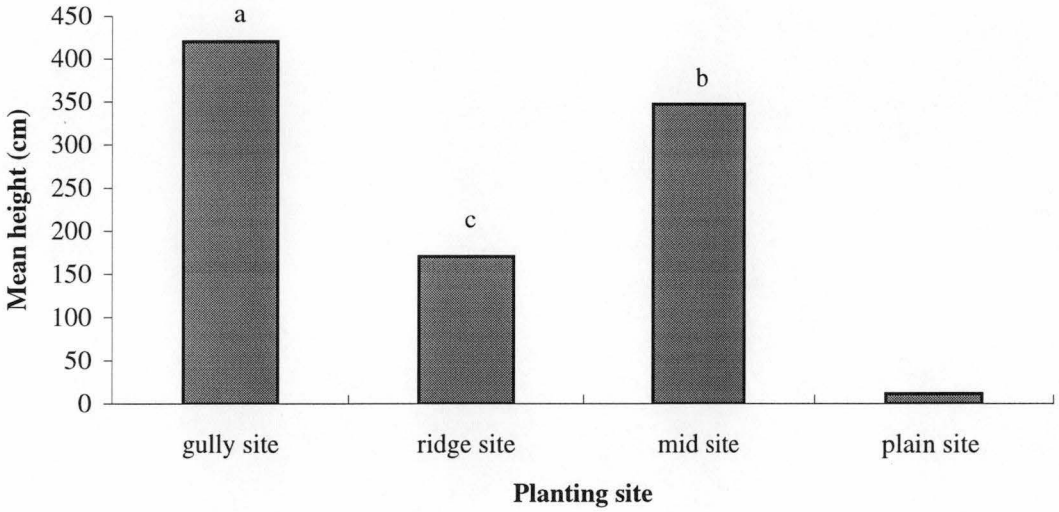


Figure 3.12 Mean height of four populations at age 45 months across three planting sites at Forestier Gully, Forestier Ridge and Lune Mid. (Identical letters indicate non-significant subsets p>0.05)

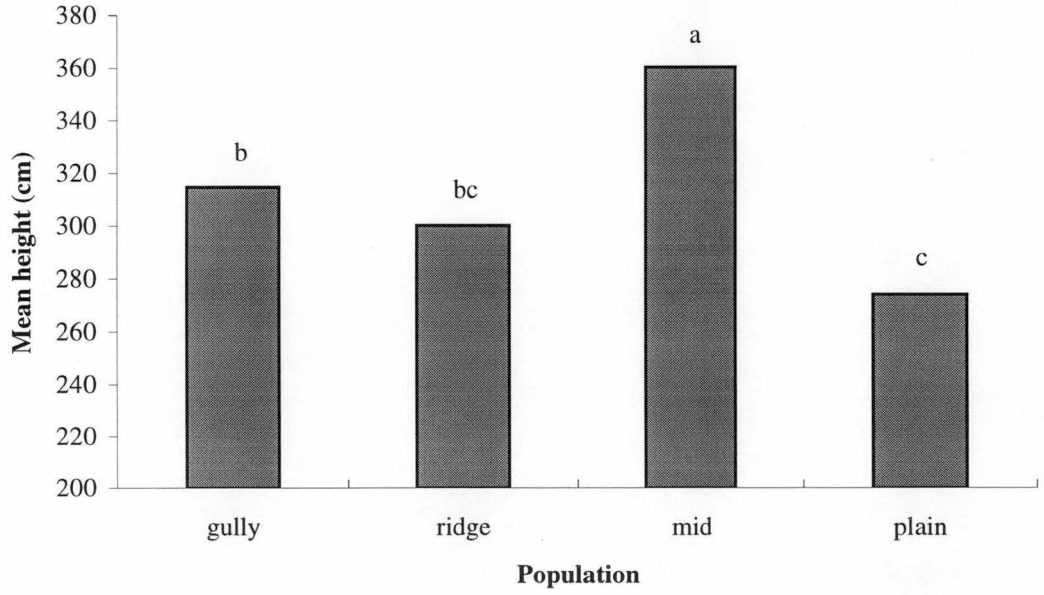


Figure 3.13 Mean height of populations planted at the Forestier Gully planting site. Identical letters indicate non-significant subsets within each age class (p>0.05).

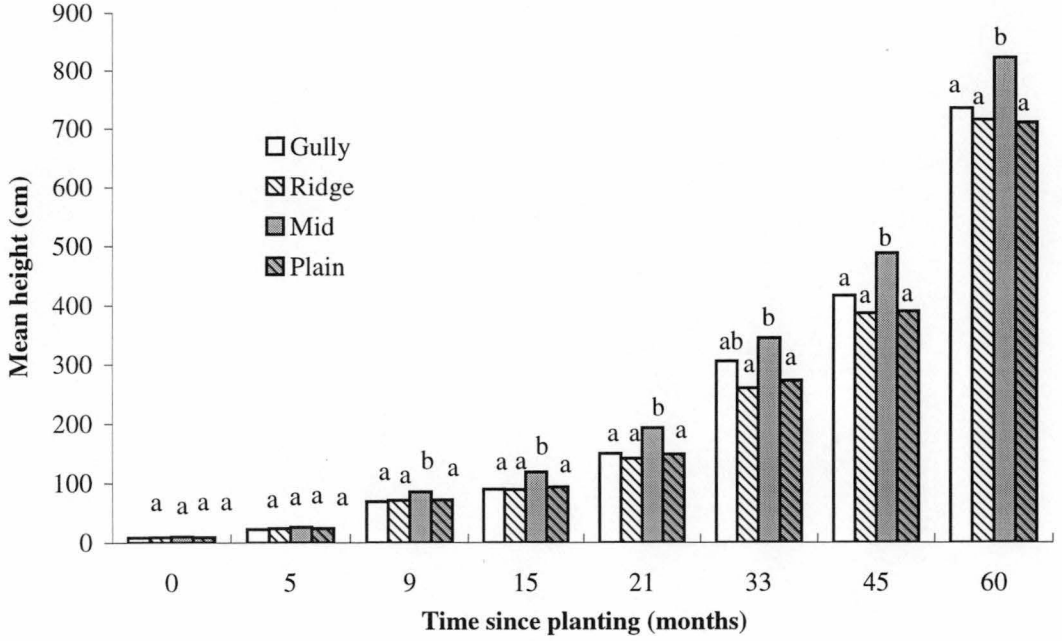


Figure 3.14 Mean height of populations planted at the Forestier Ridge planting site. Identical letters indicate non-significant subsets within each age class (p>0.05).

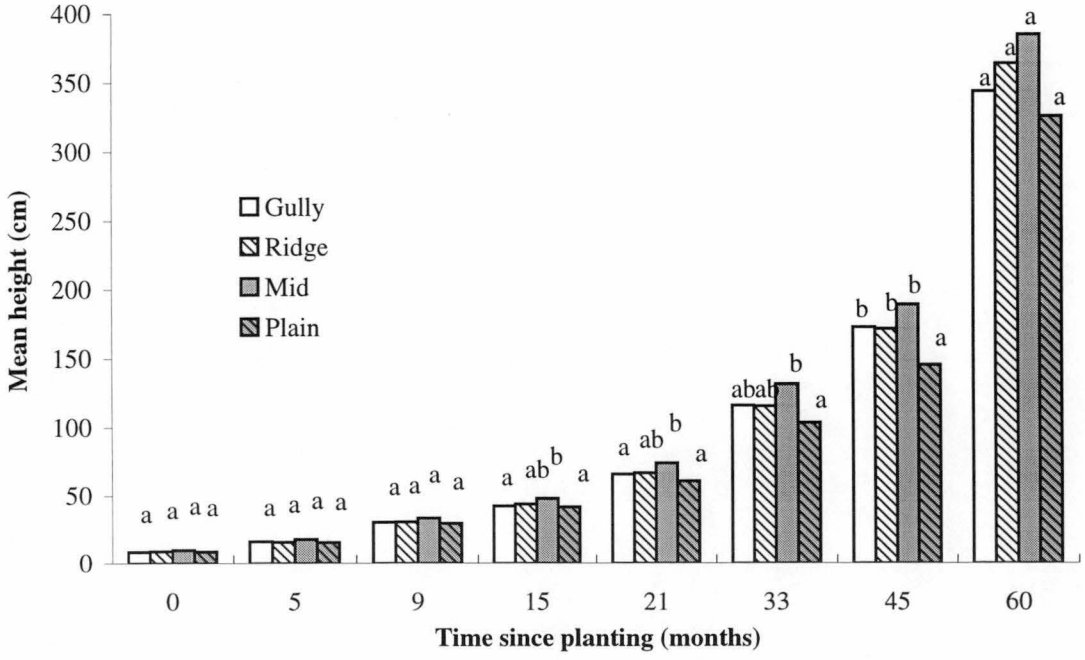


Figure 3.15 Mean height of populations planted at the Lune Mid planting site. Identical letters indicate non-significant subsets within each age class (p>0.05)

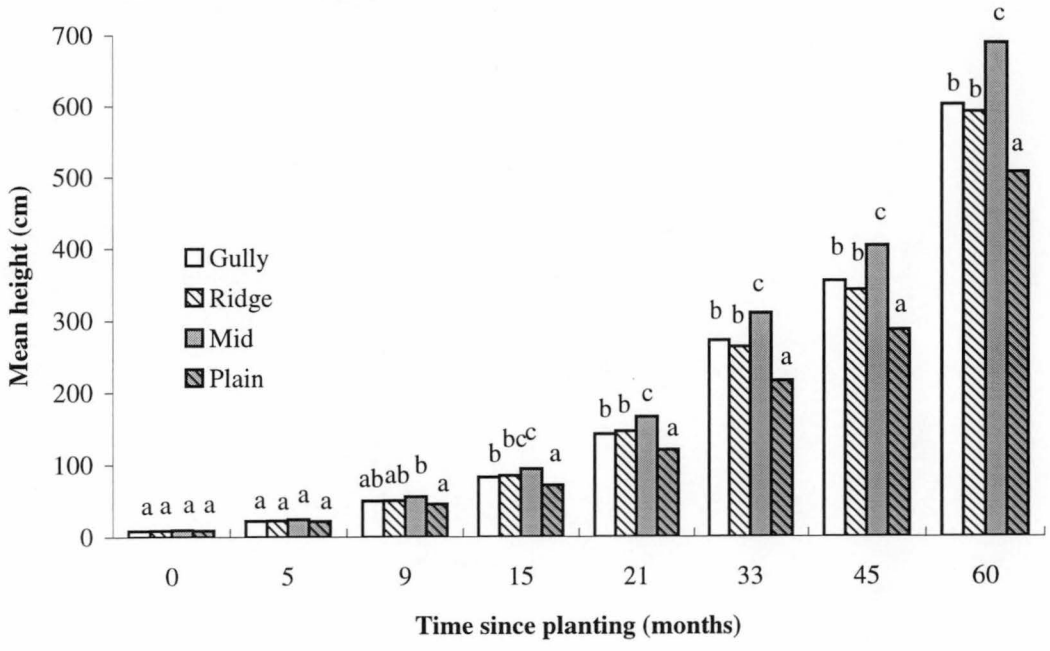


Figure 3.16 Mean height of populations planted at the Lune Plain planting site (differences between populations are not significant (p>0.05))

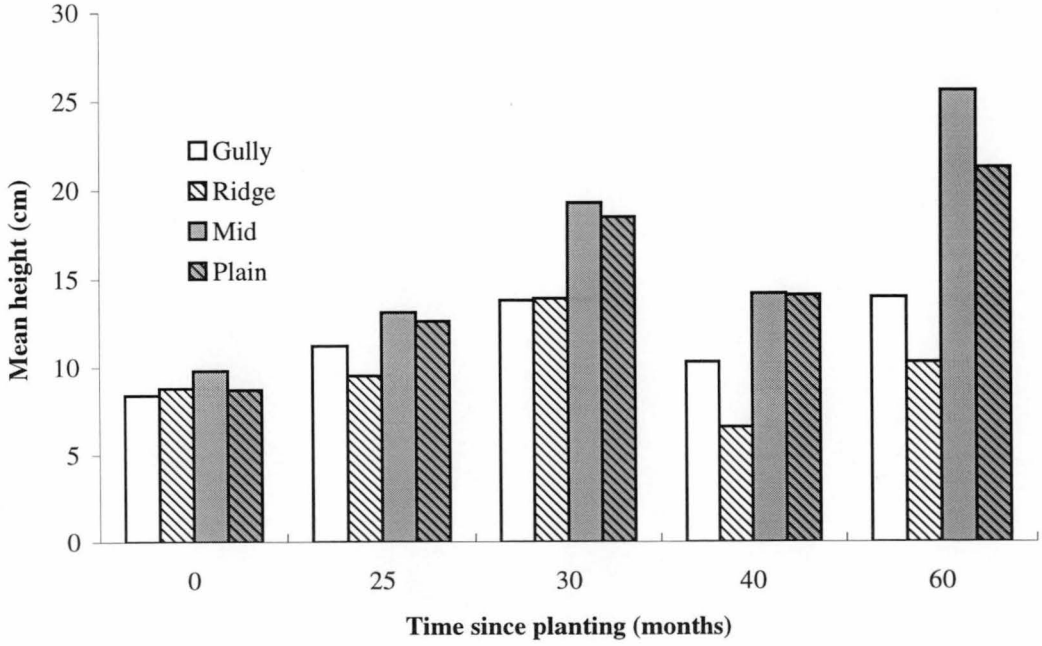
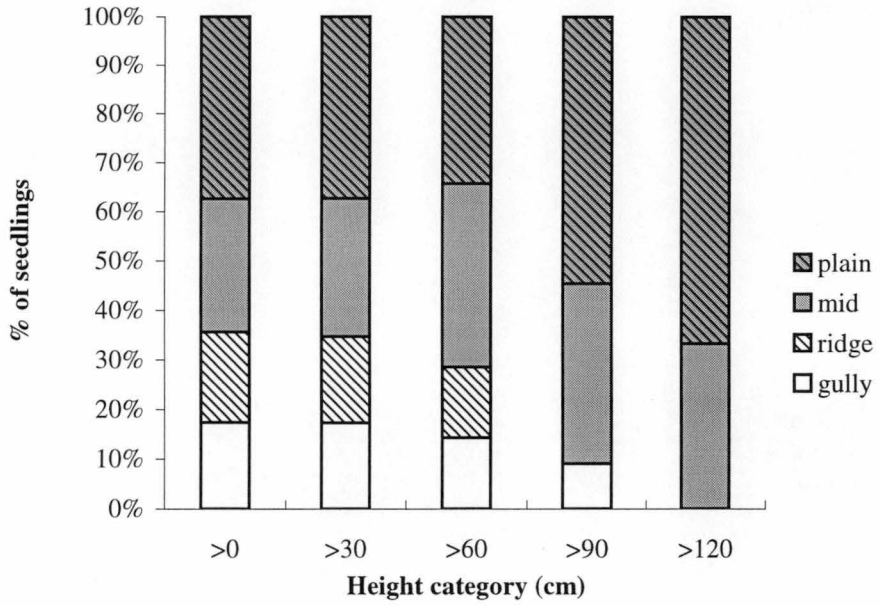


Figure 3.17 Proportion of seedlings from four populations within various height categories at age 60 months in the Lune Plain planting trial



Total volume production

Total volume production varied significantly between planting sites, with the Forestier Gully site producing 14 times the volume of the nearby Ridge site at age 45 months (Figure 3.18). Growth at the Lune Plain site was insufficient to allow for the calculation of volume. Across the other planting sites, there were significant differences between populations, with the Mid population producing the highest volume (Figure 3.19). There was also a significant interaction between populations and sites (Table 3.6). This interaction mainly affected the magnitude of differences, rather than the ranking of populations at each site. In particular, the difference between the Mid and Plain populations was much larger at the Lune Mid site than at other planting sites (see below).

Differences in volume production were recorded at the Forestier Gully site at 33 and 45 months after planting (Table 3.10, Figure 3.20). At these ages, the Mid population had produced significantly more volume than the other populations. These differences became less apparent after thinning, and at age 60 months there were no differences in the mean volume of the retained trees.

Volumes produced by the Mid, Gully and Ridge populations were not significantly different at the Forestier Ridge site at age 45 months (Table 3.10, Figure 3.21). However, the Plain population produced significantly less volume than the Mid and Ridge populations. This pattern was maintained after thinning to age 60 months.

Large differences in volume production were recorded at the Lune Mid site (Table 3.10, Figure 3.22). At age 33 months, the highest volume was produced by the Mid population. Further differentiation occurred by age 45 months, when the Mid population had produced significantly more volume, and the Plain population significantly less volume than the other populations. At this age, volume production by the Plain population was only 50% of that produced by the Gully and Ridge populations, and only 31% of that produced by the Mid population. These significant differences continued to be maintained after thinning to age 60 months.

Analysis of the variance components for volume indicated that the majority of variation occurred between trees (81%) and plots (14%) (Table 3.9). Within the individual planting trials, variation between families within populations was generally not significant and represented less than 18% of the total random variation. (Table 3.10).

Table 3.9 Estimated variance components for the height and volume of four populations at age 45 months on three planting sites (Forestier Gully, Forestier Ridge and Lune Mid).

Random term	Height			Volume		
	Component	S.E.	% of variation	Component	S.E.	% of variation
site.block	1288	683	5	0.403	0.222	4
population.family	724	365	3	0.096	0.097	1
site.population.family	684	416	3	0.109	0.148	1
site.block.population.family (plot)	4000	538	16	1.465	0.224	14
units (tree)	18668	539	74	8.696	0.251	81

Table 3.10 Results from REML variance components analysis of data for the total volume at various ages on the Gully, Ridge and Mid planting sites (results are for the total volume of unthinned plots at 33 and 45 months after plantings, and for the volume of the retained tree on thinned plots at 60 months after planting)

Age (mths)	Planting site	Fixed term	d.f.	Wald statistic [#1]		Random term	Variance components ± SE [#2 #1]				
33	Forestier	block	3	38.4	**	pop.fam	2.22	±	1.57	(12%)	ns
	Gully	pop	3	15.6	**	block.pop.fam	18.17	±	2.26		
		block x pop	9	19.5	*						
	Lune Mid	block	3	9.2	*	pop.fam	0.91	±	1.26	(5%)	ns
		pop	3	18.2	**	block.pop.fam	17.40	±	2.16		
		block x pop	9	12.0	ns						
45	Forestier	block	3	38.5	**	pop.fam	32.2	±	15.2	(18%)	*
	Gully	pop	3	11.9	**	block.pop.fam	143.6	±	17.7		
		block x pop	9	21.8	**						
	Forestier	block	3	31.4	**	pop.fam	0.159	±	0.213	(4%)	ns
	Ridge	pop	3	10.1	*	block.pop.fam	3.933	±	0.484		
		block x pop	9	11.6	ns						
	Lune Mid	block	3	5.9	ns	pop.fam	5.9	±	8.3	(5%)	ns
		pop	3	27.5	**	block.pop.fam	117.1	±	14.4		
		block x pop	9	15.5	ns						
60	Forestier	block	3	29.2	**	pop.fam	8.8	±	8.2	(8%)	ns
	Gully	pop	3	5.4	ns	block.pop.fam	105.6	±	13.0		
		block x pop	9	3.7	ns						
	Forestier	block	3	46.0	**	pop.fam	0.208	±	0.307	(4%)	ns
	Ridge	pop	3	8.3	*	block.pop.fam	5.505	±	0.685		
		block x pop	9	16.2	ns						
	Lune Mid	block	3	5.1	ns	pop.fam	2.83	±	5.54	(4%)	ns
		pop	3	27.0	**	block.pop.fam	80.41	±	9.97		
		block x pop	9	16.4	ns						

[#1] * = significant at p<0.05, ** = p<0.01, *** = p<0.001, ns = not significant (p>0.05)
[#2] percentage of total random variation shown in brackets

Figure 3.18 Mean volume of all populations at age 45 months on four sites. Identical letters indicate non-significant subsets at p>0.05 (plain site not included in analysis)

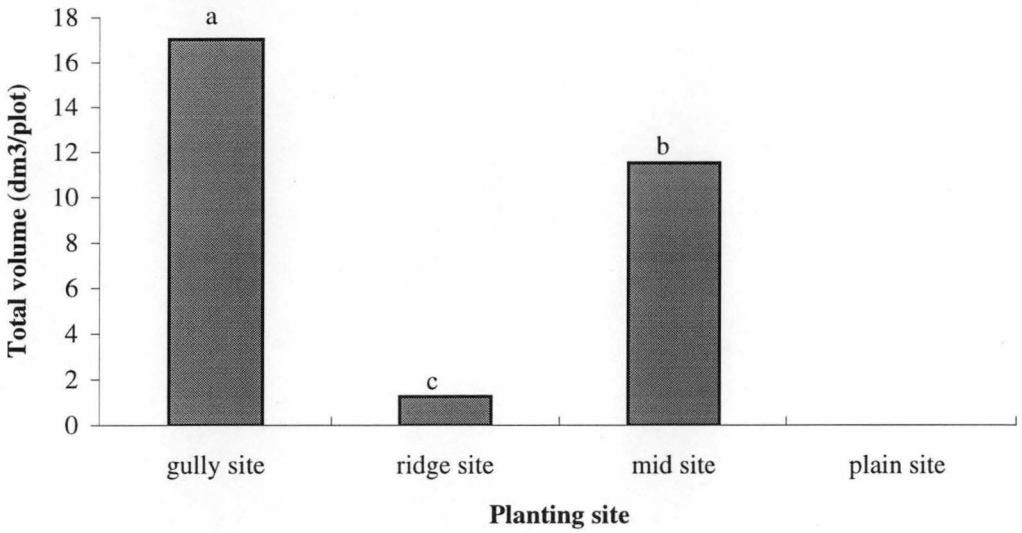


Figure 3.19 Mean volume of four populations at age 45 months across three planting sites at Forestier Gully, Forestier Ridge and Lune Mid. (Identical letters indicate non-significant subsets at p>0.05)

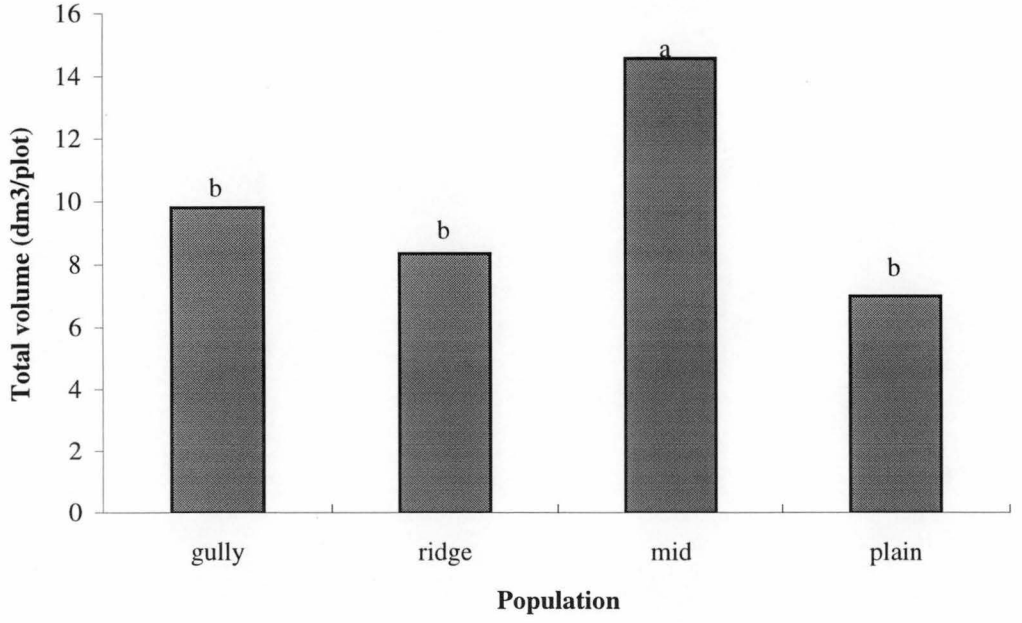


Figure 3.20 Mean volume at ages 33#, 45# and 60## months for populations planted at the Forestier Gully planting site (# = mean total plot volume, ## = mean tree volume after thinning). Identical letters indicate non-significant subsets within each age class (p>0.05).

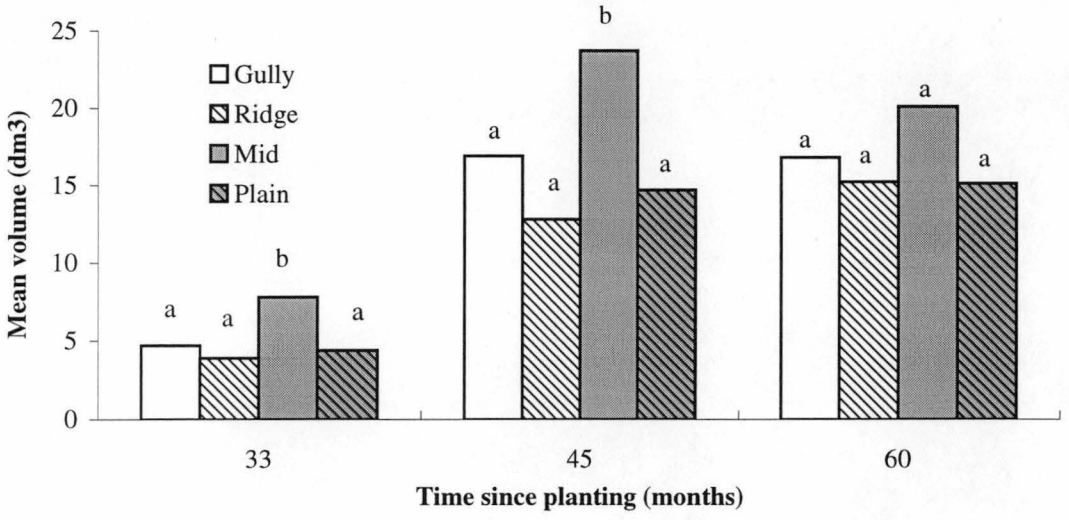


Figure 3.21 Mean volume at ages 45# and 60## months for populations planted at the Forestier Ridge planting site (# = mean total plot volume, ## = mean tree volume after thinning). Identical letters indicate non-significant subsets within each age class (p>0.05).

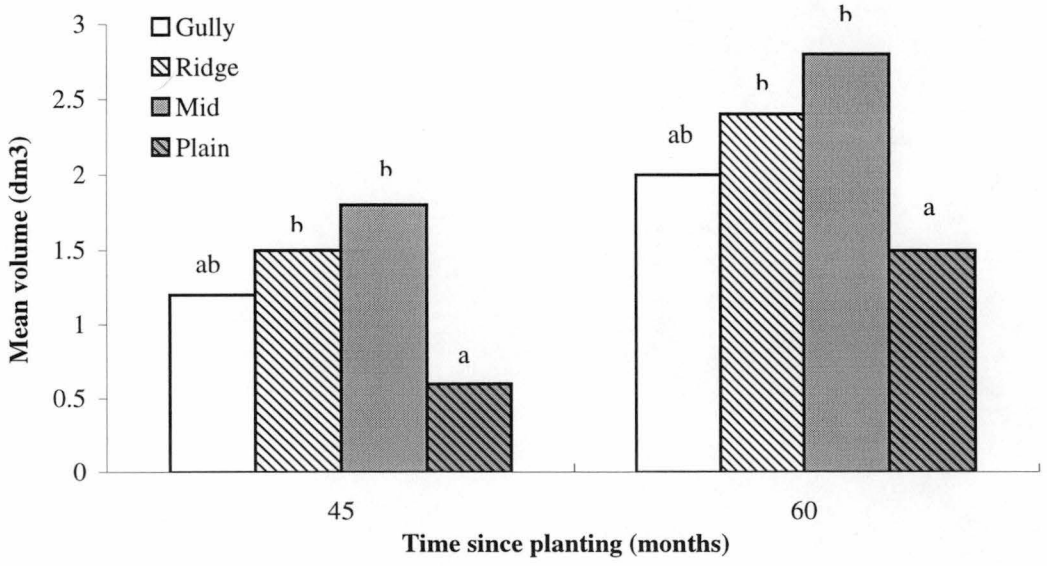
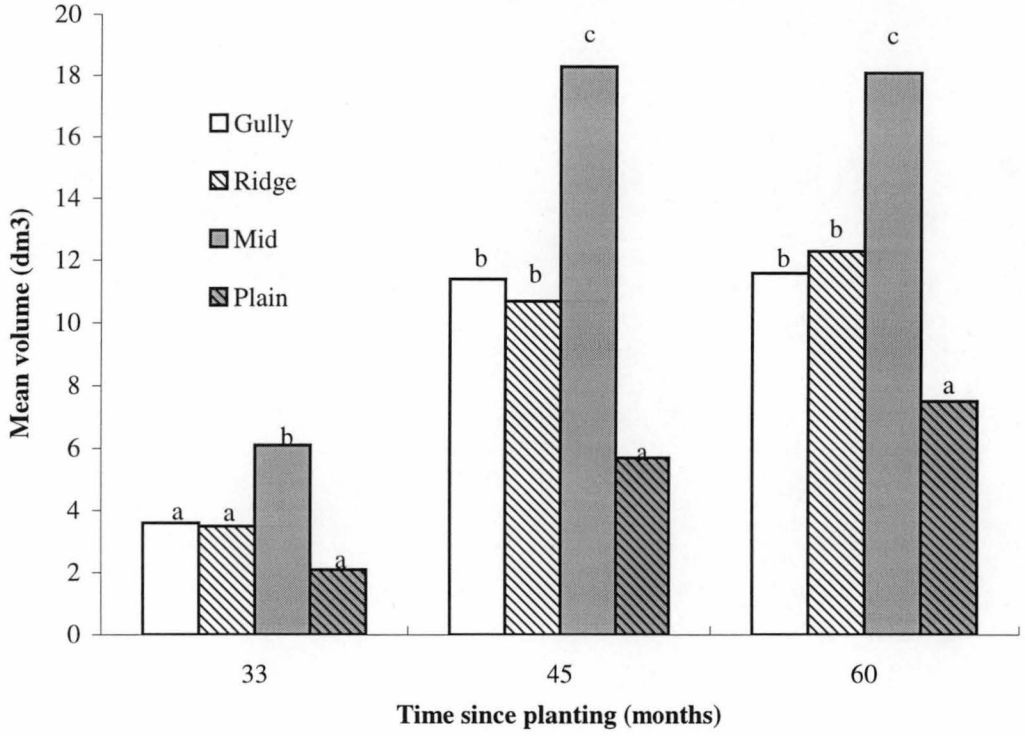


Figure 3.22 Mean volume for populations at 33#, 45# and 60## months after planting at the Lune Mid site (# = mean total plot volume, ## = mean tree volume after thinning). Identical letters indicate non-significant subsets within each age class (p>0.05)



4. Discussion

Current results from the Lisle and Taranna trials indicate that there may be large differences in the growth of populations from different sites within a small geographical area. However, the interpretation of these differences is difficult, as they could be random, as a consequence of high intra-provenance variation, or alternatively may reflect differential selection pressures within each site. Support for site differentiation could be indicated by the results at the Lisle site where the low site index population (Strathblane 18) had significantly poorer growth than the medium site index populations (Strathblane 29 and 34). In contrast, at the Taranna site, the low site index population had higher survival and volume than the medium site index populations, although these differences were not significant. Differences in the relative rankings of these populations could possibly be explained by differences in the growing environments between the Lisle and Taranna sites. The Lisle site was a high rainfall, fertile site conducive to rapid growth rates, whereas the Taranna site was initially subjected to poor drainage and very wet soil conditions.

The growth of the highest site index population (Strathblane 50) was much poorer than the medium site index populations at both planting sites. This result suggests that selection for fast growth has not been a factor on the highest quality site. Alternatively, the slower growth of the highest site index population may be associated with a higher proportion of '*E. regnans*' types (35% of all trees) than for the medium site index populations (25.5%). The presence of putative *E. regnans* genotypes was clearly evident on the basis of bark characteristics by the age 19 years measurement. Only the 'pure' forms of *E. regnans* (trees with gum bark type persisting to within one metre of the ground) were recorded. The assessment of '*E. regnans*' types may therefore have underestimated the total effect of other levels of hybridisation between *E. regnans* and *E. obliqua*. Clearly, the introduction of hybridisation adds another dimension to the interpretation of results from these trials. As a result, it is not possible to demonstrate a direct relationship between growth and site factors from the origin of the seed source.

Results from the new collections of populations from Forestier Gully, Forestier Ridge, Lune Mid and Lune Plain indicated that there were no significant differences in the early growth of seedlings under glasshouse conditions. In contrast, Green (1971) found that significant differences across the height of seedlings from 22 provenances were expressed by age 6 weeks. Using the same provenances, Brown *et al.* (1976) demonstrated a high correlation (60%) between glasshouse heights at 37 weeks and the height of field plantings at age 3.5 years.

Differences in height growth between populations in the new trials were not expressed on productive sites (Forestier Gully and Lune Mid) until the end of the first growing season, 9 months after planting. Differences on the lower productivity site at Forestier Ridge were not significant until 15 months after planting. Once established, the differences in height growth were maintained on the productive sites to at least age 60 months. On these sites, the Mid population was significantly taller than the other populations, providing clear evidence of population differentiation at the local site level between this population and the nearby Plain population. In contrast, there were no differences between the Gully and Ridge populations, indicating a lack of differentiation for growth, at least on productive sites.

Differences between populations were less pronounced at the lower productivity (Forestier Ridge) site, where after 33 months, the Mid population was not significantly different to the Gully and Ridge populations, suggesting that attributes other than inherent fast growth may be important for success on this harsher site. There was also no differentiation between the Gully and Ridge populations, indicating that both populations were equally well adapted to

grow under the conditions experienced over the 60 months since planting. Rainfall on this site for the first three years after planting was within 98% of the long term average (Chapter 2). In addition, the seedlings had minimal levels of competition during the first four years, and subsequent thinning maintained a low level of competition after age four years. Thus the plants were unlikely to have been exposed to stress, such as severe competition for moisture, during the period of the experiment and longer term trends may be particularly important on this site.

Total volume production is regarded as a useful index of growth potential because it combines the measures of survival, diameter and height growth. As such, the pre-thinning volumes at age four years in the current trials are a more relevant measure of total productivity than the post-thinning volumes of single tree plots at age 60 months. Across all sites the Mid population produced more than twice the volume of the Plain population. This difference was most apparent at the Lune Mid site where the volume produced by progeny of the 'on-site' Mid population was more than three times that of the progeny from the nearby Plain population. Observations from the two highly productive sites (Forestier Gully and Lune Mid) suggest that the successful growth of populations was related to their ability to rapidly achieve site occupancy, by establishing a competitive advantage over the understorey species. Early competition from understorey species can lead to significant growth suppression of eucalypt seedlings (Wilkinson and Neilsen 1990). Vigorous understorey growth of woody species and sedges occurred at both sites (chapter 2), causing suppression of weaker eucalypt seedlings. In contrast, the understorey stratum was virtually absent under the dense crowns of vigorous, well-stocked plots.

The Gully population produced higher volume than the Ridge population at the Gully site and less volume than the Ridge population at the Ridge site. These results indicate the possibility of differential site adaptation, although the differences at age four years were not significant ($p>0.05$).

Overall, the results from the planting trials at the Gully, Ridge and Mid sites provide evidence of genetic differentiation between some populations for height and volume growth. The variation due to the site of origin represented a relatively small component of the total experimental variation due to very high levels of variation between plots and between trees within plots. The limited number of populations included in the current trial does not allow a more accurate assessment of the relative importance of variation at this level to the total variation likely to occur within the range of the species.

Survival and growth at the Lune Plain planting trial were very low, reflecting the severity of local climatic and edaphic factors at this site. High mortality of seedlings was associated with very low temperatures due to cold air drainage across the plain. Frosts as low as -7°C were recorded at the Plain site, compared to a minimum recorded temperature of only -1 °C at the nearby Mid planting site (chapter 2). Temperatures of -5 °C or less were commonly recorded at the Plain site during winter and such temperatures have been shown to be within the lethal range for the hardened leaf tissue of seedlings (chapter 5). During summer, seedlings within plain sites may be exposed to moisture stress due to the drying of organic surface horizons (Jarman *et al.* 1988). Summer droughting may be exacerbated by a shallow depth of root development due to high water tables, with few roots extending below the A1 horizon (chapter 2). Additional constraints on growth are imposed by the low nutrient availability and low pH of the peat surface horizons (Jarman *et al.* 1988, chapter 2).

As a result of these environmental factors, the natural stocking of eucalypts on the plain sites is very low, with mature trees providing less than 5% crown cover (chapter 2) or about 10 to 20 stems ha^{-1}. Survival of seedlings after 60 months at the Plain site averaged less than 10%, reducing the stocking level from 5,000 to 500 stems ha^{-1}. The survival of seedlings was continuing to decrease and a high proportion of the remaining seedlings were very small and unhealthy. Seedlings less than 90 cm in height were assessed to have a very low chance of future survival because of severe competition from the dense understorey of buttongrass and other sedges which was beginning to overtop and smother the smaller seedlings (Photograph 3.1). As a result, it was considered that only seedlings greater than 90 cm in height were likely to survive over the next few years, and the most likely long term survivors would be recruited from seedlings that were more than 120 cm in height (Photograph 3.2). There was the equivalent of 190 and 52 stems ha^{-1} in the >90 cm and >120 cm height classes respectively. The Ridge population was totally absent from both height classes and the Gully population was absent from the taller class, indicating that the Forestier provenance is not likely to be represented in the future stand. The most likely population to survive appeared to be the Plain population, which represented 55% of the seedlings in the >90 cm class (Mid = 36%) and 67% of the seedlings in the >120 cm class (Mid = 33%).

Photograph 3.1 Small seedling at the Plain site three years after planting. The seedling has been completely overtopped by the dense understorey and its future survival is unlikely.

Photograph 3.2 Taller seedling at the Plain site at age three years. This seedling has successfully competed with the understorey.

5. Conclusions

Significant differences in height and volume growth were recorded between progeny of *E. obliqua* originating from the Forestier Gully, Forestier Ridge, Lune Mid and Lune Plain populations. There was evidence of substantial genetic differentiation between the Mid and Plain populations, with progeny from the Mid population producing up to three times the volume of the progeny from the nearby Plain population. In contrast, there was less evidence of differentiation for growth between progeny from adjoining populations at the Gully and Ridge.

The results indicate that differentiation for growth may occur between some populations over the very small geographical scale of a few hundred metres.

CHAPTER 4 SEED AND GERMINATION

1. Introduction

Eucalypt seed readily germinates in response to suitable conditions of temperature, moisture availability and light (Boland *et al.* 1980). Germination characteristics appear to be at least partially under genetic control, although there have been very few studies of population variation (Turnbull and Doran 1987). Doran and Boland (1984) found that there was very little variation in the optimum temperature of germination between provenances of *E. microtheca*. Slight differences in the germination response to temperature were noted between provenances of *E. cloeziana*, but there was little difference in response to moisture stress and light (Turnbull and Shepherd 1984). Battaglia (1993) found that populations of *E. delegatensis* had a similar germination rate response to temperature and moisture stress, but that the germination capacity differed significantly in response to moisture stress, with populations from dry sites least sensitive to moisture stress. In *E. viminalis*, both the germination rate and germination capacity were shown to be significantly higher for high rainfall populations than for low rainfall populations (Ladiges 1974a). Differences in the germination response between species and provenances of *E. camaldulensis*, *E. nitens* and *E. obliqua* have also been implicated as adaptations to the moisture conditions associated with the populations' natural environments (Gibson and Bachelard 1987). Seed dormancy is present in some species and variation between populations is commonly reported (Boland *et al.* 1980). Doran and Gunn (1979) found that dormancy was highly variable within populations of *E. glaucescens* but the pattern of variation did not appear to be correlated with the environmental conditions of the parent trees. In contrast, Battaglia (1993) demonstrated that populations of *E. delegatensis* from cold sites were more likely to be associated with greater degrees of dormancy than populations from mild sites.

Cold, moist stratification is a standard technique for promoting the germination of dormant seed and for increasing the rate of germination (Boland *et al.* 1980). Battaglia (1993) demonstrated a linear relationship between the rate of germination of *E. delegatensis* and the duration of stratification, suggesting that the increase in the rate of germination is a result of progress towards germination during stratification. In contrast, Green (1971) tested 22 populations of *E. obliqua* and found that only one (Powelltown, Victoria) showed a significant response to stratification.

The successful germination of a seed will depend upon the coincidence of favourable climatic factors, the availability of a suitable seedbed and protection from seed-damaging animals and diseases. Sites may be highly heterogeneous, both in terms of time (season) and space (from a scale of the micro-site to the broader environment) (Battaglia 1993). The germination characteristics of populations may be important in maximising the opportunities for the successful establishment of seedlings on a particular site. The current experiments were designed to investigate variation in the germination rate and capacity between populations of *E. obliqua.*

2. Methods

2.1 Seed viability and germination

The germination of seed was studied at three temperatures and for two levels of pre-treatment as indicated in Table 4.1.

Table 4.1. Treatments applied in the germination experiments

Experiment		Germination temperature	Pre-treatment
1		20°C	stratification
2	a	15°C	stratification
	b	15°C	no stratification
3	a	13°C	stratification
	b	13°C	no stratification

Seed within each of the 48 families (chapter 2) was well mixed to ensure a uniform distribution of seed and chaff and a sample of seed was removed from the centre of the seed bag using a spatula. For the initial study (experiment 1), an equal amount of seed (0.6g per replicate) was taken from each family. Results from this study were used to calculate the weights of seed required to yield approximately 30 germinants for each family replicate. These quantities of seed were used in subsequent experiments in order to overcome the problem associated with the large variability in germination capacity between families. Each replicate of seed was weighed out and evenly distributed across a 9cm filter paper.

In experiment 1 (stratification + 20°C) the filter papers were placed on cotton towelling within plastic containers and distilled water was added until the towelling and filter papers

were thoroughly moist but not over-saturated. The plastic containers were sealed within plastic bags to prevent moisture loss. The experiment contained four replicates of each family (12 families x 4 populations x 4 replicates = 192 tests). The containers held 12 filter papers and families were randomly allocated within each replicate of 4 containers. A cold, moist stratification treatment was imposed by placing the containers in a constant temperature cool room at a temperature of 2°C (+/- 1°C) for 4 weeks. Following stratification, the containers were placed on shelves within a germination room at a constant of 20°C (+/- 1.5°C) under a regime of 12 hours fluorescent light per day.

Experiments 2 and 3 were conducted in a germination cabinet in which size limitations necessitated the use of individual petri dishes. These were prepared by placing a 9cm filter paper upon moist vermiculite within a glass petri dish. The petri dishes were randomly located on shelves within the germination cabinet. All families were used in experiment 2, with four replicates, giving a total of 384 tests (12 families x 4 populations x 2 treatments x 4 replicates). Experiment 3 comprised four families randomly selected from each population and there was three replicates of the stratification treatment and six replicates of the non-stratification treatment, giving a total of 144 tests (4 families x 4 populations x 2 treatments x 3 or 6 replicates = 144). The stratification treatments were imposed by placing the petri dishes in cardboard boxes within the cool room as described above. Germination temperatures were maintained within a germination cabinet to within +/- 0.2°C. A 12 hour period of illumination per day was provided by fluorescent lights within the cabinet. Distilled water was added to the vermiculite during the germination period as required to maintain a moist filter paper.

During the course of the germination tests, seeds were sprayed as required with a 0.5g L^{-1} solution of benomyl, a fungicide that prevents the growth of moulds and has no phytotoxic effect on eucalypt seed. Germination was defined as the clear extension of the radicle from the seed coat. Forceps were used to carefully remove germinated seeds from the filter paper. Germination counts were made after the commencement of germination and continued at regular intervals until all germination had appeared to cease. Squash tests were then conducted on the remaining seed and a count was made of any healthy embryos that had failed to germinate. Total seed viability was defined as the sum of the germinated seeds and the viable non-germinated seeds, expressed as the number of viable seeds per g. Germination capacity was calculated as the percentage of the viable seeds that had germinated by the end of the test period, 30 to 40 days after the commencement of germination (Boland *et al.* 1980). Germination energy was determined as the proportion of viable seeds that had germinated at various times during the germination period (Boland *et al.* 1980).

Total seed viability was ~~determined~~ analysed by ANOVA of the number of viable seeds per g. Percentage germination data were transformed to log(-log(P)) values where P= percentage germination/100. Preliminary analyses indicated that the cumulative germination data were generally sigmoidal in shape and the log(-log) transformation ensured the homogeneity of variances and the normal distribution of residuals. All statistical analyses were performed on the mean values of the replicates for each family. The germination energy of each population was determined by ANOVA of the transformed values for percentage germination at various times after the commencement of germination. Multifactor ANOVA was performed on the data from experiments 2 and 3 in order to evaluate the effects of population, stratification and two-way interactions. The rate of germination was determined by regression analysis of the family mean log(-log(P)) values with log(T), where T= time in days for germination to occur, taken from the commencement of germination. Differences between populations and treatments were determined by paired comparisons of the estimated intercepts and slopes for the regressions.

2.2 Seed size

The seed of *E. obliqua* is very small and viable seeds cannot be visually distinguished from chaff material. Seed size was assessed by separating 10g of seed from each family into three size classes using sieves with the following apertures : 0.05mm, 1.0mm and 1.2mm. The seed material (i.e. mix of viable seeds and chaff) from each size class was weighed and 0.5g was then distributed onto filter papers and placed on wet towelling within plastic trays. The trays were placed in a germination room at 20°C and germination was counted after 10 days and after 20 days when germination appeared to have ceased. Squash tests were conducted on the remaining seed material to detect the presence of any ungerminated but viable seeds.

The proportion of seed material and the proportion of viable seeds within each size class were calculated for each family. The mean size of viable seeds was determined as the weighted mean of the proportion of seed in each size class (as defined by the aperture size above). The family mean values were subjected to one-way ANOVA and differences between populations were compared with the value for LSD at p=0.05.

3. Results

Total seed viability and seed size showed significant variation between populations (Table 4.2). The Forestier populations, Gully and Ridge, had more than twice the total number of viable seeds per g than the Lune populations, Mid and Plain (Figure 4.1). The mean size of the seed material was correlated with the mean size of viable seeds (r^2= 34%, $p<0.001$). The Mid population had a significantly higher proportion of large seed particles than the other populations (Table 4.2 and Figure 4.2) and a significantly higher proportion of large size viable seeds than the Gully and Ridge populations (Table 4.2 and Figure 4.3). Overall, the Gully had the lowest mean seed size (Table 4.2 and Figure 4.4).

Seed size had a significant effect on germination energy, with larger seeds germinating faster than smaller seeds (Table 4.3 and Figure 4.5). There were also significant differences in the germination energy of populations within various size classes (Table 4.4 and Figure 4.6).

Germination capacity averaged 99% across the experiments (Figure 4.7) and there were no significant differences between populations or treatments. Temperature had a significant effect on the rate of germination for *E. obliqua* (Table 4.5, Figures 4.7 and 4.8). Germination at 20 °C commenced four days earlier than at 15 °C and 13 °C and the initial rate of germination was more rapid. As a result, a germination rate of 80% was attained seven or 10 days earlier at 20 °C than at 15 °C and 13 °C respectively.

Germination at 20 °C following stratification was very rapid, and there were no significant differences in germination between populations (Table 4.6, Figures 4.9 and 4.10). In contrast, significant differences were indicated for germination energy at 20 °C without prior stratification (Table 4.4 and Figure 4.6).

Significant differences between populations were also detected in experiments 2 and 3, with increasing magnitude and longevity of response at the lower temperature. At 15 °C, the Gully population had a significantly higher germination energy during the main period of germination (Table 4.7 and Figure 4.11). In addition, the rate of germination for the Gully population was different to that for the Plain and Ridge populations (Figure 4.12). Significant differences in germination energy at 13 °C were indicated for the Gully v Ridge, Gully v Mid, Gully v Plain and Mid v Plain populations (Table 4.8 and Figure 4.13). The

rate of germination for the Plain population was also found to differ from that of the Gully and Mid populations (Figure 4.14).

The effect of stratification on germination was not consistent across the experiments. Significant differences were detected at 13 and 15 °C in both the commencement and rate of germination. At 13 °C, the germination of the stratified seed commenced two days earlier than the stratified seed (Figure 4.15) but proceeded at a slower rate (Table 4.5 and Figure 4.16), resulting in equivalent germination after 20 days. However, at 15 °C, the stratified seed was slower to commence germination (Figure 4.17) and once germination commenced the rate remained slower than that of the non-stratified seed (Figure 4.18). There were no significant interactions between the populations and stratification treatments in either experiment (Tables 4.7 and 4.8). Stratification resulted in more rapid and uniform germination at 20 °C with an average germination of more than 87% after 9 days (Figure 4.9) compared to an average germination for unstratified seed of 56% after 10 days (Figure 4.6).

Table 4.2 Results from one-way ANOVA for the weight of seed material, total number of viable seeds and proportion of viable seeds within three size classes and for the mean size of viable seeds

Factor	Size class	Source	d.f	M.S.	F-ratio	Significance level
Weight of seed material (proportion of total seed weight)	1.2mm	population	3	873.08	6.231	***
		residual	44	140.21		
	1.0mm	population	3	184.32	1.33	ns
		residual	44	138.16		
	0.5mm	population	3	1097.32	2.86	*
		residual	44	383.28		
Total number of viable seeds/g	1.2mm	population	3	1858.81	4.53	**
		residual	44	410.23		
	1.0mm	population	3	5837.35	5.74	**
		residual	44	1016.44		
	0.5mm	population	3	1537.43	7.21	***
		residual	44	213.38		
	all seeds	population	3	24299.20	8.26	***
		residual	44	2940.91		
Proportion of viable seeds within each size class	1.2mm	population	3	1311.88	4.06	**
		residual	44	322.81		
	1.0mm	population	3	464.39	3.32	*
		residual	44	139.73		
	0.5mm	population	3	285.25	4.00	**
		residual	44	71.23		
mean seed size (mm)		population	3	0.0525	4.18	**
		residual	44	0.0125		

(ns = not significant at p=0.05; * = significant at p<0.05; ** = p<0.01; *** = p<0.001)

Figure 4.1 Comparative seed viability, expressed as the total number of viable seeds per g within three size classes (identical letters for the adjoining portions of histogram indicate non-significant subsets for the number of viable seeds within each size class; letters at the top of each histogram are for total viable seeds)

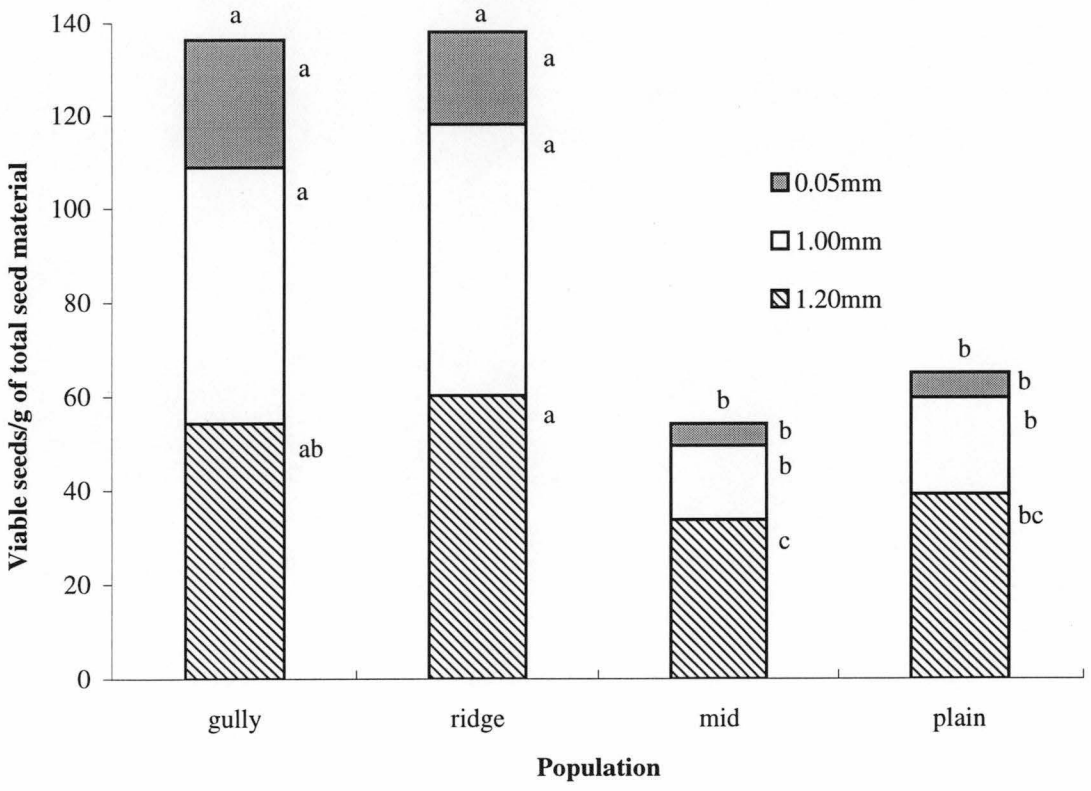
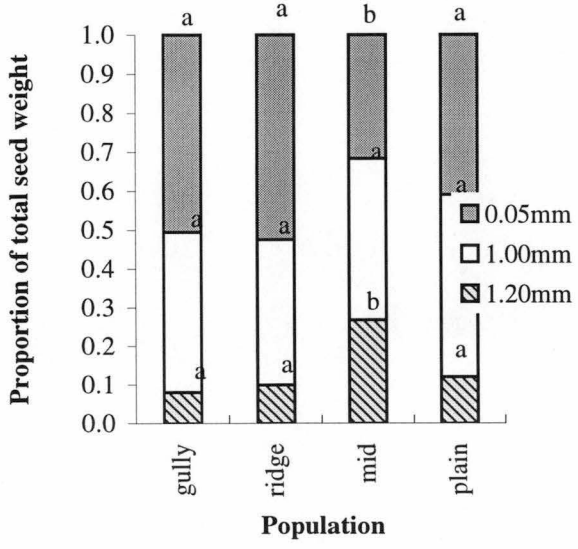


Figure 4.2 Proportion by weight of seed particles within each size class

Figure 4.3 Proportion of total number of viable seeds within each size class

(identical letters indicate non-significant subsets for each size class)



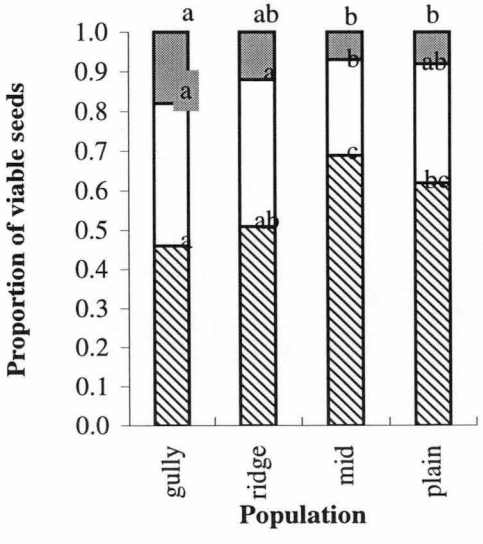


Figure 4.4 Mean size of viable seeds (identical letters indicate non-significant subsets)

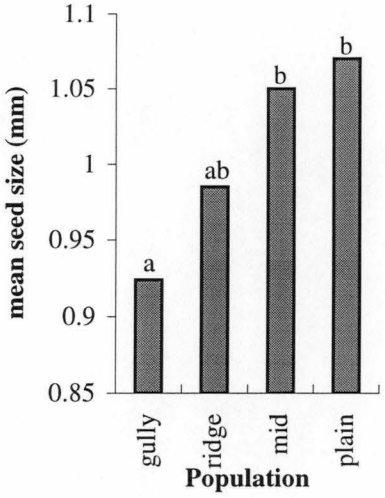


Figure 4.5 Relationship between seed size and germination energy after 10 days at 20°C (r^2=38%)

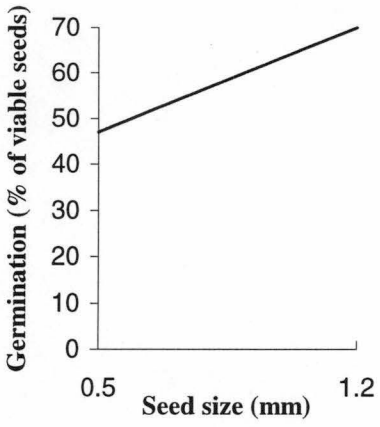


Figure 4.6 Germination energy after 10 days at 20°C for viable seeds within each size class (identical letters indicate non-significant subsets for each size class)

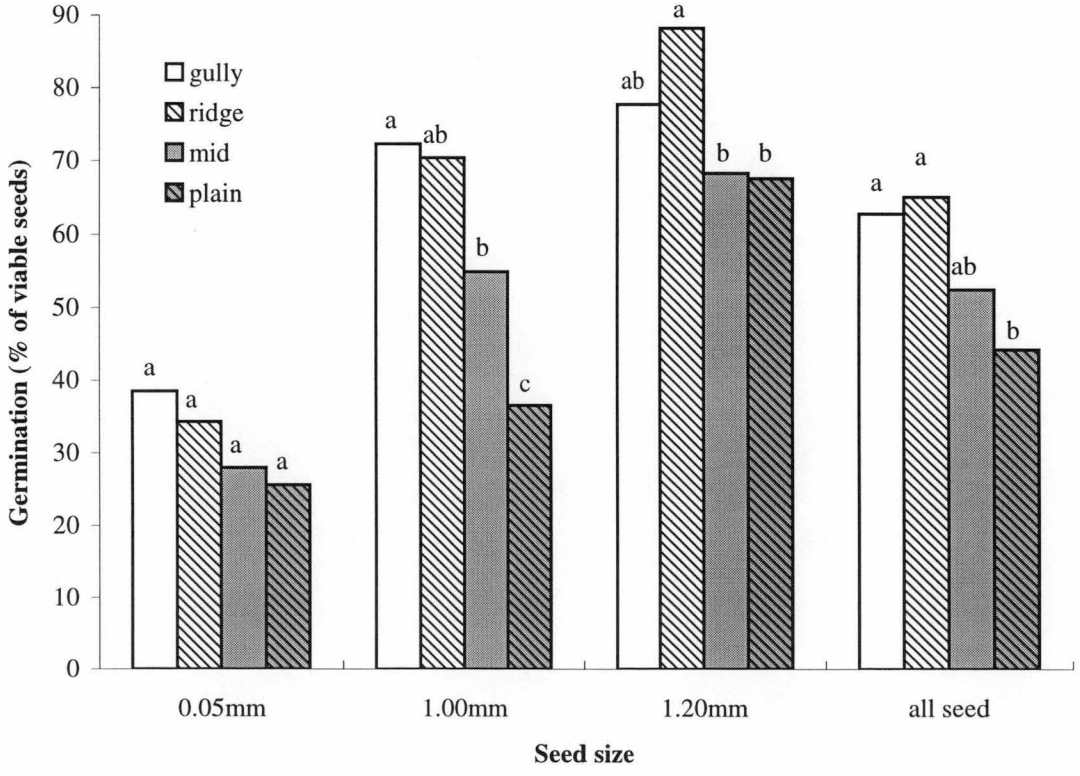


Table 4.3 Relationship between seed size and germination energy after 10 days at 20 °C determined from regression analysis for seed from four populations

Source	Linear model	Significance level	r^2(%)
gully	Y = 36.99 + 34.45X	***	49.9
ridge	Y = 31.28 + 44.10X	***	60.9
mid	Y = 25.56 + 33.01X	***	35.5
plain	Y = 21.27 + 29.11X	***	28.3
all populations	Y = 29.33 + 34.67X	***	37.8

(# Y= germination energy; X= seed size; *** = significant at p<0.001)

Table 4. 4 ANOVA for the germination energy after 10 days at 20 °C for seed from various size classes

Seed size	Source	d.f.	M.S.	F-ratio	Significance level
1.2mm	population	3	1116.63	3.42	*
	residual	44	326.13		
1.0mm	population	3	3306.13	8.79	***
	residual	44	375.94		
0.05mm	population	3	372.40	0.80	ns
	residual	44	466.67		
all seed	population	3	3220.54	4.50	**
	residual	44	715.14		

(ns = not significant at p=0.05; * = significant at p<0.05; ** = significant at p<0.01; *** = significant at p<0.001)

Figure 4.7 Comparative germination rate of *E. obliqua* seed at various temperatures

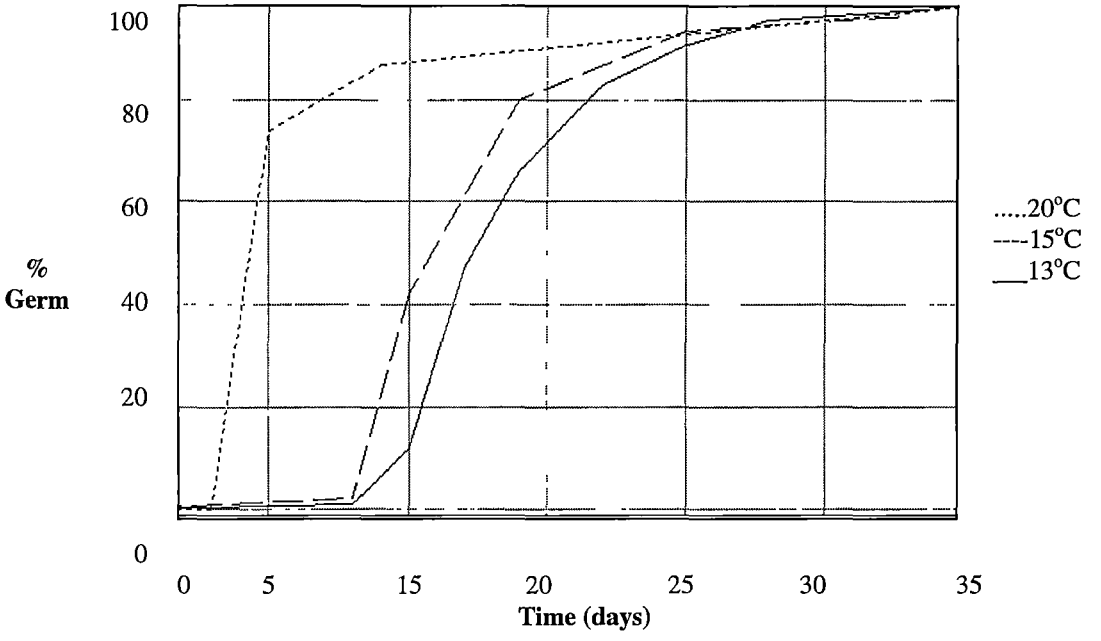


Figure 4.8 Rate of germination of *E. obliqua* seed at various temperatures expressed as the upper and lower confidence limits (CL) of the regressions of transformed values for germination percentage against time

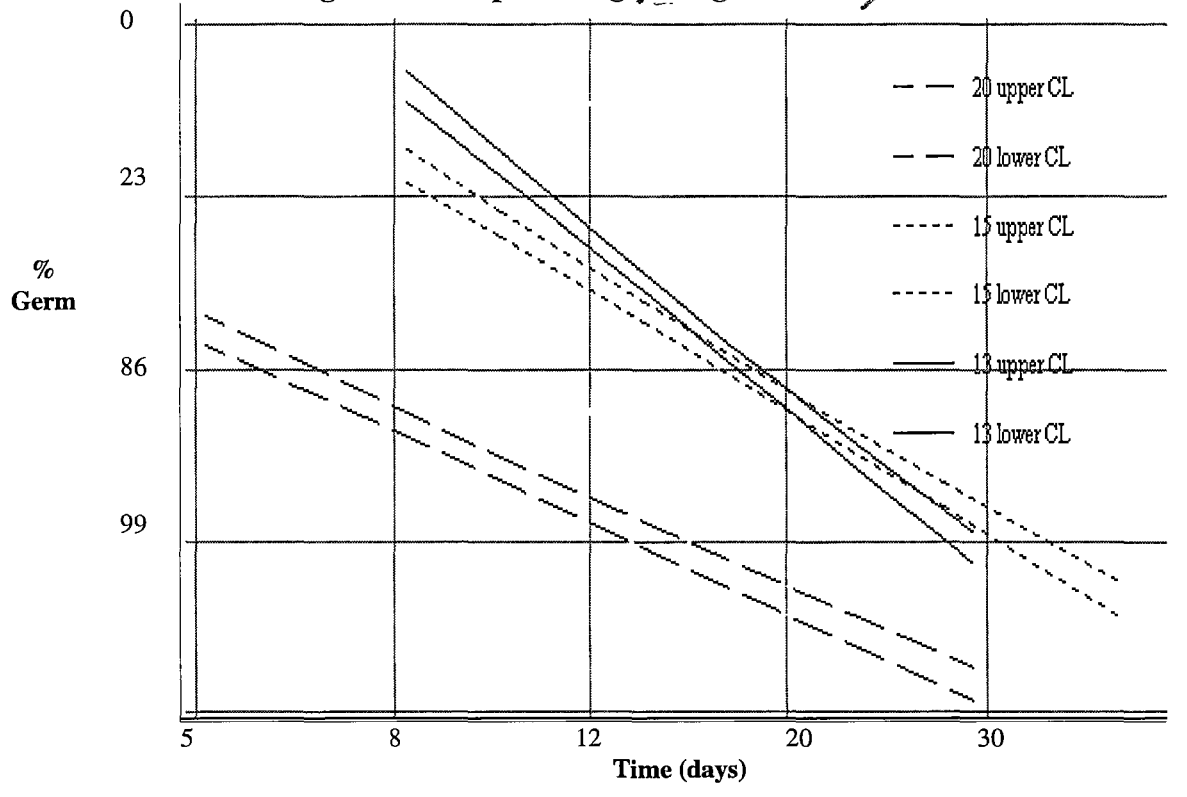


Table 4.5 Estimated values and SE for the intercept (a), slope (b) and r^2 of linear models determined for the rate of germination from regression analysis (the models are of the form Y=a + bX where Y=-log(log) transformed value of germination percentage and X=log transformed values of time (days for germination))

Experiment	Source	Intercept			Slope			r^2 (%)
1	gully	-1.810	±	0.054	-2.473	±	0.166	61.0
	ridge	-1.616	±	0.052	-2.483	±	0.159	63.1
	mid	-1.932	±	0.066	-2.670	±	0.210	55.5
	plain	-1.733	±	0.054	-2.948	±	0.164	69.5
2	gully	-0.506	±	0.049	-3.556	±	0.145	80.9
	ridge	-0.312	±	0.052	-3.432	±	0.151	78.5
	mid	-0.337	±	0.054	-3.667	±	0.156	79.6
	plain	-0.294	±	0.054	-3.319	±	0.157	75.8
	stratification	-0.547	±	0.048	-3.147	±	0.138	68.6
	no stratification	-0.537	±	0.037	-3.398	±	0.115	75.4
3	gully	-0.133	±	0.072	-4.935	±	0.279	76.9
	ridge	0.040	±	0.056	-5.034	±	0.218	85.1
	mid	-0.054	±	0.053	-4.494	±	0.204	83.7
	plain	0.081	±	0.043	-4.175	±	0.164	87.4
	stratification	-0.343	±	0.057	-3.964	±	0.218	72.5
	no stratification	0.238	±	0.042	-5.310	±	0.153	84.4
combined	germination at 20 °C	-1.773	±	0.030	-2.645	±	0.091	85.6
	germination at 15 °C	-3.715	±	0.035	-3.149	±	0.046	93.4
	germination at 13 °C	-0.016	±	0.036	-4.659	±	0.140	92.1

Table 4.6 ANOVA of transformed values of germination percentage at various times for populations of *E. obliqua* at 20 °C (experiment 1).

Time (days)	Source	d.f.	M.S.	F-ratio	P-value
5	population	3	0.0808	1.08	ns
	error	44	0.0747		
9	population	3	0.2362	0.80	ns
	error	44	0.2969		
30	population	3	0.7657	2.68	ns
	error	44	0.2857		

Figure 4.9 Germination of the four populations at 20°C following stratification (identical letters indicate non-significant subsets for each time period)

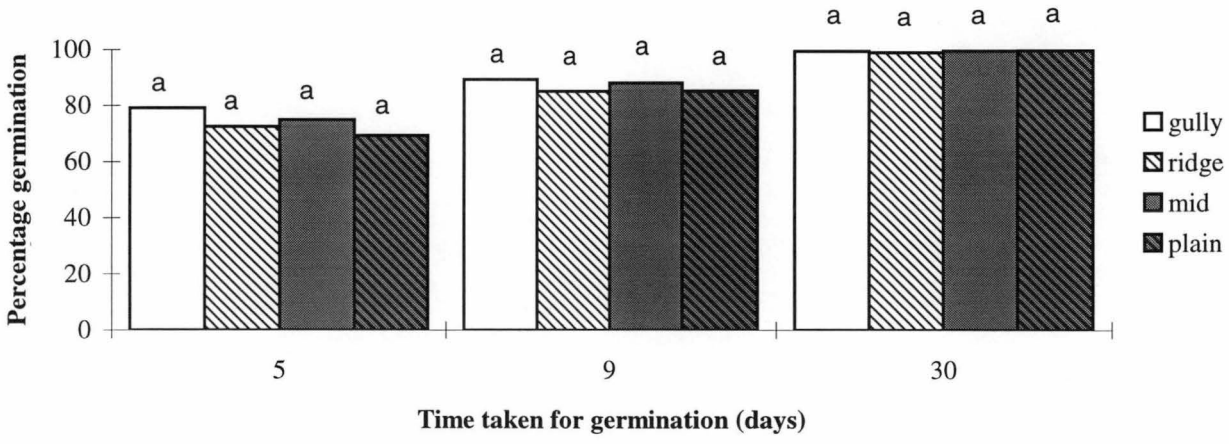


Figure 4.10 Rate of germination after stratification of the four populations at 20°C expressed as the upper and lower confidence limits (CL) of the regressions of the transformed values of germination percentage against time

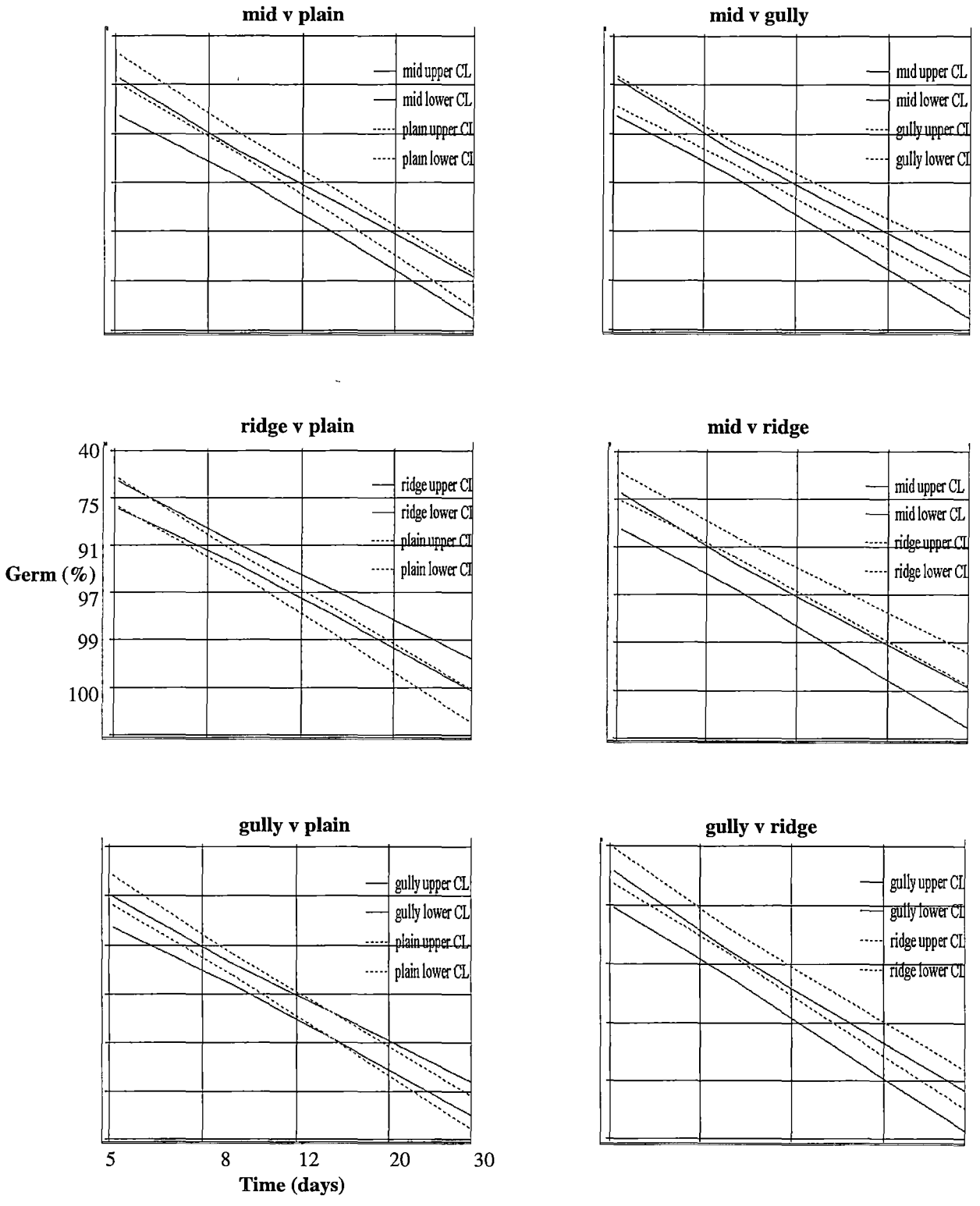


Table 4.7 ANOVA of transformed values of germination percentage at various times for populations of *E. obliqua* at 15 °C (experiment 2).

Time (days)	Source	d.f.	M.S.	F-ratio	P-value
8	population	3	0.0338	0.96	ns
	treatment	1	6.2624	177.86	***
	interaction (pxt)	3	0.0338	0.96	ns
	total	95			
10	population	3	0.4402	5.23	**
	treatment	1	0.4241	5.04	**
	interaction (pxt)	3	0.1461	1.73	ns
	total	95			
14	population	3	0.4521	3.69	*
	treatment	1	0.7113	5.81	*
	interaction (pxt)	3	0.2152	1.76	ns
	total	95			
20	population	3	0.4928	2.10	ns
	treatment	1	0.0004	0.00	ns
	interaction (pxt)	3	0.1373	0.58	ns
	total	95			
28	population	3	0.6397	2.22	ns
	treatment	1	0.0552	0.19	ns
	interaction (pxt)	3	0.1763	0.61	ns
	total	95			
42	population	3	0.4934	1.74	ns
	treatment	1	0.9934	3.51	ns
	interaction (pxt)	3	0.0316	0.11	ns
	total	95			

(ns = not significant ($p>0.05$); * = $p<0.05$; ** = $p<0.01$, *** = $p<0.001$)

Figure 4.11 Comparative germination energy of the four populations at 15°C (identical letters indicate non-significant subsets within each time period)

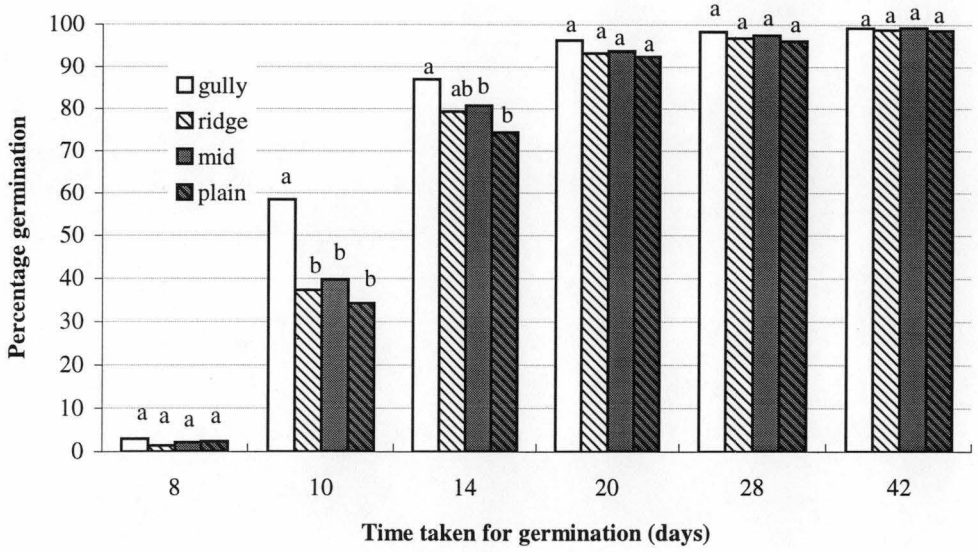


Figure 4.13 Comparative germination energy of the four populations at 13°C (identical letters indicate non-significant subsets within each time period)

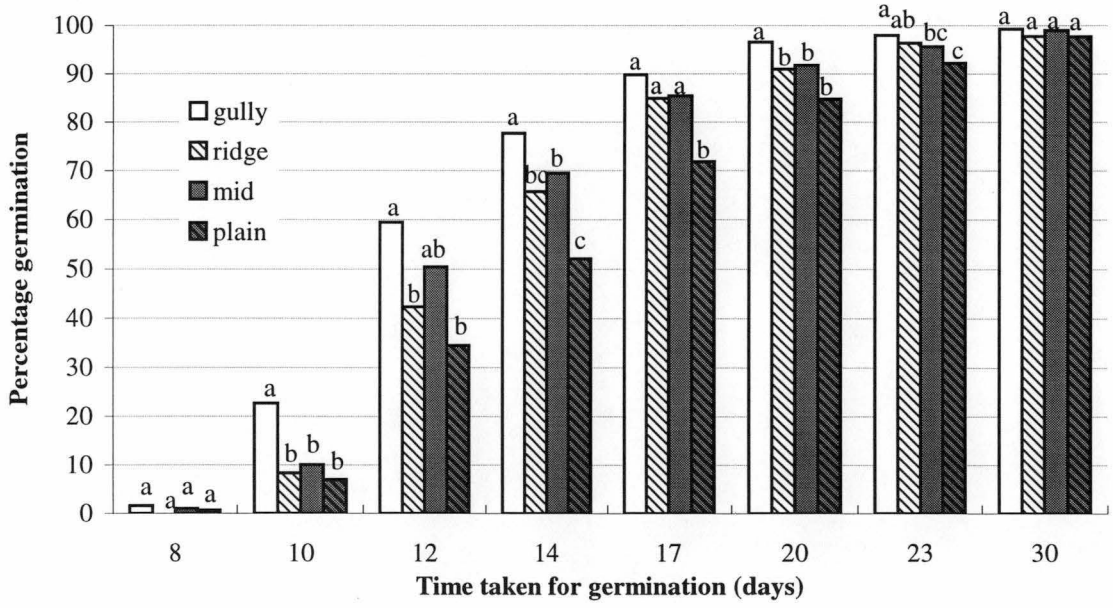


Figure 4.12 Rate of germination of the four populations at 15°C expressed as the upper and lower confidence limits (CL) of the regressions of the transformed values of germination percentage against time

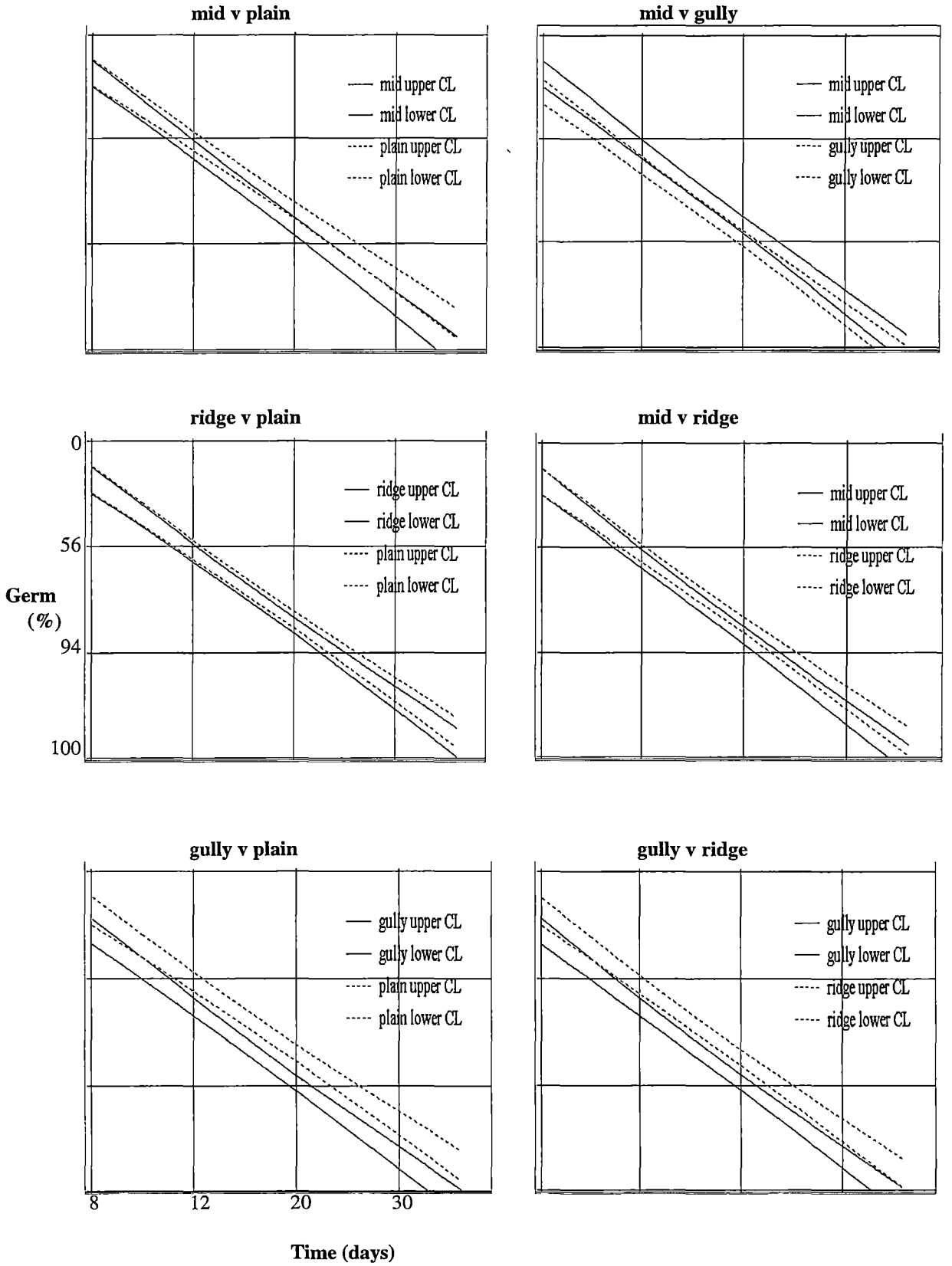


Table 4.8 ANOVA of transformed values of germination percentage at various times for populations of *E. obliqua* at 13 °C (experiment 3).

Time (days)	Source	d.f.	M.S.	F-ratio	P-value
8	population	3	0.0116	2.21	ns
	treatment	1	1.3091	249.03	***
	interaction (pxt)	3	0.0116	2.21	ns
	total	47			
10	population	3	0.1388	3.54	*
	treatment	1	4.5041	114.99	***
	interaction (pxt)	3	0.0313	0.80	ns
	total	47			
12	population	3	0.1904	2.95	*
	treatment	1	2.8022	43.36	***
	interaction (pxt)	3	0.0371	0.57	ns
	total	47			
14	population	3	0.2887	3.80	*
	treatment	1	1.7766	23.40	***
	interaction (pxt)	3	0.0409	0.54	ns
	total	47			
17	population	3	0.4402	6.78	***
	treatment	1	0.3957	6.10	*
	interaction (pxt)	3	0.1016	1.57	ns
	total	47			
20	population	3	0.8354	6.80	***
	treatment	1	0.3973	3.23	ns
	interaction (pxt)	3	0.3234	2.63	ns
	total	47			
23	population	3	0.6446	3.88	*
	treatment	1	0.3275	1.97	ns
	interaction (pxt)	3	0.1634	0.98	ns
	total	47			
30	population	3	0.6917	1.50	ns
	treatment	1	0.1710	0.37	ns
	interaction (pxt)	3	0.1450	0.32	ns
	total	47			

(ns = not significant at p=0.05; * = significant at p<0.05; ** = significant at p<0.01; *** = significant at p<0.001)

Figure 4.14 Rate of germination of the four populations at 13°C expressed as the upper and lower confidence limits (CL) of the regressions of the transformed values of germination percentage against time

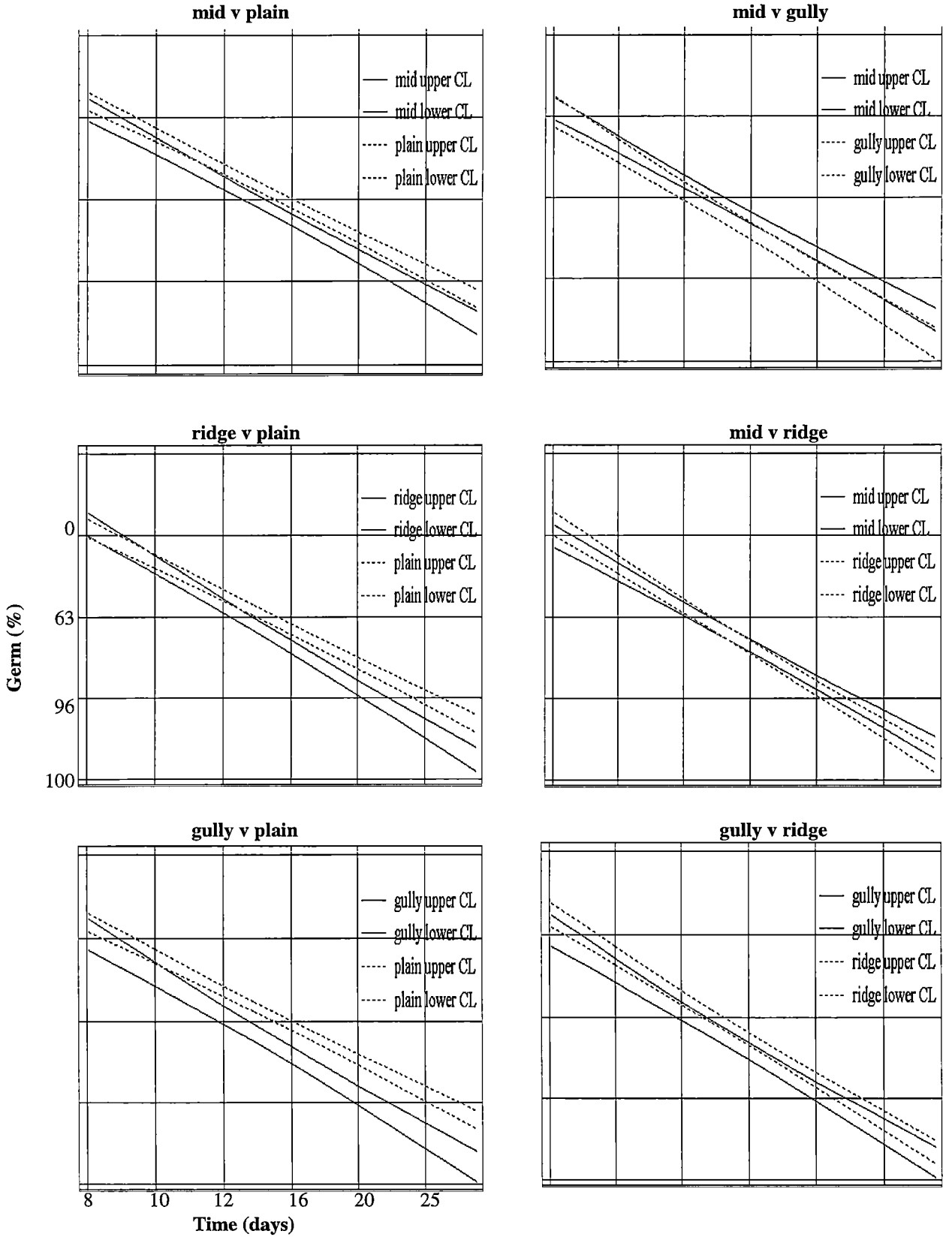


Figure 4.15 Effect of stratification on the germination energy of seed at 13 °C (identical letters indicate non-significant subsets within each time period)

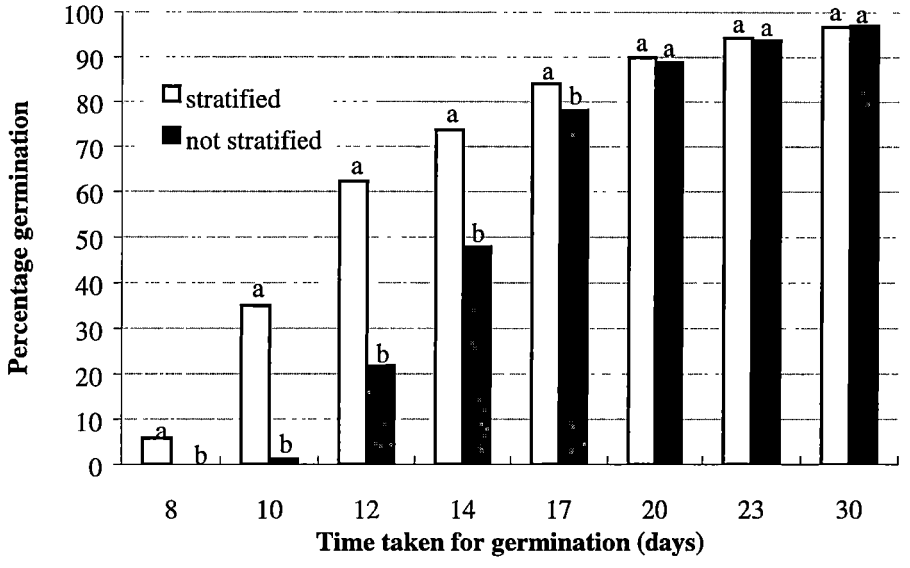


Figure 4.16 Effect of stratification on the rate of germination of seed at 13 °C, expressed as the upper and lower confidence limits (CL) of the regressions of the transformed values of germination percentage against time

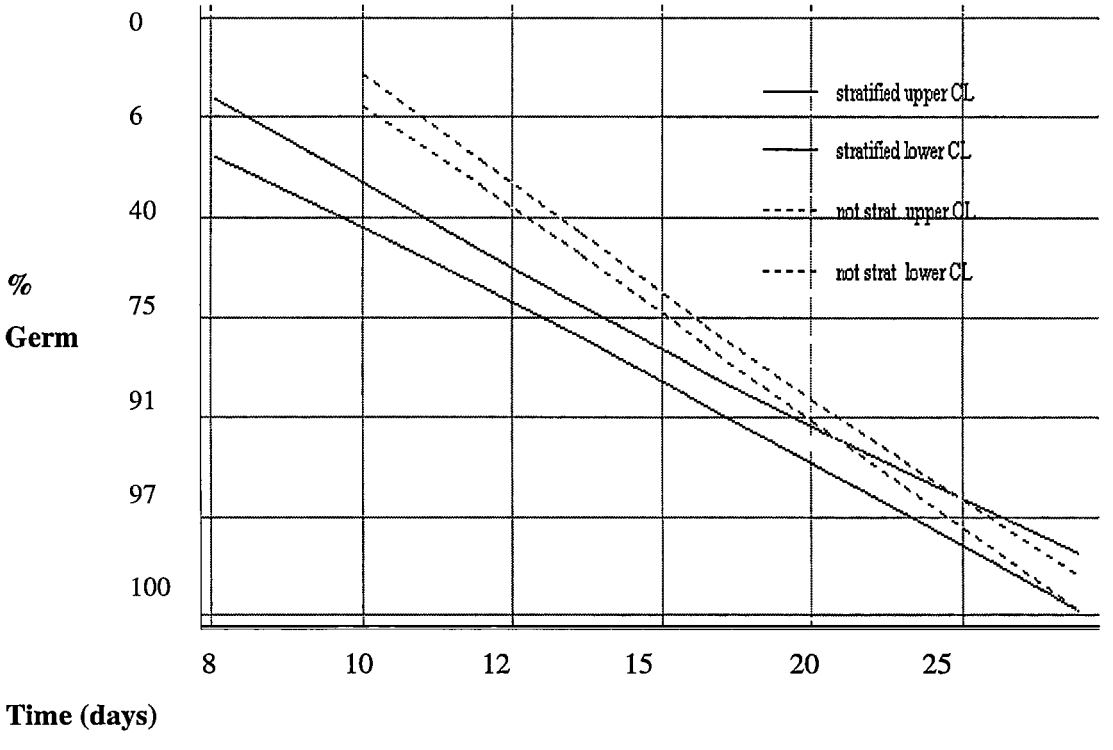


Figure 4.17 Effect of stratification on the germination energy of seed at 15°C (identical letters indicate non-significant subsets within each time period)

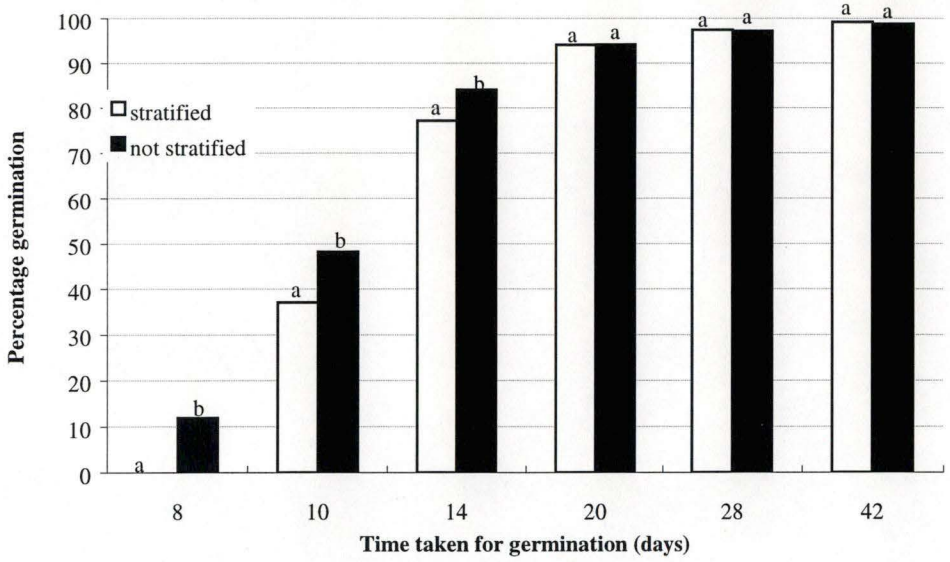
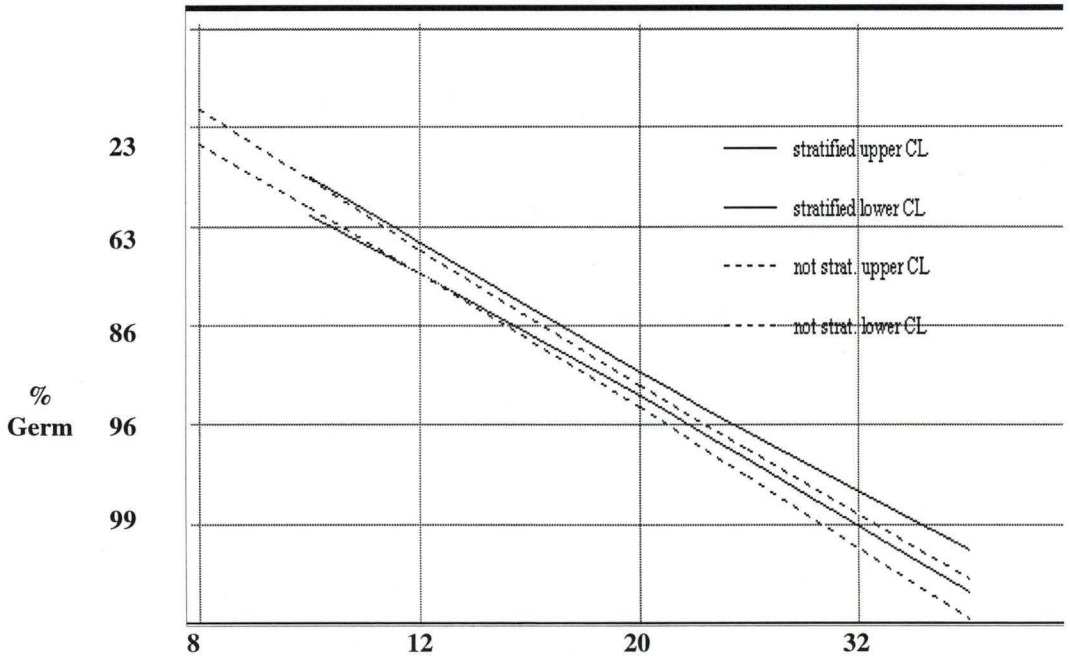


Figure 4.18 Effect of stratification on the rate of germination at 15°C, expressed as the upper and lower confidence limits (CL) of the regressions of the transformed values of germination percentage against time



4. Discussion

Seed viability from natural stands of eucalypts is highly variable and appears to be primarily influenced by environmental factors such as stand density, crown size and health, weather conditions, the availability and efficiency of pollinating animals, and predation by insects and fungi (Cremer *et al.* 1978). Seed viability from individual trees can also demonstrate considerable variation from one year to another (Pederick 1960). Environmental differences in pollination and/or predation between the Forestier and Lune sites are the most likely factors to have contributed to the significant phenotypic variation that was demonstrated for seed viability.

Germination capacity across all populations and treatments was almost 100%, confirming that dormancy is generally absent within *E. obliqua*. However, stratification had a significant effect on the rate of germination, with earlier germination at 13 °C and 20 °C than for unstratified seed. The delay in the germination of seed at 15 °C following stratification is difficult to explain and suggests that more than one factor may be involved in the mechanism controlling pre-germination conditioning.

Germination energy of seed showed significant variation between the populations of *E. obliqua* used in the current experiments. At the lowest temperature (13 °C) there was strong evidence of population differentiation at the local site level, with the populations from the wet forest sites (Forestier Gully and Lune Mid) having significantly higher germination energy than the adjoining open forest sites (Forestier Ridge and Lune Plain respectively). Temperature is regarded as the most important environmental cue responsible for the synchronisation of germination with conditions suitable for seedling establishment (Probert 1992). Most species have a range within which germination may occur, with a pronounced peak towards the optimum temperature. For *E. obliqua*, the optimum temperature for germination has been reported as 21 °C (70 °F) by Grose (1962) and 15 °C by Boland *et al.* (1980). The fastest and most uniform rates of germination for the *E. obliqua* populations were at 20 °C, with significantly slower, and more variable germination at 15 and 13 °C. Slower or delayed germination may be of adaptive value on sites such as Forestier Ridge and Lune Plain that are subject to periodically dry soils or cold temperatures. In contrast, rapid and uniform germination may be necessary to establish a competitive advantage over competing vegetation on wet forest sites such as Forestier Gully and Lune Mid. Similar

results were obtained by Ladiges (1974a) who found that dry forest populations of *E. viminalis* had a slower germination rate than wet forest populations.

Environmental or maternal factors may influence characteristics such as germination capacity and germination energy through effects on traits such as seed size and seed coat thickness (Perry 1976). The mechanical resistance of the seed coat has been identified as the primary cause of seed dormancy in *E. delegatensis* and *E. pauciflora* (Bachelard 1967). Variation in seed size within eucalypts has been related to differences in both germination capacity and energy, with large seeds associated with higher values than small seeds (Grose and Zimmer 1958, Aktar 1973). Large seeds have been associated with larger cotyledons and faster early growth than small seeds (see chapter 7). Differences in seed size and seed coat morphology have also been linked to variation in the germination response of eucalypt species and provenances to moisture stress (Bachelard 1985, Gibson and Bachelard 1987). Maternal effects may be regarded as partly phenotypic, reflecting the environmental conditions to which the mother plant is exposed during seed maturation (Wulff 1986, Gutterman 1992), and partly genetic since the seed coat is derived from maternal diploid tissue. Variation in seed characteristics such as seed size and germination responses also appears to be genetically determined independent of maternal influences (Wulff 1986, Probert 1992). Gutterman (1992) argues that the genotype determines the basic conditions under which germination may occur, whilst phenotypic influences, including environmental and maternal effects, confer a degree of variability that ensures that germination is dispersed over a greater period of time. Selection at the microsite level may also help to maintain genetic diversity within the population. High diversity may be of adaptive value given the variation in germination conditions between seasons and micro-sites (Battaglia 1993). The separation of genetic effects from environmental and maternal effects is not possible in studies of seed derived from natural stands of eucalypts and most workers cite variation in the germination responses of different populations as evidence of genetic differentiation (e.g. Ladiges 1974a, Gibson and Bachelard 1987).

The significance of population differences in germination responses is likely to depend upon the comparative selection forces acting at both the germination and seedling establishment phases of the regeneration process. Variation in germination characteristics may be critical in a harsh environment where the survival of a species may depend upon dispersal of germination over time in order to maximise the opportunities for establishment (Gutterman 1992). Selection pressures during the germination phase are likely to be important in

eucalypt species, but may be of a lower order of magnitude than the very high selection pressures that are exerted during the post-germination phase of establishment (Battaglia and Reid 1993a).

5. Conclusions

Significant differences in seed viability and seed size were found between the Forestier and Lune provenances. Full germination capacity was achieved within all populations under various regimes of stratification and temperature, confirming the general absence of dormancy previously reported for *E. obliqua*. Both temperature and stratification had significant effects on the commencement and rate of germination. Germination was significantly later and slower at the lower temperatures, and the differences between populations were more pronounced. The results provide evidence of genetic differentiation between populations at the local scale, with the wet forest Gully and Mid populations having significantly higher germination energy at low temperatures than the adjoining open forest populations of the Ridge and Plain respectively.

CHAPTER 5 FROST TOLERANCE

1. Introduction

Frost damage has been cited as one of a suite of environmental factors that can lead to high mortality and poor growth rates in eucalypt stands (McKimm and Flinn 1979, Webb *et al.* 1983). Eucalypts appear to be most sensitive to damage during the early cotyledonary and seedling stages (Battaglia and Reid 1993b), although exceptional frosts may also kill pole stage trees, resulting in changes to species distributions and dominance relationships (Davidson and Reid 1985).

Variation in frost resistance has been reported to occur between and within the populations of many eucalypt species (see review in chapter 1, section 4.2). Clinal patterns of variation are usually correlated with altitudinal (temperature) gradients. Discontinuous patterns may occur at the broad provenance level, or at a finer scale such as in topographic depressions associated with cold air drainage (Harwood 1980).

Most eucalypt species are relatively sensitive to low temperatures and the difference between a lethal and a non-damaging temperature may be as little as 1-2 °C (Raymond *et al.* 1986, Tibbits and Reid 1987a). Intra-specific variation appears to exhibit a similar order of magnitude. Tibbits and Reid (1987a) found that differences in frost resistance between populations of *E. nitens* was equivalent to only 0.3 °C for unhardened seedlings, and about 2.3 °C for hardened seedlings. Similarly, a difference of 2.7 °C in the mean lethal temperature for hardened seedlings was recorded between provenances of *E. delegatensis* by Hallam and Reid (1989).

Extreme temperature gradients may occur over short distances during a radiation frost. On a sub-alpine site in Tasmania, Davidson and Reid (1985) measured a difference in minimum temperature of 7.3 °C between the base of a depression and a ridge-top site 200m away. Davidson and Reid indicated that such temperature gradients may be a major selective force in determining the distribution pattern of species in a sub-alpine environment. Differences of a lower order of magnitude would be expected on more temperate sites. For example, a temperature difference of about 2 °C can be associated with an altitudinal range of 300m or with a change in aspect (Australian Bureau of Statistics 1994, Hocker 1979). In addition,

differences of 2-4°C can occur at the microsite level as a result of variation in the degree of overstorey crown cover (Keenan 1986).

Patterns of variation in frost resistance are likely to reflect the scale of differences in minimum temperatures between sites. Significant variation in frost resistance has been recorded for populations from a wide geographic or altitudinal range (Paton 1972, Tibbits and Reid 1987a). In species such as *E. nitens* and *E. fastigata* the variation between provenances may be 3 to 4 times greater than the variation between families (Wilcox 1982, Tibbits and Reid 1987a), suggesting substantial differences exist between the selection pressures exerted at each provenance site. However, high levels of variation have also been reported to occur between and within families, indicating that selection for frost tolerance may also be important at a finer scale (Paton 1972). The small range in frost sensitivity within most populations means that even very small variations to normal temperature regimes may exert a significant selective pressure. Such pressure may lead to the maintenance of high levels of intra-population variation and may also lead to population differentiation based on very small, but ecologically significant, differences in frost tolerance.

Previous studies of frost resistance within the Series *Obliquae* have included the species *E. regnans, E. delegatensis, E. pauciflora* and *E. fastigata* but information on *E. obliqua* is lacking. Similarly, most studies have investigated variation at the broad provenance level, and the ecological significance of variation at the site level (*sensu* Zobel and Talbert 1984) has not been examined. The present field and laboratory studies were therefore undertaken in order to investigate variation in the frost tolerance of *E. obliqua* populations at the site level.

2. Methods

2.1 Field Studies

Thermometers installed at the Lune Plain planting site indicated that the seedlings were subjected to frosts as low as -4.5 °C during the first week after planting. Frost damage was evident as necrotic tissue on the leaf lamina four weeks after planting. This damage was assessed on an ocular basis as the proportion of the total lamina area maintained as green tissue on each seedling. Seedlings were assessed and assigned to a percentage damage class

(after Wilcox *et al.* 1982, Tibbits and Reid 1987a) as follows:

Score	*% of green leaf tissue*
0	<10%
20	11-30%
40	31-50%
60	51-70%
80	71-90%
100	>90%

Plot means were calculated and the data were subjected to REML variance components analysis, using the following models:

Random model: population.family

Fixed model: constant + population + blocks + population.blocks.

The Wald test was used to determine the significance of fixed effects. Differences between the means of the populations were compared with the standard error of the difference using the *t*-test.

2.2 Laboratory studies

Two series of frosting experiments were conducted using the conductivity method described by Hallam (1986) and Tibbits and Reid (1987a). The first series further investigated the frost resistance of seedlings, using leaves from seedlings grown within a glasshouse. The second series evaluated the frost resistance of leaves from four year old saplings within the planting trials at the Forestier Gully and Ridge sites (chapter 2).

Leaves were collected from 9 month old glasshouse seedlings in July and from the same seedlings at age 12 months in October. The temperature range within the glasshouse during July averaged 10/4°C, with overnight minima as low as -1.5 °C. The seedlings had ceased active growth and were assumed to have developed maximum hardiness. Prior to the October trials, the glasshouse was heated for three weeks to maintain a minimum temperature of

15 °C, resulting in the seedlings resuming active growth. For both trials, three leaves were collected from each family by removing one leaf from each of three seedlings. Sampling aimed to collect fully expanded leaves of equivalent age, nodal position, size and health, although there was some variation due to differences in the development and health of seedlings.

Leaves from the current season's growth were collected from saplings in the Forestier Gully and Forestier Ridge planting sites in April, May and July 1994, four years after planting. One block was selected at random for sampling within each of the Forestier planting sites Within this block, four families were randomly selected from each population and leaves were collected from the tallest three trees within each family. Three leaves were collected from each tree, giving a total of 96 sets of leaves (2 sites x 4 populations x 4 families x 3 trees). The leaves were removed during the early morning and immediately placed into plastic bags and stored within an insulated container. The inside of the container was cooled by the insertion of freezer blocks that had been wrapped in newspaper to avoid direct contact with the leaf material. The leaves were stored overnight in a cool room at 2°C and removed the following morning for the frosting experiments. Previous studies have demonstrated that excised leaves may be stored for at least 24 hours without any significant effect on frost resistance (Raymond *et al.* 1986).

In the laboratory, the leaves were removed from the plastic bags and carefully wiped with distilled + deionised water to remove any surface contamination. Discs 6mm in diameter were excised from the centre of each leaf using a sharp hole punching tool. Two discs from each set of three leaves (a total of six discs) were placed directly into a 100mm x 25mm glass vial and 0.2ml of distilled + deionised water and approximately 0.1mg of AgI were added to facilitate freezing. The vials were loaded into a wire container, placed into a freezing chamber and the temperature was lowered from room temperature(c. 18°C) to 2°C at the rate of 1°C per minute, and thereafter to the test temperature at the rate of 0.2°C per minute. Frost temperatures selected for each of the experiments are indicated in Table 5.1. In addition, a control set of discs was prepared for each experiment and subjected to all treatments other than a frosting. The frost temperature was maintained for 40 minutes and the temperature was then allowed to gradually rise to about 10°C after which time the vials were removed from the chamber and allowed to stand at room temperature for 30 minutes. Ten ml of distilled + deionised water were added to each vial and the vials were shaken on an agitator for about 20 hours. A microprocessor conductivity meter was used to measure the initial

conductivity (CI) of the bathing medium to 0.1 μS cm^{-1}. After measurement, the vials were immersed into a water bath, boiled for 10 minutes to kill the leaf tissue and placed on the agitator for a further 20 hours. The total conductivity (CT) of the bathing solution was then measured and the relative conductivity (CR) was calculated as CR=CI/CT% (Tibbits and Reid 1987b). The relative conductivity values were plotted against frost temperature and the relative frost resistance was determined as the temperature resulting in a loss of 50% of the cellular electrolytes (T50) (Tibbits and Reid 1987b).

The T50 values were analysed by multifactor ANOVA. The CR values for the control (unfrosted) samples were also analysed by multifactor ANOVA in order to evaluate the differences in the natural levels of electrolyte leakage between the different populations and sites for each period of testing.

Table 5.1 Dates of frosting experiments and temperatures applied in frost chamber

Experiment	Date of frost treatment	Minimum temperatures applied (°C)			
Seedlings	25 July	-4.5	-5.0	-5.5	nil (control)
	10 October	-3.0	-3.5	-4.5	nil (control)
Saplings	13 April	-2	-3	-4	nil (control)
	3 May	-5	-6.5	-8	nil (control)
	18 July	-5.5	-6.5	-7.5	nil (control)

3. Results

There were significant differences between populations for the frost resistance of seedlings planted at the Lune Plain site (Table 5.2). Seedlings from the Plain population had the lowest levels of frost damage (Figure 5.1). Significant differences were also attributed to blocks and there were no interactions between populations and blocks (Tables 5.2 and 5.3).

In contrast, there were no significant differences between populations for the T50 values of leaf discs taken from one year old seedlings in the glasshouse (Table 5.4 and Figure 5.2). Seedling leaves appeared to be seriously damaged by frosts lower than -3.5 to -4.5°C, and there was no evidence of hardening between the experimental frosts conducted in July and early October.

Table 5.2 Wald statistics for fixed terms of REML variance components analysis of frost damage to seedlings at the Lune Plain planting site

Fixed term	Wald statistic	d.f.	Significance level
population	9.9	3	*
blocks	8.7	3	*
interaction (population x block)	13.9	9	ns

* = $p<0.05$, ns = not significant ($p>0.05$)

Table 5.3 Mean frost damage (±SE) of seedlings within four blocks at the Lune Plain planting site, expressed as the percentage of leaf area covered by necrotic tissue

Block	Leaf damage (%)
1	54.1 ± 2.9
2	53.6 ± 2.9
3	59.3 ± 2.9
4	50.7 ± 2.9

Figure 5.1 Mean frost damage (± SE) for seedlings at the Lune Plain planting site, expressed as the percentage of leaf area covered by necrotic tissue

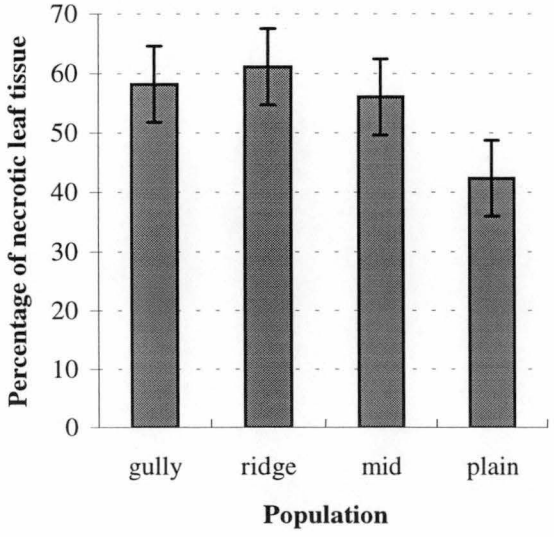


Figure 5.2 Temperatures causing 50% loss of electrolytes (T50) from leaf discs taken from seedlings at two times of the year (vertical bars are for LSD at p=0.05)

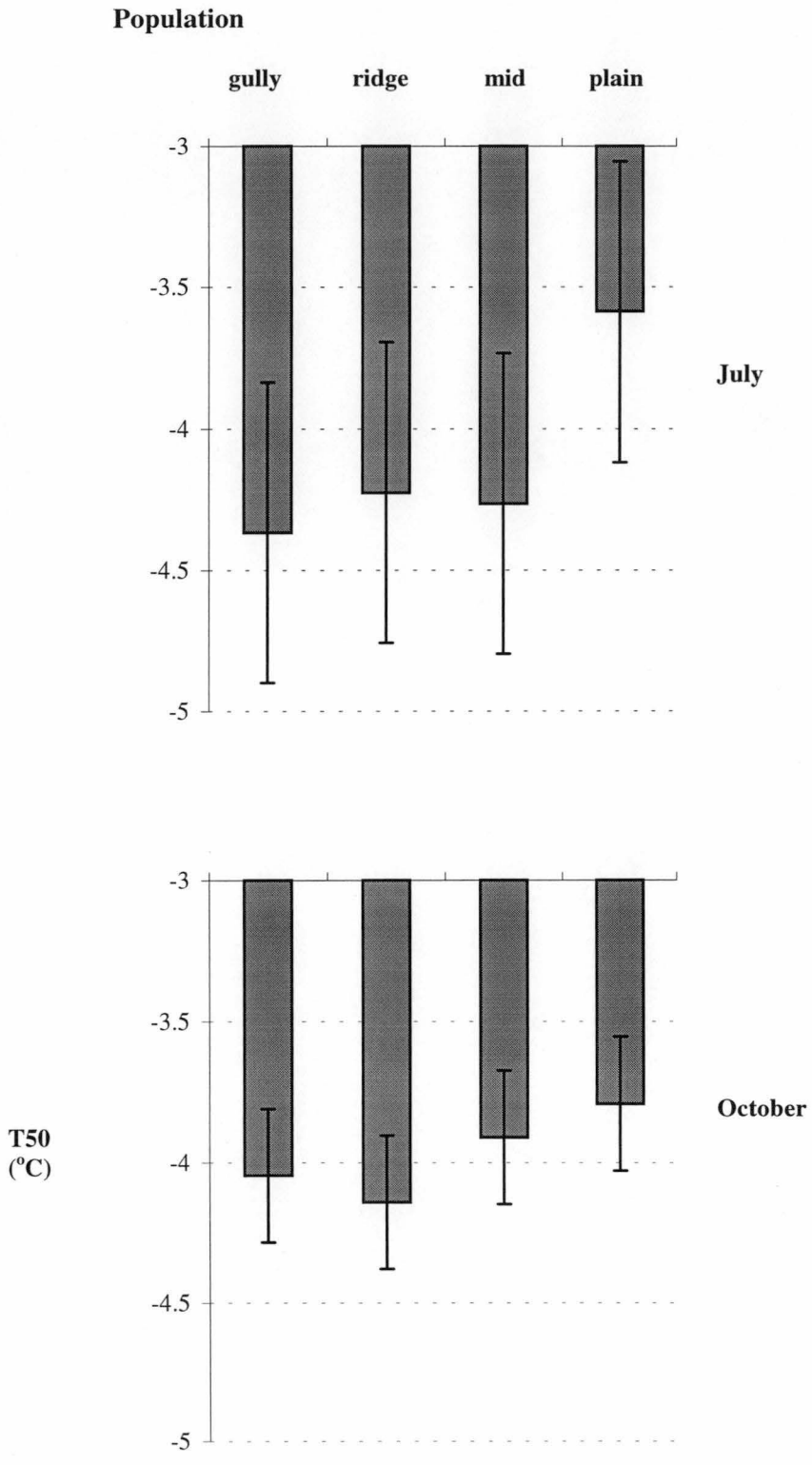


Table 5.4 ANOVA table for T50 values of frosted leaf discs and CR values of control (non-frosted) discs from glasshouse seedlings

Factor	Month of frost treatment	Source	d.f.	M.S.	F-ratio	Significance level
T50	July	population	3	1.5100	0.90	ns
		residual	44	1.6751		
	October	population	3	0.2826	0.85	ns
		residual	44	0.3329		
CR (control)	July	population	3	6.1072	5.65	**
		residual	44	1.0816		
	October	population	3	11.9918	6.96	***
		residual	44	1.7239		

** = $p<0.01$, *** $p<0.001$, ns = not significant ($p>0.05$)

No significant differences were found between populations or sites for the T50 values of leaf discs from four year old saplings planted at the Forestier Gully and Ridge sites (Table 5.5 and Figure 5.3). The ranking of the populations was similar for each of the three times of year, with the Plain and Ridge populations respectively demonstrating the highest and the lowest frost resistance. Leaves from the saplings were not seriously damage by temperatures down to about -5.4°C in April and May and the differences between populations were less than 0.2°C. In contrast, differences of up to 2.0 °C were recorded between populations during July. The overall variability in frost resistance increased between April (LSD=0.17), May (LSD=0.28) and July (LSD=2.28). The T50 values were generally lower for the Ridge planting site compared to the Gully planting site (Figure 5.4).

Significant variation was found for the CR value of control (non-frosted) leaf material. The lowest CR values for glasshouse seedlings were recorded by the Plain population during July and by the Gully population during October (Table 5.6). Leaf tissue from saplings at the Gully planting site had a significantly lower CR value than tissue from the Ridge planting site during May and July (Table 5.7). On these sites, leaf tissue from the Ridge population had lower CR values than seedlings from other populations. There was a trend of decreasing CR values (for saplings) from April to July, and increasing values (for seedlings) from July to October (Tables 5.6 and 5.7).

Table 5.5 ANOVA table for T50 values of frosted leaf discs and CR values of control (non-frosted) discs from saplings at age four years

Factor	Month of frost treatment	Source	d.f.	M.S.	F-ratio	Significance level
T50	April	population	3	0.0221	0.78	ns
		site	1	0.0187	0.66	ns
		interaction (p x s)	3	0.0277	0.98	ns
		residual	24			
	May	population	3	0.0444	0.60	ns
		site	1	0.2251	3.04	ns
		interaction (p x s)	3	0.0356	0.48	ns
		residual	24			
	July	population	3	5.4523	1.12	ns
		site	1	2.5690	0.53	ns
		interaction (p x s)	3	6.2872	1.29	ns
		residual	24			
CR (control)	April	population	3	15.5703	1.82	ns
		site	1	1.6787	0.20	ns
		interaction (p x s)	3	3.1416	0.37	ns
		residual	24	8.5350		
	May	population	3	14.5982	2.00	ns
		site	1	100.1250	13.72	***
		interaction (p x s)	3	5.4936	0.75	ns
		residual	24	7.2966		
	July	population	3	0.9997	0.69	ns
		site	1	50.8509	35.10	***
		interaction (p x s)	3	1.6198	1.12	ns
		residual	24	1.4487		

*** = $p<0.001$, ns = not significant ($p>0.05$)

Table 5.6 CR values of control (non-frosted) leaves from glasshouse seedlings at two times of the year across four populations (identical letters indicate non-significant subsets within each time period)

Time of year	Population	CR value	
July	Gully	13.67	a
	Ridge	13.60	a
	Mid	13.77	a
	Plain	12.27	b
October	Gully	14.73	a
	Ridge	15.71	ab
	Mid	17.13	c
	Plain	16.21	bc

Figure 5.3 Temperatures causing 50% loss of cellular electrolytes (T50) from leaf discs taken from four year old saplings at three times of the year.
I. Differences between populations (vertical bars are for LSD at p=0.05)

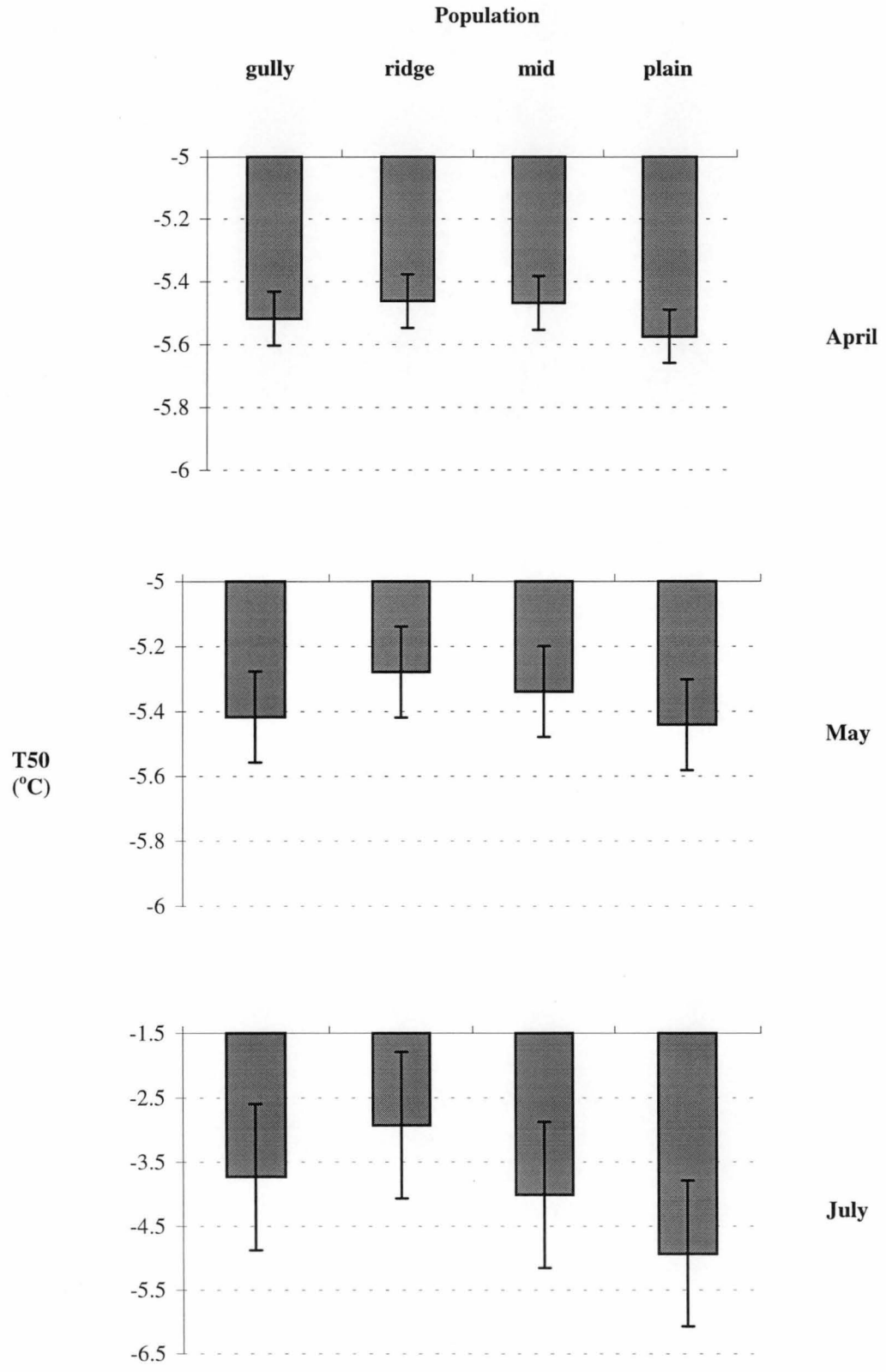


Figure 5.4 Temperatures causing 50% loss of cellular electrolytes (T50) from leaf discs taken from four year old saplings at three times of the year.
II. Differences between planting sites (vertical bars are for LSD at p=0.05)

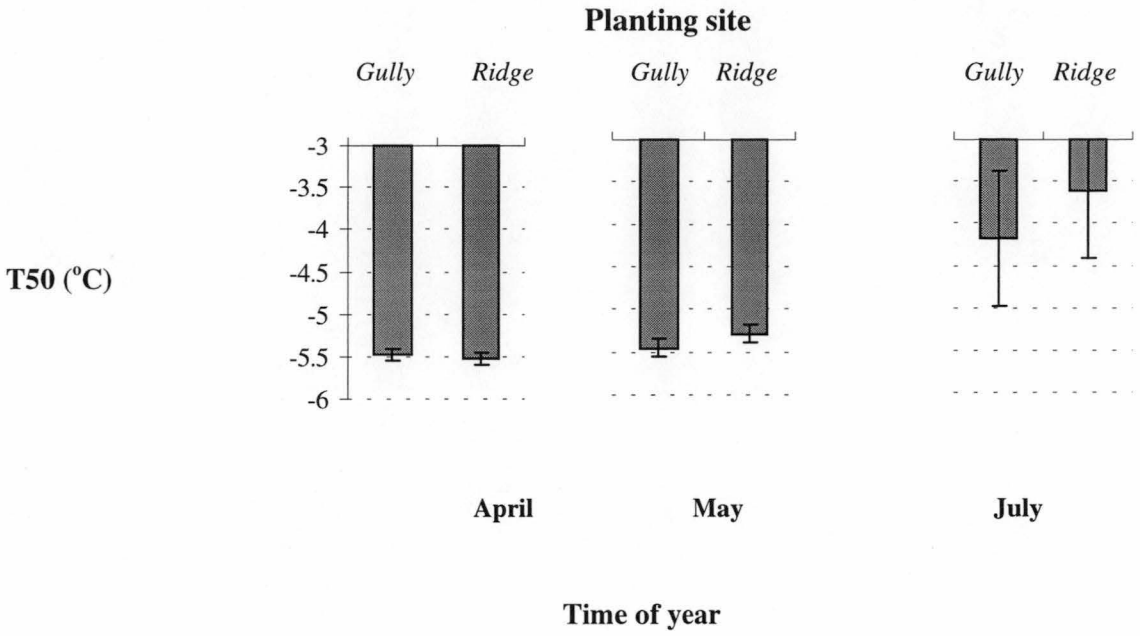


Table 5.7 CR values of control (non-frosted) leaves from four year old saplings at three times of the year across four populations and two sites (identical letters indicate non-significant subsets within each time period and factor)

Time of year	Factor		CR value	
April	planting site	gully	21.46	a
		ridge	21.92	a
	population	Gully	23.34	a
		Ridge	19.97	a
		Mid	21.45	a
		Plain	22.01	a
May	planting site	gully	18.65	a
		ridge	22.18	b
	population	Gully	19.58	a
		Ridge	19.14	a
		Mid	22.15	a
		Plain	20.79	a
July	planting site	gully	13.56	a
		ridge	16.08	b
	population	Gully	14.74	a
		Ridge	14.66	a
		Mid	15.34	a
		Plain	14.55	a

4. Discussion

The results did not provide a consistent trend for frost tolerance across the four populations. Significant differences between populations were only obtained in the field studies, where the plain population exhibited the highest level of frost tolerance. A similar pattern was evident for the frost tolerance of leaves taken from four year old saplings. In this study, there was an indication of increasing differentiation between populations over the period from April to July, with differences between populations of up to 2°C. In contrast, the frost tolerance of leaves taken from glasshouse seedlings was highly variable, and there was no evidence of differentiation between the populations.

Previous studies of frost tolerance have indicated a good correlation between field frosts and artificial techniques such as the electrical conductivity method (Raymond *et al.* 1986, Tibbits and Reid 1987b). However, Wilcox (1982) found that the ranking of provenances from field frosts did not always agree with the results from artificial frost experiments, suggesting that there may be important interactions between the genotype and factors such as seedling physiology and morphology. Ontogenetic variation in the frost resistance of *E. delegatensis* has been reported by Battaglia and Reid (1993b) who found significant differences in frost damage between tissues from leaves and seedlings of different age and from leaves collected from different leaf nodes.

In the current studies, the seedlings exposed to the field frost were very uniform in terms of ontogenetic and height development (chapter 3). Significant differences between planting blocks indicated that the frosting environment was locally variable, probably in response to the small differences in air drainage taken into account during the design of the trial. However, there were no interactions between blocks and populations and the Plain population was clearly the most frost resistant. In contrast, the glasshouse seedlings were older (9-12 months old) than the planted seedlings (5 months old) and were more variable in terms of ontogenetic and height development. In addition, the seedlings were crowded within containers and the leaves were therefore exposed to variable shading and sheltering. As a result, these factors may have contributed to higher levels of variation for leaves from the glasshouse seedlings than for leaves from the open conditions of the planted seedlings. Similarly, despite careful selection of leaves, there is likely to be inherent variation between leaves from the sapling stands because of differences in the age (or development) of the leaf, height from the ground and other positional effects such as relative exposure. The apparent

lack of hardening of the sapling leaves over the three sampling periods may also reflect variation due to these factors, rather than a lack of hardening *per se*.

Significant variation in the relative conductivity of non-frosted leaf tissue occurred within the current studies. Relative conductivity is a measure of the loss of cellular electrolytes, and thus provides an indication of the permeability and integrity of cellular membranes. When plants are under stress, the cell membranes contract and the membrane lipid configuration alters, resulting in the increased leakage of cell contents (Kuhns *et al.* 1993). High leakage is often associated with moisture stress (Tibbits and Reid 1987b) and there is significant seasonal variation which appears to be related to the ability of the plant to develop drought hardiness (Kuhns *et al.* 1993). Differences at the generic level have been recorded for Tasmania rainforest trees by Read and Hill (1988). More recent work has demonstrated intra-specific differences in membrane leakage within *Quercus macrocarpa* (Kuhns *et al.* 1993) and *Populus deltoides* (Gebre *et al.* 1994). These studies indicated that populations from mesic sites may have higher leakage rates than plants from more xeric sites. Differences in membrane leakage may also result from stresses imposed by factors such as cold temperatures (Raymond *et al.* 1986), suggesting that differences between plants at any site may involve more than one environmental stress.

Highly significant differences occurred between populations for the relative conductivity of non-frosted leaf material from glasshouse seedlings. Seedlings from the Plain population had the lowest values during July, suggesting that this population was under the lowest level of stress during the coldest period of the year. This pattern was altered by October with the resumption of active growth by some seedlings.

Differences in membrane leakage also occurred between the leaf material of saplings from the two planting sites. Higher leakage from plants at the Ridge planting site may indicate higher stress levels than at the Gully site. The Ridge site has a north-westerly aspect and would be exposed to higher maximum temperatures, higher evapo-transpiration stress and lower minimum temperatures than the Gully site which has a sheltered, south-easterly aspect (Chapter 2, section 4.2). Rainfall for the three months prior to the April test was 51% below average (Bureau of Meteorology 1994), and this deficit may have exacerbated the moderate moisture stress that would normally occur within the season of late-summer to early autumn. A significant reduction in membrane leakage occurred at subsequent measurements, after reasonable rainfall fell during the cooler late-autumn to winter period. At the Gully site, the leakage index decreased significantly at each of the three measurement times from April to

July, suggesting that stress levels were progressively reduced as increased rainfall and reduced evapo-transpiration demand ameliorated the moisture stress on the plants. Recovery was slower on the Ridge site, with comparable leakage indices recorded for the April and May measurement dates, and no significant decrease until the July measurement. Rainfall for the four months prior to the May measurement was 42% below average, and May is normally the first month in the year in which rainfall exceeds evaporation (Bureau of Meteorology 1994). Thus the continuation of relatively dry conditions may have resulted in higher moisture stress at the drier Ridge site than at the more mesic Gully site. In addition to possible moisture stress, seedlings at the Ridge site were also exposed to a much greater temperature range than that experienced at the Gully site. Temperatures recorded at the planting sites during the period between the April and May measurements showed that the Ridge site was exposed to a temperature range of -2 to 23 °C, whilst the Gully received 4 to 20°C. The frosts during this period at the Ridge site were the first for the season and may therefore have imposed a stress on the seedlings. Regular frosts occurred from the beginning of May, and by the July measurement it could be expected that the stress likely to be imposed by a mild frost would be reduced by physiological changes within the seedlings associated with factors such as frost hardening or a reduction in growth rate.

5. Conclusion

The results provide an indication of population differentiation, with the Plain population demonstrating the highest level of frost tolerance. This is not unexpected, given the high frequency of severe frosts associated with cold air ponding within the Lune Plain environment (chapter 2). Further testing of leaf material under different conditions of ontogeny and environment would be necessary to more fully evaluate the significance of frost as a selection pressure for populations of *E. obliqua*.

CHAPTER 6 LEAF BROWSING AND INFECTION

1. Introduction

Parasitic damage has been proposed as an important factor contributing to niche differentiation within species of eucalypts (Burdon and Chilvers 1974). Host specificity at the subgeneric level was identified by Heather (1971) who found that the fungi *Phaeoseptoria eucalypti* and *Septoria normae* were restricted to the subgenus *Symphyomyrtus* while infections of *Aulographina eucalypti* only occurred within the *Monocalyptus*. Burdon and Chilvers (1974) isolated 23 different species of fungi from four sites and found that all but one species exhibited field specificity toward one or more subgenera. They noted that parasitic damage was highly variable between individual trees and species, and indicated that host specificity could extend to groups of species within subgenera. Later work has provided evidence of intra-specific variation in the susceptibility of populations to disease. Harris *et al.* (1985) demonstrated that mortality due to infection by *Phytophthora cinnamomi* showed significant variation at both the provenance and family levels within *E. regnans*. Significant variation within and between provenances of *E. nitens* has also been recorded for susceptibility to the leaf spotting fungus *Mycosphaerella nubilosa* (Purnell and Lundquist 1986).

Leaf diseases can lower plant productivity by impairing the photosynthetic machinery within diseased tissue and by causing the premature shedding of badly infected leaves, thus reducing the total photosynthetic area of the plant (Burdon and Chilvers 1974, Purnell and Lundquist 1986). The growth of *E. nitens* was found to be negatively correlated with disease intensity when more than 20% of the crown was defoliated (Purnell and Lundquist 1986). Burdon and Chilvers (1974) found that the natural suite of leaf parasites within sapling stands of eucalypts would result in average levels of effective leaf loss of more than 20% and they concluded that leaf parasites could have a significant impact on the growth and selection of individual trees. Chronic infection by *Mycosphaerella cryptica* (Cooke) Hansf. has caused tip dieback and malformation of eucalypt saplings planted in New Zealand (Cheah and Hartill 1987).

Leaf diseases are relatively common in young eucalypt stands within Tasmania, although infections are generally limited to leaves weakened by natural ageing (Wardlaw 1990). Infection by species of *Mycosphaerella* is favoured by warm, wet weather and severe

outbreaks have been reported to affect *E. nitens*, *E. regnans*, *E. globulus* ssp. *pseudoglobulus* and *E. obliqua* (Marks *et al.* 1982). Severe infection of seedlings by leaf diseases has been noted in some seasons and on some sites where "off-site" species or provenances have been sown (Wardlaw 1990). However, the absence of comparable seedlings from local species and provenances under the same conditions on the same site has precluded a rigorous evaluation of comparative infection levels (T. Wardlaw, Forestry Tasmania, pers. comm.).

Browsing of young tree seedlings by animals can eliminate or alter the relative composition and growth of individual plant species within a forest stand (Gill 1992). In North American forests, browsing by deer has had a major effect on the distribution and abundance of species such as Ponderosa pine (*Pinus ponderosa* Laws) and Atlantic white cedar (*Chamaecyparis thyoides* (L.) B.S.P.) (Adams 1951, Little and Soames 1965). Within Tasmanian eucalypt forests, severe browsing by native animals remains a primary cause of understocking and poor growth within eucalypt regeneration and plantation areas (Mollison 1960, Gilbert 1961, Neilsen and Wilkinson 1995).

Browsing damage within young eucalypt stands has been observed to be highly variable, with individual seedlings often subjected to very different levels of defoliation (L. Edwards, Forestry Tasmania, pers. comm.). Inter-specific differences in susceptibility to browsing have been commonly observed (Neilsen 1990) but formal studies have not been conducted. Preferential browsing at the intra-specific level has also been suggested as the reason for the poor growth of specific populations of *E. globulus* and *E. nitens* in some provenance trials (P. Kube, Forestry Tasmania, pers. comm.). These observations suggest a genetic component to the susceptibility of eucalypt seedlings to browsing damage. Such differences may indicate that browsing has directly acted as a selection force on some sites. Alternatively, preferential browsing may reflect differences in factors such as leaf chemistry that are associated with separate selection forces.

2. Methods

2.1 Assessment of leaf spotting

Moderate levels of infection by *Mycosphaerella cryptica* were sustained by the two year old seedlings in the planting trials at the Lune Mid, Forestier Gully and Forestier Ridge trials

during the summer of 1993 (Photograph 6.1). Assessments of leaf spotting disease were made by an ocular assessment of the proportion of necrotic leaf tissue on each seedling in the planting sites. Ocular assessment is an accurate, non-destructive method of measuring disease damage, providing that damage classes are carefully calibrated to represent a normal distribution of damage levels across all seedlings, and a single observer is used to ensure consistency within and between each site (T. Wardlaw, Forestry Tasmania, pers. comm.). Alternative methods, such as dry weight/fresh weight ratio or direct measurement by planimeter, are impractical for very large samples and generally necessitate destructive sampling, which compromises the future value of growth studies. Damage classes selected for the assessments were as follows:

Leaf spotting rating	*Percentage of leaf covered by necrotic tissue*
1	0%
2	1-10%
3	11-20%
4	21-30%
5	31-75%
6	>75%

Assessments were carried out at the planting sites during March 1993, when the seedlings were in their third growing season. Results were analysed separately for each site. The ratings for leaf spotting were considered a multinomial response and were analysed by fitting an ordinal regression using composite link functions and REML (Genstat Committee 1987). Initially, class probabilities were determined from a logistic distribution with the nominal class intervals dividing up the percentage scale to produce cut-points of 10, 20, 30 and 75. The nominal cut-points were then used as initial values to estimate cut-point parameters in a random effects, proportional-odds ordinal regression model (Candy 1995, Candy and Wilkinson 1995). Population and blocks were considered to be fixed effects and family within population as a random effect. The model used in the algorithm developed by Candy and Wilkinson (1995) was as follows:

$$Pr_j = [\exp(\beta_j + \eta)/1 + \exp(\beta_j + \eta)] - [\exp(\beta_{j-1} + \eta)/1 + \exp(\beta_{j-1} + \eta)]$$

where: j = class 2,...4; and cut-points for the 5 classes are given by $\beta^T = (\beta_1, \beta_2, \beta_3, \beta_4)$ (where $0<\beta_1<\beta_2<\beta_3<\beta_4$)and η comprises fixed and random effects.

Differences between population effects were compared with the standard error of the difference using the *t*-test. The model was run with and without the addition of

population x block as a fixed term; the difference in the conditional residual deviance between the two models was compared with the corresponding value of χ^2 to determine the significance of the interaction.

The effect of seedling height on susceptibility to leaf spotting was evaluated by covariate analysis, using the mean height of the seedlings at age 2 years (i.e. the approximate height of the seedlings during the period of initial leaf infection) as a covariate. Leaf spotting was analysed as the plot mean across the four blocks, using families as random effects. The relationship between the height and the infection severity of individual seedlings was determined by regression analysis, using the nominal cut-points for percentage damage of 0, 10, 20, 30, 75 and 100 for ratings 1 to 6 respectively.

Photograph 6.1
Necrotic patches on leaves of seedling caused by low level of leaf spotting infection

2.2 Assessment of browsing damage

Fences erected at the Forestier Ridge, Lune Mid and Lune Plain sites totally excluded browsing animals, whilst at the Forestier Gully site, the fence successfully excluded the major browsing species, Bennetts wallaby (*Macropus rufogriseus* (Desmarest)) and pademelon (*Thylogale billardierii* (Desmarest)), but was unable to prevent access by the brushtail possum (*Trichosurus vulpecula* (Kerr)). Seedlings at the Forestier Gully site sustained very light levels of browsing during the first season of growth but the levels of defoliation became moderate to severe during the winter and spring of the second year of growth. Browsing intensity was determined by an ocular assessment of all seedlings in November, 1991 (15 months after planting). The assessment method used was as follows:

Browsing rating	*Intensity of defoliation*
0	nil
1	loss of growing tips
2	<50% loss of foliage
3	>50% loss of foliage

The probability of browsing was determined by analysing the browsing data as a binomial distribution of unbrowsed (rating 0) and browsed (ratings 1-3) using the generalised linear mixed model (GLMM) and REML analyses of GENSTAT (Genstat Committee 1987). The GLMM analysis used the Logit link function with the following model:

model = [families (random term)] + [constant + population + block +
population.block (fixed terms)].

The probability of a seedling being browsed (P_b) was determined as:

$$P_b = [(\exp (\text{model})] / [1 + \exp (\text{model})]$$

Differences between the populations were compared with the standard error of the difference using the *t*-test. The severity of browsing was determined by ANOVA of the highest defoliation class (>50% loss of foliage) using the mean for each family of the number of seedlings in this class across the four blocks.

Browsing damage was also noted on stumps remaining after the thinning of trees at age four years in the Forestier Gully planting site (chapter 7). A substantial reduction in the number

of stumps supporting coppice shoots occurred during the winter following thinning, and this was attributed to heavy browsing pressure. The methodolgy and analysis of the assessment of coppicing are provided in chapter 7. Results for the impact of browsing on coppice growth are provided in this chapter.

3. Results

3.1 Leaf spotting

The severity of leaf spotting was highest at the Lune Mid planting site where 64% of all seedlings had necrotic tissue covering greater than 20% of the total leaf surface area (Figure 6.1). Across the three planting sites, there were significant differences in damage levels between populations (Figures 6.2 to 6.5). The plain population had significantly higher damage levels than the Mid and Gully populations at all sites. Significant differences were also recorded between the Gully and Ridge, and Ridge and Plain populations at the Lune Mid site. Block x population interactions were significant at the Gully site but not at the Ridge and Mid sites (Table 6.1). The interaction at the Gully site was mainly due to differing severity between blocks, with lower levels of infection in one block.

Covariate analysis indicated that mean plot height was not significantly correlated with the mean damage rating for leaf infection (Table 6.2.). Regression analysis across all populations at the Lune Mid site demonstrated that the relationship between individual tree height and damage level was highly significant ($p<0.001$) but the correlation was relatively low ($r^2=16\%$) (Figure 6.6). This relationship was not substantially improved for individual seedling data within each population ($r^2=13\%$ for Gully population, 10% for Ridge, 25% for Mid and 17% for Plain).

Table 6.1. Population x block interactions for leaf spotting infection at the Forestier Gully, Forestier Ridge and Lune Mid planting sites

Planting site	Deviance	d.f.	Significance level
Forestier Gully	36.0	9	0.01
Forestier Ridge	6.5	9	ns
Lune Mid	11.3	9	ns

Figure 6.1 Frequency of seedlings within various classes of leaf spotting disease at the Forestier Gully, Forestier Ridge and Lune Mid planting sites

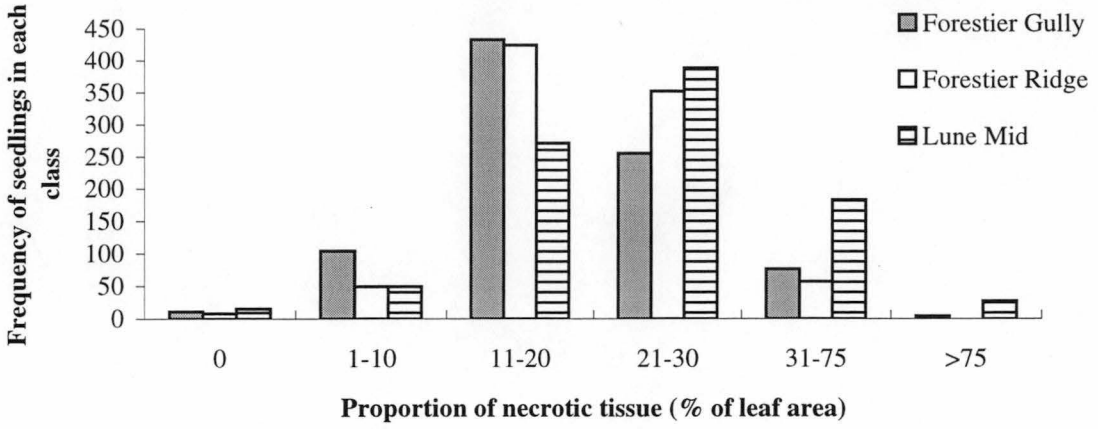


Figure 6.2 Calculated damage score and SE of differences for leaf spotting damage sustained by four populations at three planting sites

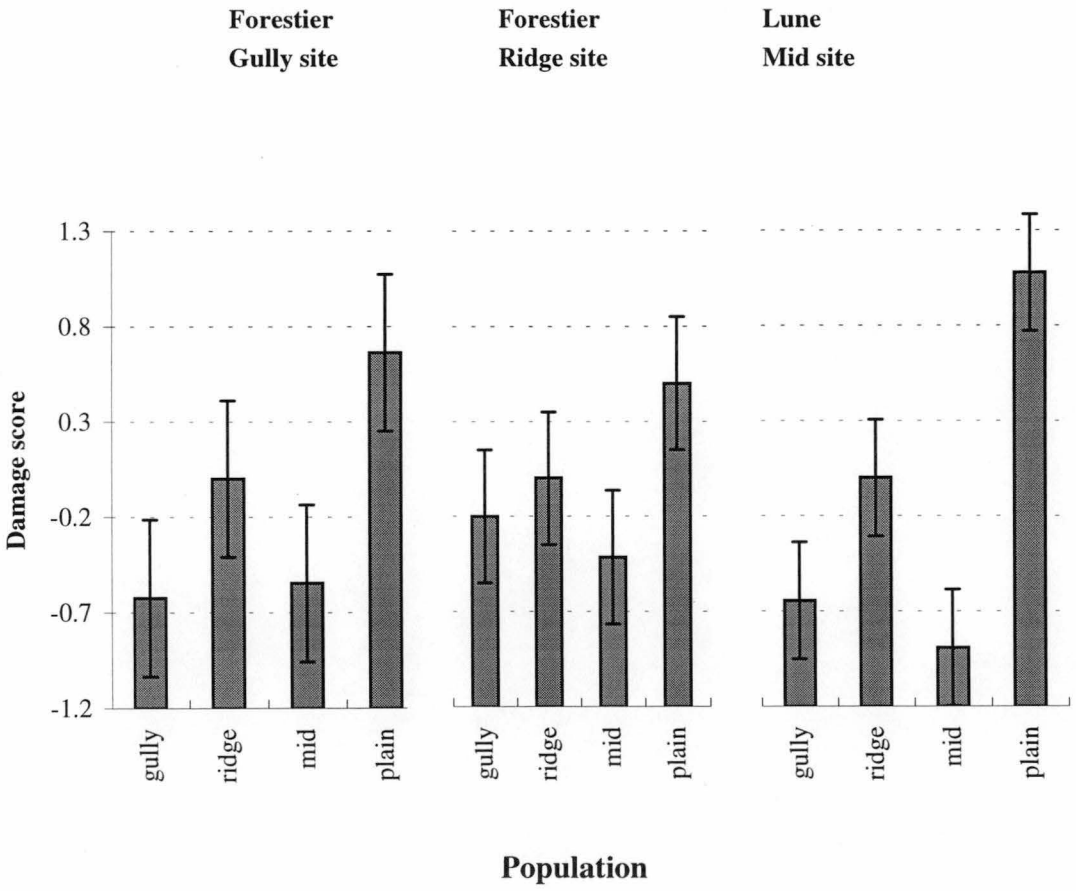


Figure 6.3 Frequency of seedlings from four populations within various classes of leaf spotting damage at the Forestier Gully site

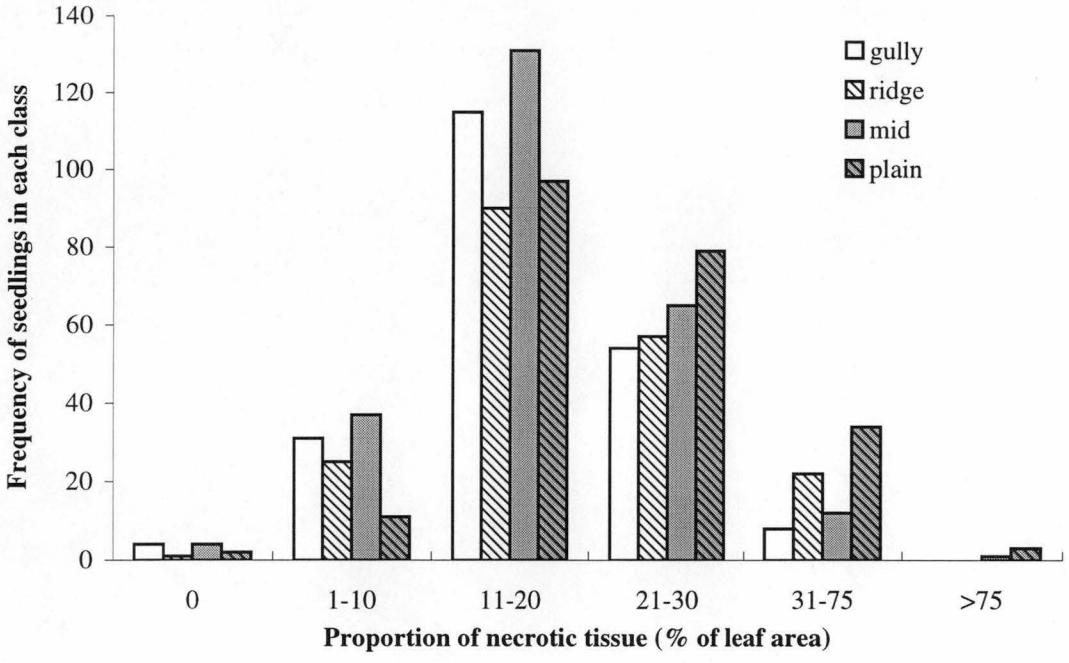


Figure 6.4 Frequency of seedlings from four populations within various classes of leaf spotting damage at the Forestier Ridge site

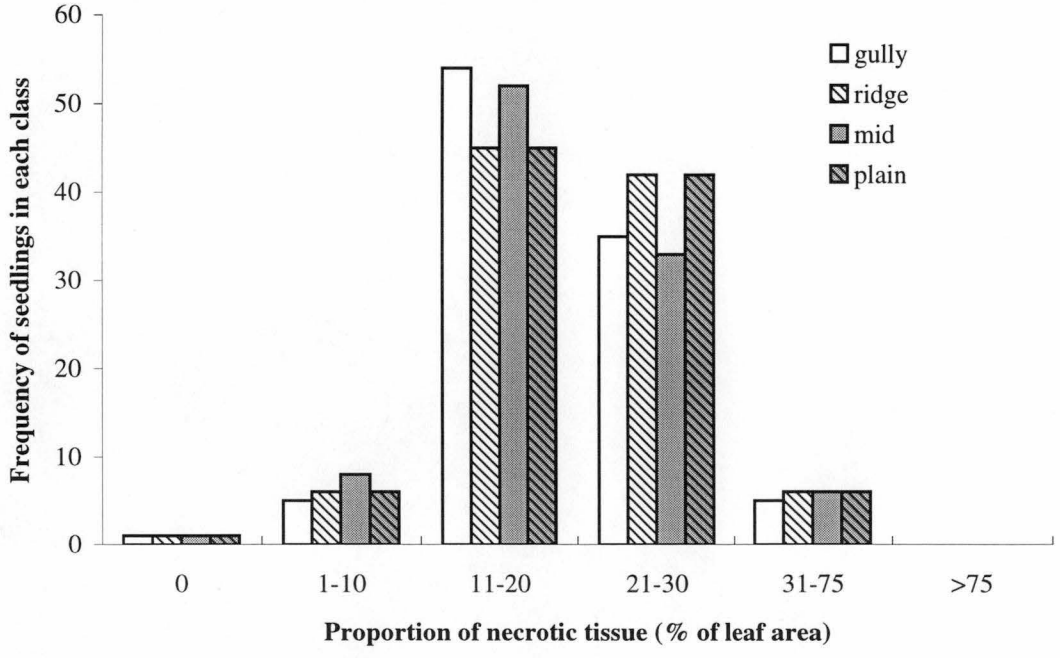


Figure 6.5 Frequency of seedlings from four populations within various classes of leaf spotting disease at the Lune Mid site

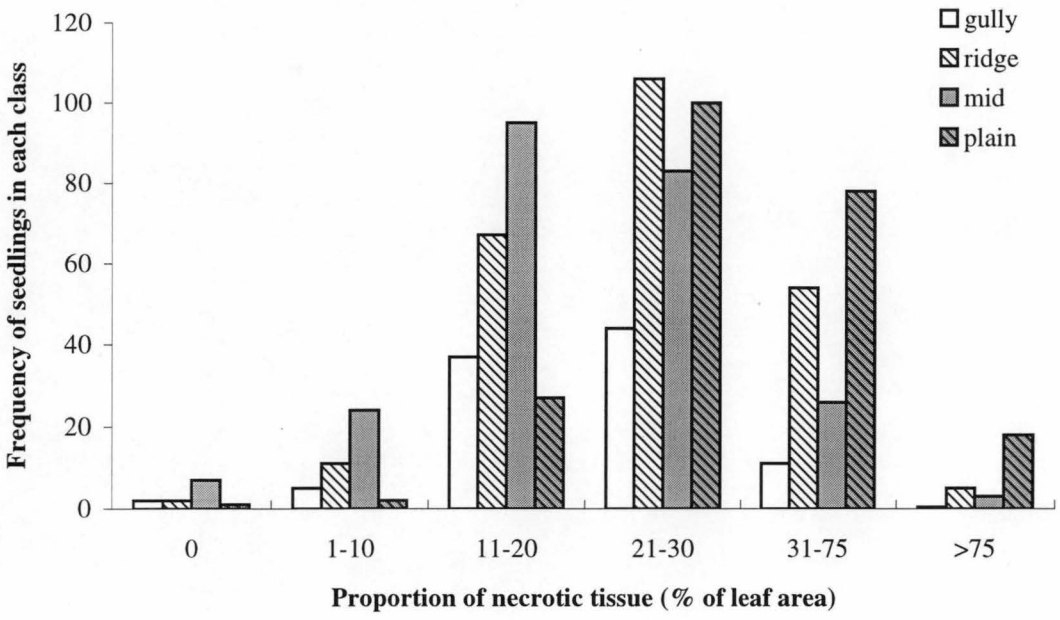


Figure 6.6 Regression of height against leaf spotting for individual seedlings across all populations at the Lune Mid site

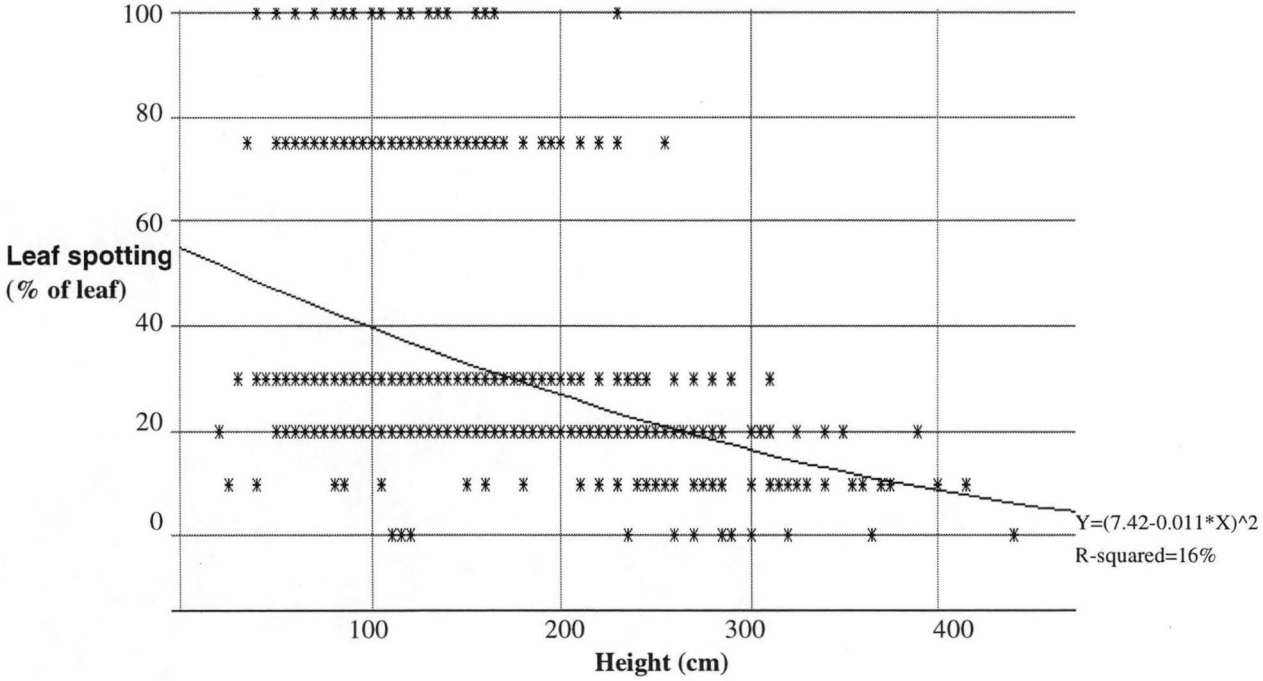


Table 6.2. Covariate ANOVA for mean plot leaf spotting rating at the Lune Mid site

Source	d.f.	M.S.	F-ratio	Significance level
COVARIATE				
height	1	0.000336	0.01	ns
MAIN EFFECT				
population	3	0.399213	6.28	0.001
RESIDUAL	43	0.063592		

3.2 Browsing

The probability of a seedling being browsed varied significantly between populations (Figure 6.7). The 'local' populations (Gully and Ridge) were more than twice as likely to be browsed as the two 'non-local' populations (Mid and Plain). In addition, the Ridge population had a significantly higher probability of being browsed than the Gully population.

The severity of browsing followed a similiar pattern, with the Ridge population sustaining the highest level of severely browsed seedlings (Table 6.3, Figure 6.8).

Browsing also had a significant effect on coppice growth (chapter 7). Coppice produced by the Gully, Ridge and Mid populations was more heavily browsed than coppice produced by the Plain population (Figure 6.9).

Table 6.3 ANOVA for seedlings subjected to severe browsing at the Forestier Gully site

Source	d.f.	M.S.	F-ratio	Significance level
population	3	13.5	2.85	0.05
error	44	4.73		

Figure 6.7 Probability of seedlings being browsed. Identical letters indicate non-significant subsets (p>0.05)

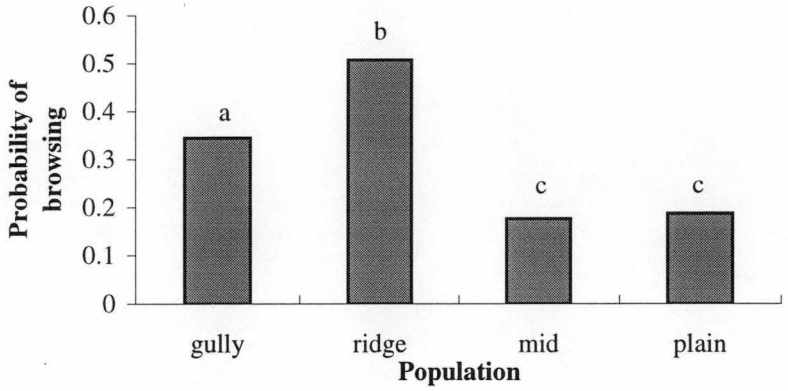


Figure 6.8 Proportion of seedlings subjected to severe browsing. Identical letters indicate non-significant subsets (p>0.05)

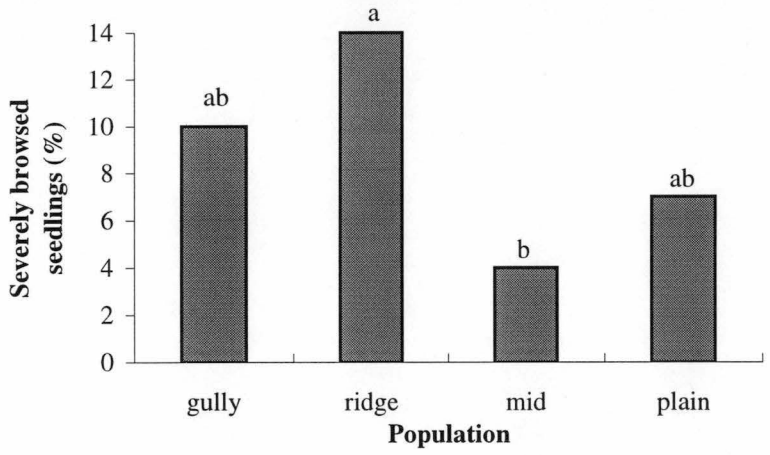
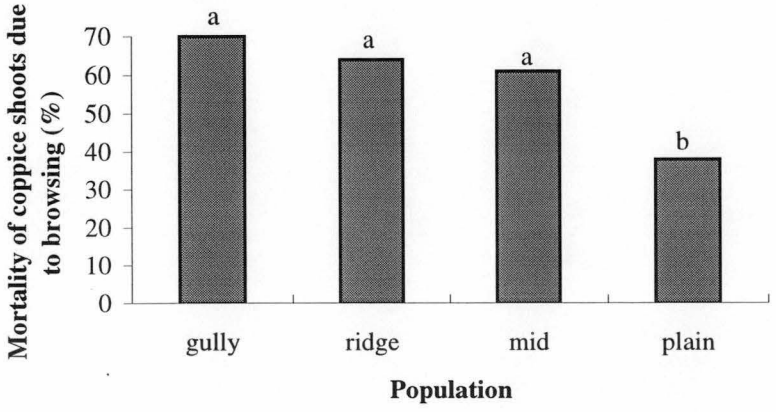


Figure 6.9 Mortality of coppice shoots attributed to browsing. Identical letters indicate non-significant subsets (p>0.05)



4. Discussion

Levels of leaf infection within the three planting sites were within the normal range for seedlings of this developmental stage (T. Wardlaw, Forestry Tasmania, pers. comm.). Despite these moderate levels of infection, there were highly significant differences between populations. These differences were most pronounced at the Lune Mid site which recorded the highest frequency of severely damaged leaves. At this site, there was evidence of differentiation between adjoining populations, with the wet forest populations from Forestier Gully and Lune Mid populations sustaining significantly lower damage levels than the open forest populations from Forestier Ridge and Lune Plain respectively.

Susceptibity of eucalypt leaves to infection by *Mycosphaerella cryptica* is greatest when young leaves are rapidly expanding under warm, wet conditions (Park 1988). Older leaves are increasingly more resistant to infection but may provide an important source of inoculum, which adds to the large number of ascospores produced from the fallen leaf litter (Cheah and Hartill 1987). The presence of moisture is the principal factor governing the release of ascospores (Cheah and Hartill 1987). Hence, high infection levels are more likely to occur to young leaves that are positioned within the sheltered, moist conditions of the lower canopy. The relative height of a seedling may therefore be an important factor in determining its susceptibility to leaf infection. In the current results, there was a significant but relatively weak relationship between tree height and infection level, possibly because the spacing of the planted seedlings resulted in the maintenance of relatively open crowns at age two years. Conditions more conducive to high infection levels are more likely to occur within the lower canopy of very dense stands resulting from seeded regeneration. Such stands are most likely to arise in wet forests such as the Forestier Gully and Lune Mid sites, where resistance to leaf infection, and hence the maintenance of vigorous growth, may be of substantial adaptive value.

Fences erected at all of the planting sites were constructed and maintained to an equivalent standard and the failure of the fence to totally exclude possums at the Forestier Gully site was attributed to substantially higher browsing pressure at this site. As a result, seedlings at the Gully site were subjected to moderate levels of defoliation whilst those at the other planting sites suffered no losses. Differential browsing pressure between sites was also implicated by the results of the coppice studies (chapter 7) in which a substantial reduction in coppice shoots occurred only at the Gully site, even though the fences at all sites had been removed

prior to the thinning operations. These results demonstrate the variability in browsing pressure that can be exerted at different sites, even over the short distance of 500m that separated the Forestier Gully and Forestier Ridge planting sites.

Within the Forestier Gully site, possums demonstrated preferential browsing, with the 'off-site' populations from Lune (Mid and Plain) the least likely to be browsed. Of the two 'local' populations, the Gully progeny were less likely to be browsed than progeny from the nearby Ridge population, suggesting a relationship between the more intense browsing pressure within the Gully site and the higher 'resistance' of the Gully population to that pressure.

Selection in favour of seedlings less likely to be browsed would be of particular adaptive value on wet forest sites such as Forestier Gully. Eucalypts are well adapted to recover from partial defoliation (Jacobs 1955). However, severe defoliation, such as the loss of more than half of the foliage, can significantly reduce survival and long term growth (Wilkinson and Neilsen 1995). These authors found that browsed seedlings had been unable to establish site occupancy due to competition from unbrowsed seedlings and the vigorous (wet forest) understorey vegetation. Such growth suppression is more likely on wet forest sites (Forestier Gully) than on dry forest sites with a more open understorey (Forestier Ridge).

The pattern of browsing for the leaves on coppice shoots was different to that observed for seedling leaves. Coppice leaves were subjected to much higher levels of browsing, with 60-70% of individual coppice stumps severely defoliated, compared to 4-14% of seedlings. The higher intensity of browsing may reflect differences in alternative food sources over the period between the browsing of the seedlings at age 15 months and subsquent browsing of the coppice shoots at age 4.5 years. However, changes in leaf ontogeny have been associated with marked differences in the palatablity of some tree species to mammals (Gill 1992). Landsberg (1990) found that the epicormic foliage of eucalypts was more heavily grazed by insects than adult leaves. Such differences could be due to variation in leaf nutrition and putative defence compounds such as tannins, phenols and terpenes (Fox and Macauley 1977, Stone and Bacon 1994).

Browsing of coppice shoots was lower for the Plain population than for the other populations. Seedling recruitment is very difficult on the harsh Plain site (chapter 3) and once established, the eucalypts are subjected to higher frequencies of fire than trees in the adjoining wet forests

sites (Jackson 1968). The persistence of coppice may be important for maximising the opportunities for maintaining site occupancy following events such as fire, and selection for low palatability would therefore be advantageous for the Plain population.

Differentiation on the basis of leaf chemistry has been demonstrated for adjoining populations of *E. camaldulensis*, and the differences were highly correlated with insect herbivory (Stone and Bacon 1994). Gill (1992) argues that such differences in leaf chemistry may occur as a consequence of factors unrelated to browsing, such as nutrient availability and distribution within the plant. However, Haukioja *et al.* (1991) suggest that natural selection could directly favour traits, such as low nutritional quality of leaves, which provide a defence against browsing. Thus, selection for low palatability could occur directly, as a result of heavy browsing pressure, or indirectly as a result of separate (parallel) selection pressures.

5. Conclusions

Population differentiation was evident for susceptibility to infection by the leaf-spotting fungus *Mycosphaerella cryptica*, and browsing by the brush-tail possum. Leaf-spotting disease is most likely to reach severe levels within very dense stands on wet forest sites and there was evidence that the populations from wet sites had higher resistance to infection than the adjoining populations from open forest sites. Population differences in palatability to possum browsing may also be attributed to differential selection forces. Seedlings from 'off-site' populations (Lune Mid and Plain) were less likely to be browsed than the 'local' populations (Forestier Gully and Ridge). Within the Forestier populations, seedlings from the Gully population were more resistant to browsing than seedlings from the adjoining Ridge population, and this difference may reflect adaptation to heavier browsing pressure within the environment of the Gully forest type.

CHAPTER 7 MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERS

1. Introduction

Studies of variation in morphological characters have been undertaken in a number of eucalypt species (see review in chapter 1, section 4.6). Many characters such as height, leaf size and internode length are a function of plant vigour, and environmental influences may account for a large proportion of the total phenotypic variation observed across the range of a species (Potts 1985). However, genetic differentiation has been noted for characters such as the shape of vegetative and reproductive organs, indicating that these factors may have been subjected to strong selection forces.

Variation in some leaf characters has been cited as evidence of adaptation to site factors. For example, various hypotheses to explain differentiation in leaf glaucousness were proposed by workers such as Barber and Jackson (1957), Cameron (1970), Thomas and Barber(1974a), Paton (1981) and Floyd *et al.* (1994). Variation in leaf shape is more difficult to explain, particularly where it is complicated by heteroblastic or ontogenetic stages within the species. A trend towards narrow leaves on dry sites has been observed within populations of *E. viminalis* and Ladiges (1974a) has argued that this would give narrow leaves a competitive advantage by minimising the risk of heat stress, whereas large leaves would have a competitive advantage on wet sites by providing a greater photosynthetic area.

Phenotypic differences in seed size include a large maternal component, in which it is often difficult to distinguish between environmental and genetic effects (Gutterman 1992). Significant correlations between capsule size, seed size and factors such as germination, cotyledonary surface area and seedling growth rate have been documented for eucalypt species (Grose and Zimmer 1958, Green 1971, Boland and Dunn 1981). Differences in these factors may be of adaptive value under variable environmental conditions.

Lignotubers and coppice provide an increasingly important source of regeneration along the ecological gradient from wet to dry eucalypt forests (Jacobs 1955). The ability to develop lignotubers enables a species to persist in the presence of substantial competition, to recover from damage to its shoots by fire, insects or browsing animals, and to rapidly develop as soon as competition is reduced (Cremer *et al.* 1978).

Only a small number of eucalypt species do not develop lignotubers, and Jacobs (1955) suggested that such species tended to occur on favourable forest sites. Intra-specific variation has been reported for a number of eucalypts. Within *E. viminalis* and *E. ovata,* lignotuber development was greater for seedlings from low rainfall or waterlogged populations than from high rainfall, tall forest populations (Ladiges and Ashton 1974, Ladiges 1974a, Clucas and Ladiges 1979). Potts (1985) also noted that seedlings of *E. gunnii/archeri* from populations exposed to harsh conditions of frost and drought had greater lignotuber development than seedlings from wet forest populations that were associated with more favourable conditions for seedling development. Pronounced genetic differentiation occurs within *E. camaldulensis,* but no link between lignotuber development and environmental factors has been proposed to explain the presence of lignotubers in northern provenances but not in southern provenances (Eldridge *et al.* 1993). Variation in lignotuber development has been reported to occur between populations of *E. obliqua* , however the results provided no indication of any pattern of variability with seed source (Green 1971).

Coppice regeneration within the eucalypts occurs from epicormic shoots following the complete or partial loss of the crown due to fire, insect attack (Jacobs 1955), stem breakage or harvesting. The capacity to produce and maintain epicormic shoots is therefore a major factor in determining the ability of an individual to survive and recover from the periodic loss of foliar, branch or stem tissue.

Generally, most species that possess lignotubers tend to coppice well, while some of the non-lignotuberous species such as *E. regnans* coppice poorly (Blake 1983). There are few studies of intra-specific variation in coppicing ability. In a review of the literature on *E. camaldulensis*, Eldridge *et al.* (1993) found that there was no indication of genetic variation in coppicing ability, despite the marked differences in lignotuber development between the northern and southern provenances. Similar levels of coppicing were also reported for different provenances of *E. grandis* and *E. saligna* (Eldridge *et al.* 1993).

Results from a Tasmanian trial indicated that variation in the coppicing ability of seven year old *E. obliqua* between populations from NSW, Victoria, South Australia and Tasmania ranged from 20 to 67% (Forestry Commission 1978). There was no clear correlation of coppicing ability with environmental attributes of the provenance location, with the exception of populations from a site quality sequence at Strathblane, Tasmania (Brown *et al.* 1976) where coppicing varied from 20% for the high site quality population to 44% for the lower site quality populations (Forestry Commission 1978). However, the high site quality

population originated from a stand that included a high proportion of *E. regnans* (chapter 3), and the possibility of hybridisation between *E. obliqua* and the poorer coppicing *E. regnans* cannot be dismissed from this trial.

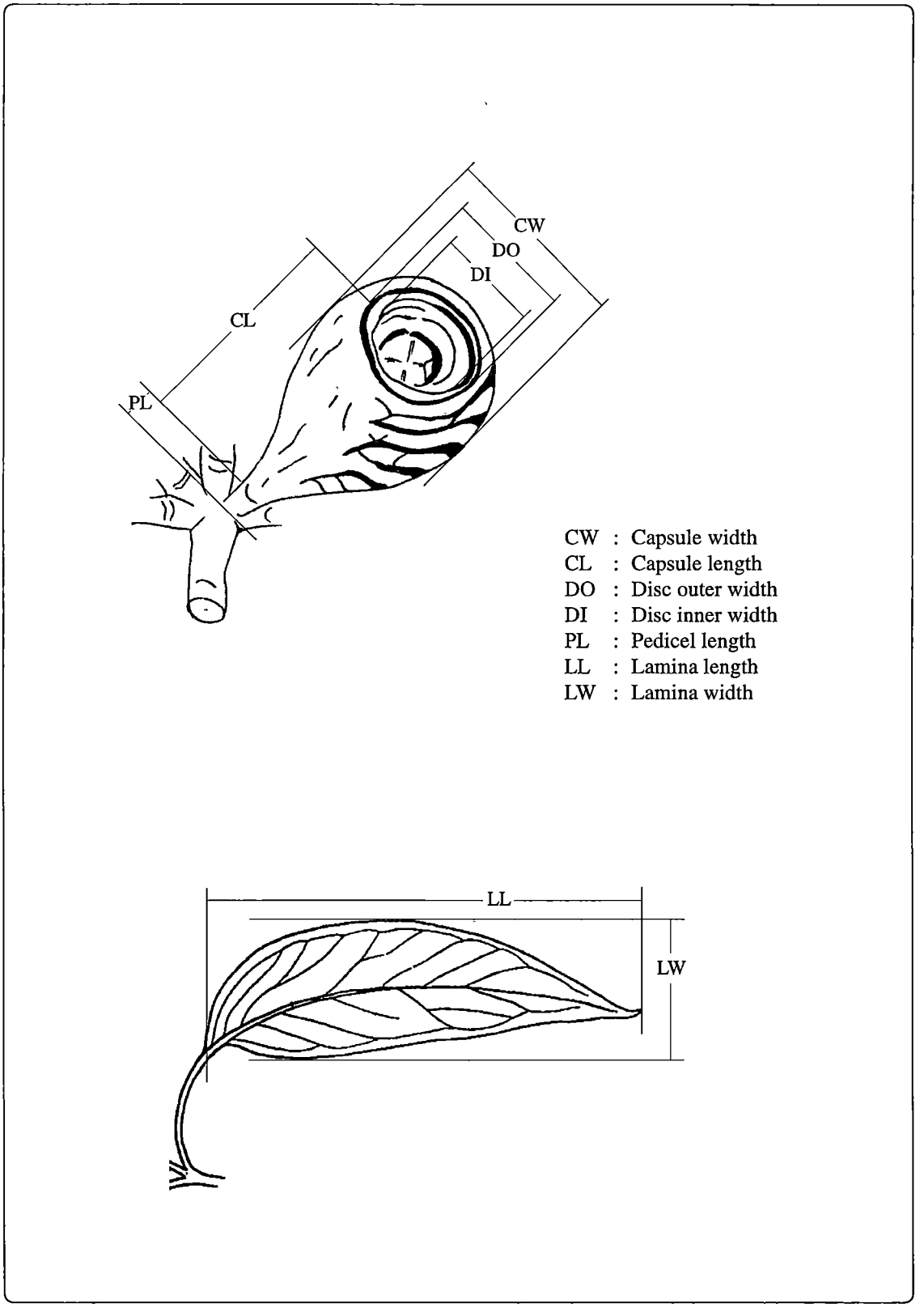
2. Methods

Adult leaves and mature capsules were collected from 48 maternal trees in March-April 1989 (chapter 2). Seven leaves were taken from one year old shoots in the upper canopy of each mother tree. Lamina length and width were measured (Figure 7.1) and lamina area was determined with the use of an electronic planimeter. The ratio of lamina length to width was used as an index of leaf shape. Ten capsules from each family were selected at random from the bulk samples collected for seed extraction (chapter 2). Dimensions for capsule length and width, pedicel length, inner disc diameter and outer disc diameter were taken with vernier callipers as indicated in Figure 7.1. Oven-dry weight was determined for 25 capsules randomly sampled from each family.

Seed size was assessed by sieving the seed and chaff mix into three size fractions as described in chapter 4. Cotyledon size was measured by germinating the seed within the three size fractions and allowing the cotyledons to fully expand until the shoot for the first true leaves began to appear. The cotyledons were then harvested by excision of the stem below the cotyledonary node. The 15 largest, healthy pairs of cotyledons were selected from the three seed size fractions within each family and were mounted and pressed for photocopying and measurement of area by electronic planimeter. The images were magnified x4 to improve the accuracy of the area measurement.

Progeny leaves were collected from four year old trees planted at the Forestier Gully and Ridge sites. Samples were fully expanded, current season leaves from an open, upper part of the crown. Six leaves were taken from each of three trees from four families within each provenance, (i.e. 2 sites x 4 populations x 4 families x 3 trees x 6 leaves =576). Leaf dimensions of lamina length, width, area and lamina length : width ratio were recorded as described above. Family means were derived for all measurements and these values were subjected to ANOVA. Mean cotyledon area for each family was determined as the weighted mean of the area of each cotyledon within each seed size class (i.e mean area x proportion of seed within each size class as documented in chapter 4). Regression analysis was used to investigate correlations between capsule size, seed size and cotyledon area.

Figure 7.1 Morphological measurements taken on capsule and leaf samples.



Seedlings planted at the Forestier Gully, Ridge and Lune Mid sites (chapter 2) were assessed for the presence or absence of lignotubers at ages 15 months and 21 months. Assessments after this time were not possible due to the occlusion of the lignotuber by the rapid stem growth of most seedlings. The proportion of surviving seedlings with a lignotuber was determined for each plot and the data were subjected to the arcsine transformation. Multifactor ANOVA was undertaken on the plot mean values averaged across the four blocks within each planting site (i.e treating the families as random terms) to determine the effects of populations, planting sites and interactions (populations x planting site). The effect of seedling height growth on the development of lignotubers was determined by covariate analysis. Comparisons between subsets were made using the least significant differences(LSD) test at the 95% confidence level where the ANOVA indicated significant differences between populations or sites (at $p<0.05$).

Coppicing ability was evaluated following the thinning of plots in the above trials. Plots were thinned to one tree per plot (i.e. a removal of up to 5 trees per plot) using a chainsaw, or secateurs for small stems. Selection for retention was based on the tree with the largest stem volume at the age 4 years (June 1994) measurement. Thinning was carried out at two times of the year, in order to study the effect of season on coppicing ability. Two blocks from each planting site were selected at random for thinning in spring (2, 4 and 9 October 1994 for the Forestier Ridge, Gully and Lune Mid sites respectively). The remaining two blocks at each site were thinned in autumn (28 March, 3, 4 April 1995 for the Forestier Ridge, Lune Mid and Forestier Gully sites respectively). Stumps were cut to a height of approximately 7cm above ground level and slash was stacked parallel to the row direction with care taken to ensure that stumps were not covered. The proportion of stumps producing coppice, and the height of the tallest shoot on coppiced stumps were recorded 10, 36 and 56 weeks after thinning for the spring fellings. Coppice production 10 weeks after the autumn fellings was limited to the production of buds, with minimal shoot growth. The proportion of stumps with coppice buds was assessed at 10 weeks and again (with height measurements) at 30 weeks.

Coppicing ability was determined as the proportion of previously living stems that had produced coppice shoots. Values were subjected to the arcsine transformation prior to analysis. Height growth of coppice was measured as the height of the tallest shoot on each stump. Results were analysed by ANOVA, using the mean of the family values across the blocks within each site. Comparisons between subsets were made using the least significant differences (LSD) test at the 95% confidence level where the ANOVA indicated significant differences between populations or sites (at $p<0.05$).

3. Results

Significant phenotypic differences were recorded for the adult leaves of the mother trees for the leaf characters of lamina width and shape (as defined by the lamina length:width ratio) but not for lamina length or area (Table 7.1). The Lune Plain mother trees had significantly narrower leaves than the mother trees at the Forestier Gully and Ridge sites (Table 7.2). Leaf shape was the most variable character, with leaves tending to be broadly lanceolate in shape at the Forestier Gully and increasingly more narrow-lanceolate in shape at the Forestier Ridge, Lune Mid and Lune Plain sites respectively.

Leaves from the four year old progeny of the four populations were not significantly different for any of the measured leaf characters (Table 7.3). However, the effect of planting site was highly significant, with leaves at the Forestier Gully planting site being longer and larger in lamina area than leaves at the Forestier Ridge site (Table 7.4).

There were significant phenotypic differences between populations for the morphology of capsules collected from each mother tree (Tables 7.5 and 7.6). Capsules from the Lune populations (Mid and Plain) were consistently larger for all morphological characters than the Forestier populations (Gully and Ridge). However the basic capsule shape (as defined by the ratio of capsule length to width) was the same for all capsules. There were significant correlations between capsule size, seed size and cotyledon area (Table 7.7) and significant differences between populations, with the largest cotyledons produced by the Mid population (Tables 7.8 and 7.9).

Table 7.1 ANOVA for the dimensions of leaves collected from 12 mother trees within each population

Character	Source	d.f.	M.S.	F-ratio	Significance level
lamina area	population	3	42.096	1.99	ns
	error	44	21.154		
lamina length	population	3	3.286	2.29	ns
	error	44	1.435		
lamina width	population	3	0.537	4.92	**
	error	44	0.109		
length:width ratio	population	3	1.846	7.82	***
	error	44	0.236		

(** = $p<0.01$, *** = $p<0.001$, ns = not significant $p>0.05$)

Table 7.2 Variation in adult leaf characters collected from 12 mother trees within each population. Identical letters indicate non-significant subsets (p>0.05)

Population	Lamina area (cm^2)		Lamina length (cm)		Lamina width (cm)		Length:width ratio	
Gully	22.46	a	10.75	a	2.92	a	3.73	a
Ridge	24.98	a	11.93	a	2.90	a	4.16	b
Mid	22.08	a	11.66	a	2.65	ab	4.44	bc
Plain	20.46	a	11.25	a	2.48	b	4.63	c

Table 7.3 ANOVA for the dimensions of leaves collected from four year old trees of progeny from four populations planted at the Forestier Gully and Ridge sites

Character	Source	d.f.	M.S.	F-ratio	Significance level
lamina area	population	3	147.63	2.42	ns
	site	1	578.61	9.50	**
	interaction (p x s)	3	35.94	0.59	ns
	residual	24	60.89		
lamina length	population	3	2.37	1.80	ns
	site	1	17.75	13.49	***
	interaction (p x s)	3	0.20	0.15	ns
	residual	24	1.32		
lamina width	population	3	0.63	1.60	ns
	site	1	1.38	3.51	ns
	interaction (p x s)	3	0.28	0.72	ns
	residual	24	0.39		
length:width ratio	population	3	0.098	1.26	ns
	site	1	0.050	0.64	ns
	interaction (p x s)	3	0.055	0.70	ns
	residual	24	0.078		

(*** = $p<0.001$, ** = $p<0.01$, ns= not significant $p>0.05$)

Table 7.4 Variation in the dimensions of leaves collected from four year old trees of progeny from four populations planted at the Forestier Gully and Ridge sites. Identical letters indicate non-significant subsets for each source of variation (p>0.05)

Source of variation		Lamina area (cm^2)		Lamina length (cm)		Lamina width (cm)		Length:width ratio	
Planting site	Forestier Gully	52.20	a	13.75	a	5.59	a	2.48	a
	Forestier Ridge	43.69	b	12.26	b	5.18	a	2.40	a
Population	Gully	46.03	a	13.03	a	5.17	a	2.53	a
	Ridge	54.37	a	13.64	a	5.78	a	2.38	a
	Mid	45.98	a	13.04	a	5.22	a	2.43	a
	Plain	45.39	a	12.31	a	5.37	a	2.31	a

Table 7.5 ANOVA for the dimensions of capsules collected from 12 mother trees within each population

Character	Source	d.f.	M.S.	F-ratio	Significance level
capsule length	population	3	198.29	6.08	**
	error	44	32.60		
capsule width	population	3	100.03	3.79	*
	error	44	26.38		
length:width ratio	population	3	0.0027	0.82	ns
	error	44	0.0033		
pedicel length	population	3	26.52	1.45	ns
	error	44	18.33		
width outer disc	population	3	392.95	8.35	***
	error	44	47.08		
width inner disc	population	3	163.25	5.56	**
	error	44	29.37		
capsule weight	population	3	0.0163	8.80	***
	error	44	0.0018		

(*** = p<0.001, ** = p<0.01, * = p<0.05, ns = not significant p>0.05)

Table 7.6 Variation in the dimensions of capsules collected from 12 mother trees within each population. Identical letters indicate non-significant subsets (p>0.05)

Population	Capsule length (mm)		Capsule width (mm)		Capsule length:width ratio		Capsule weight (g)		Pedicel length (mm)		Disc outer width (mm)		Disc inner width (mm)	
Gully	7.54	a	7.47	a	1.01	a	0.156	a	1.68	a	5.05	a	3.56	a
Ridge	7.40	a	7.54	a	0.98	a	0.165	a	1.95	a	5.11	a	3.51	a
Mid	8.08	b	7.97	b	1.01	a	0.205	b	1.62	a	6.07	b	4.21	b
Plain	8.24	b	8.03	b	1.02	a	0.236	b	1.68	a	6.07	b	4.13	b

Table 7.7 Correlations between capsule size, seed size and cotyledon area determined from regression analysis

Factors	Model	Significance level	r^2
seed size (Y) capsule size (X)	Y = 0.777 + 0.048X	***	27%
cotyledon area (Y) capsule size (X)	Y = 0.112 + 0.021 X	***	22%
cotyledon area (Y) seed size (X)	Y = 0.100 + 0.098 X	***	40%

*** = p<0.001

Table 7.8 ANOVA for the mean size of cotyledons within various seed size classes

Seed size	Source	d.f.	M.S.	F-ratio	Significance level
1.2mm	population	3	0.0124	3.4	*
	error	44	0.0036		
1.0mm	population	3	0.0088	5.24	**
	error	44	0.0017		
0.05mm	population	3	0.0028	0.87	ns
	error	44	0.0033		
all seed	population	3	0.0145	5.47	**
	error	44	0.0026		

(** = p<0.01, * = p<0.05, ns = not significant p>0.05)

Table 7.9 Mean size of cotyledons (cm^2) within various seed size classes for each population. Identical letters indicate non-significant subsets within each seed class (p>0.05)

Population	Seed size class (mm)							
	1.2mm		1.0mm		0.05mm		all seed	
Gully	0.219	a	0.175	ab	0.124	a	0.189	a
Ridge	0.240	ab	0.143	a	0.092	a	0.193	a
Mid	0.285	b	0.205	b	0.120	a	0.262	b
Plain	0.215	a	0.155	a	0.102	a	0.194	a

There were significant differences in the development of lignotubers between populations and planting sites (Table 7.10). Interactions between population and planting site were not significant for either of the time periods following planting. Covariate analysis indicated that seedling height did not have a significant effect on the proportion of lignotubers at either of the measurements at age 15 months (p=0.93) or age 21 months (p=0.60).

One year after planting, the seedlings of the Ridge population had produced significantly more lignotubers than the other populations (Figure 7.2), and in particular had produced more than twice as many as the Gully population. The Plain population also produced significantly more lignotubers than the Gully population. These trends continued in the second year after planting, although differences between the Ridge, Mid and Plain populations diminished.

The development of lignotubers was significantly affected by planting site (Figure 7.3). One year after planting, only 4% of seedlings at the Ridge site had produced lignotubers, compared to 12 and 23% at the Gully and Mid sites respectively. Over the next 12 months, the development of lignotubers at the Ridge site was significantly greater than at the other two sites, with the proportion rising to 43% at the Ridge site and 29 and 27% at the Gully and Mid sites. There were no significant interactions between population and site.

Table 7.10 ANOVA of transformed values for the proportion of stems with lignotuber development at 15 and 21 months after planting on three sites

Time period (after planting)	Source	d.f.	M.S.	F-ratio	Significance level
15 months	population	3	0.2246	5.58	***
	site	2	1.0554	26.22	***
	interaction (population x site)	6	0.0518	1.29	ns
	residual	132	0.0403		
21 months	population	3	0.1287	3.00	*
	site	2	0.5367	12.50	***
	interaction (population x site)	6	0.0331	0.77	ns
	residual	132	0.0429		

(*** = $p<0.001$, * = $p<0.05$, ns = not significant $p>0.05$)

Figure 7.2 Differences between populations in the proportion of stems with lignotubers across three planting sites. Identical letters within each time period indicate non-significant subsets (p>0.05)

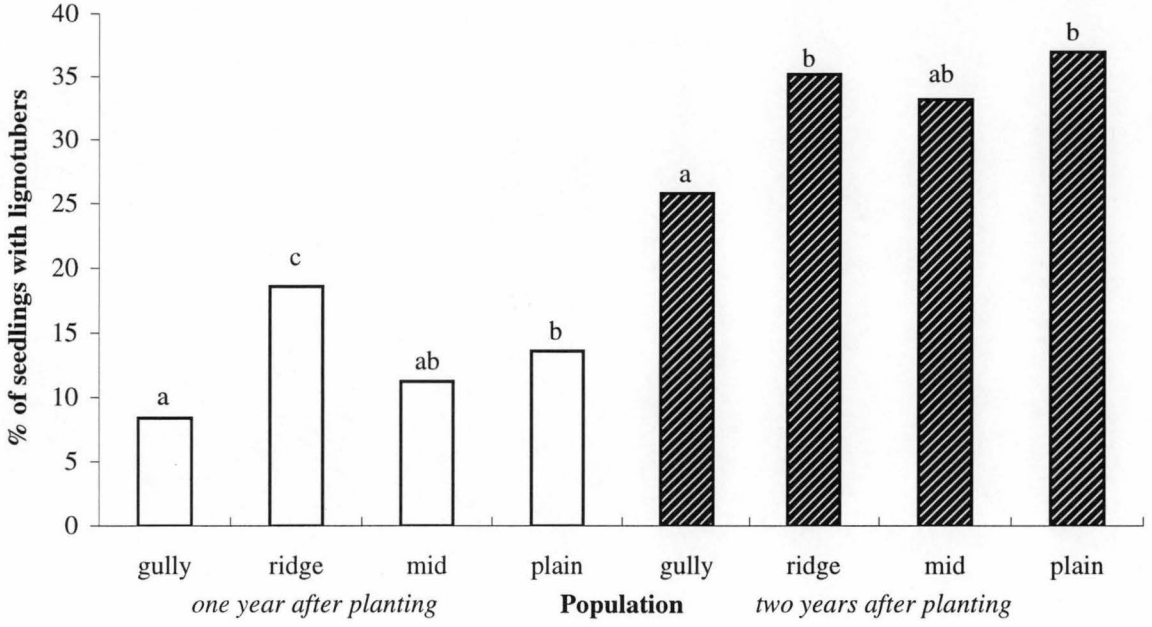
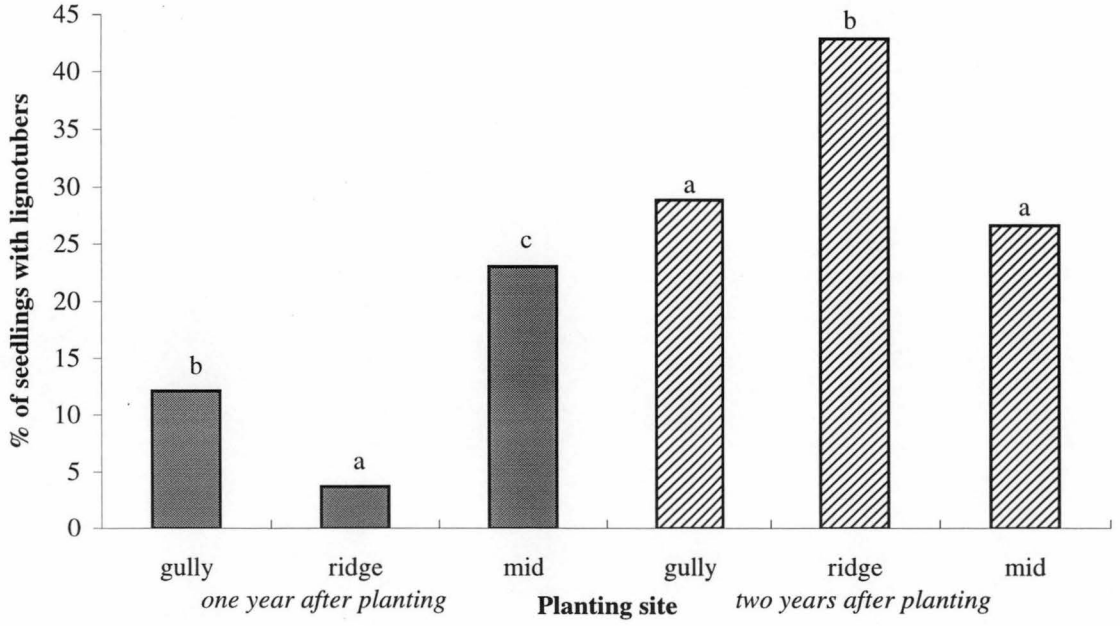


Figure 7.3 Differences between planting sites in the proportion of stems with lignotubers. Identical letters within each time period indicate non-significant subsets (p>0.05)



The production and persistence of coppice shoots on cut stumps was influenced by population, site and the season of felling, and there were no significant interactions (Table 7.11). All populations demonstrated a very high potential coppicing ability, as evidenced by the production of coppice on over 95% of all cut stumps 10 weeks after the spring felling (Figure 7.4). Significant differences between populations were apparent 36 weeks after the spring felling, with a higher persistence of coppice within the plain population than for the other populations. There were also differences between populations for the growth of coppice shoots (Figure 7.5).

The effect of site was dependent upon the time of felling and the period since felling (Figure 7.6). Virtually all stumps across the three sites produced coppice shoots 10 weeks after the spring felling. However, the initiation of coppice 10 weeks after the autumn felling varied significantly from 77% of stumps at the Forestier Ridge site to only 8% of stumps at the Lune Mid site. In addition, the initial growth of coppice following both the spring and autumn fellings was significantly better at the Ridge site than at the wet forest sites (Figure 7.7). The persistence of coppice was also highly variable, with significant differences between all sites 36 weeks after the spring felling. Mortality of coppice shoots after the spring felling was most pronounced on the wet forest sites (Gully and Mid). Repeated browsing by mammals was the major reason for the significantly lower persistence and height growth of coppice shoots at the Gully site (Figure 7.7). Differences between populations at this site were also attributed to differential susceptibility to browsing (chapter 6).

Table 7.11 Results from ANOVA of data across three sites for the proportion of cut stems containing coppice shoots (transformed values for % coppice), and the height (cm) of coppice shoots on stumps cut in spring and autumn

Time of felling	Period since felling	Character	Source	d.f.	M.S.	F-ratio	Significance level
Spring	10 weeks	% coppice	site	2	0.00008	0.01	ns
			pop	3	0.0033	0.47	ns
			site x pop	6	0.0079	1.12	ns
			residual	132	0.0071		
		height	site	2	36.03	3.08	*
			pop	3	8.21	0.70	ns
			site x pop	6	2.05	0.18	ns
			residual	132	11.68		
	36 weeks	% coppice	site	2	6.65	71.11	***
			pop	3	0.62	6.62	***
			site x pop	6	0.13	1.40	ns
			residual	132	0.09		
		height	site	2	21590.20	77.95	***
			pop	3	746.96	2.70	*
			site x pop	6	451.50	1.63	ns
			residual	132	276.96		
	56 weeks	% coppice	site	2	16.26	137.98	***
			pop	3	0.35	3.04	*
			site x pop	6	0.23	1.95	ns
			residual	132	0.12		
		height	site	2	35103.40	91.17	***
			pop	3	772.61	2.01	ns
			site x pop	6	534.98	1.39	ns
			residual	132	385.03		
Autumn	10 weeks	% coppice	site	2	14.01	153.21	***
			pop	3	0.12	1.36	ns
			site x pop	6	0.03	0.34	ns
			residual	132	0.09		
	30 weeks	% coppice	site	2	5.27	41.80	***
			pop	3	0.34	2.65	*
			site x pop	6	0.09	0.76	ns
			residual	132	0.13		
		height	site	2	348.25	34.40	***
			pop	3	22.27	2.20	ns
			site x pop	6	9.37	0.93	ns
			residual	132	10.12		

ns = not significant ($p>0.05$), * $p<0.05$, *** $p<0.001$

Figure 7.4 Differences between populations for the proportion of stumps with live coppice shoots at various periods after felling in spring and autumn across three planting sites. Identical letters indicate non-significant subsets for each time period and felling (p>0.05)

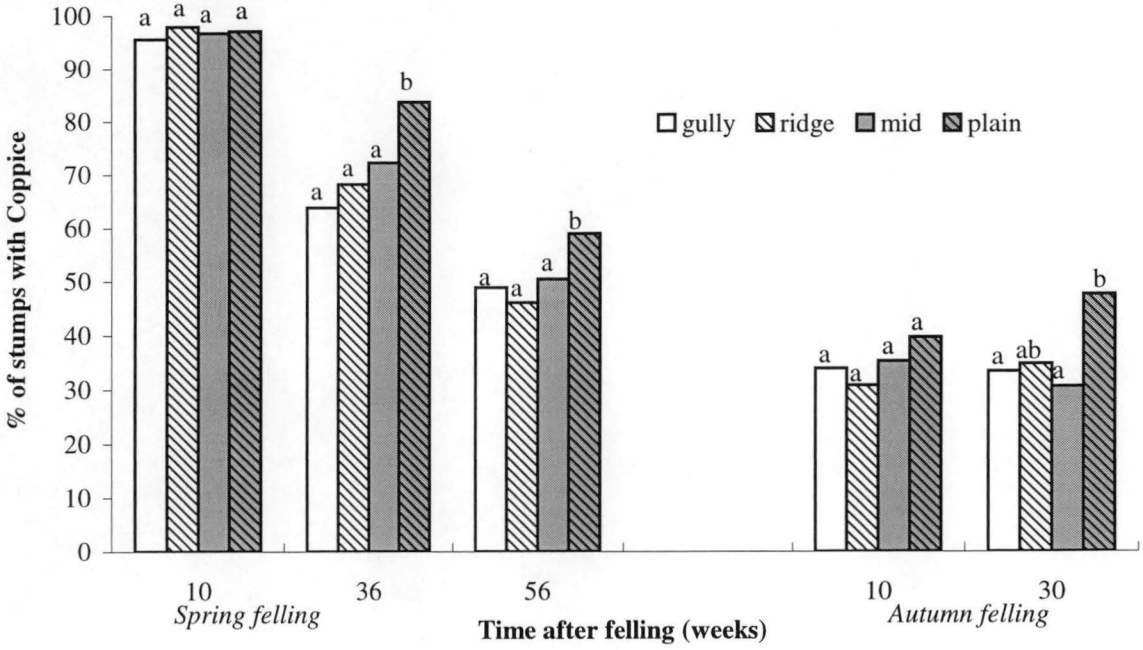


Figure 7.5 Differences between populations for the height of live coppice shoots at various periods after felling in spring and autumn across three planting sites. Identical letters indicate non-significant subsets for each time period and felling (p>0.05)

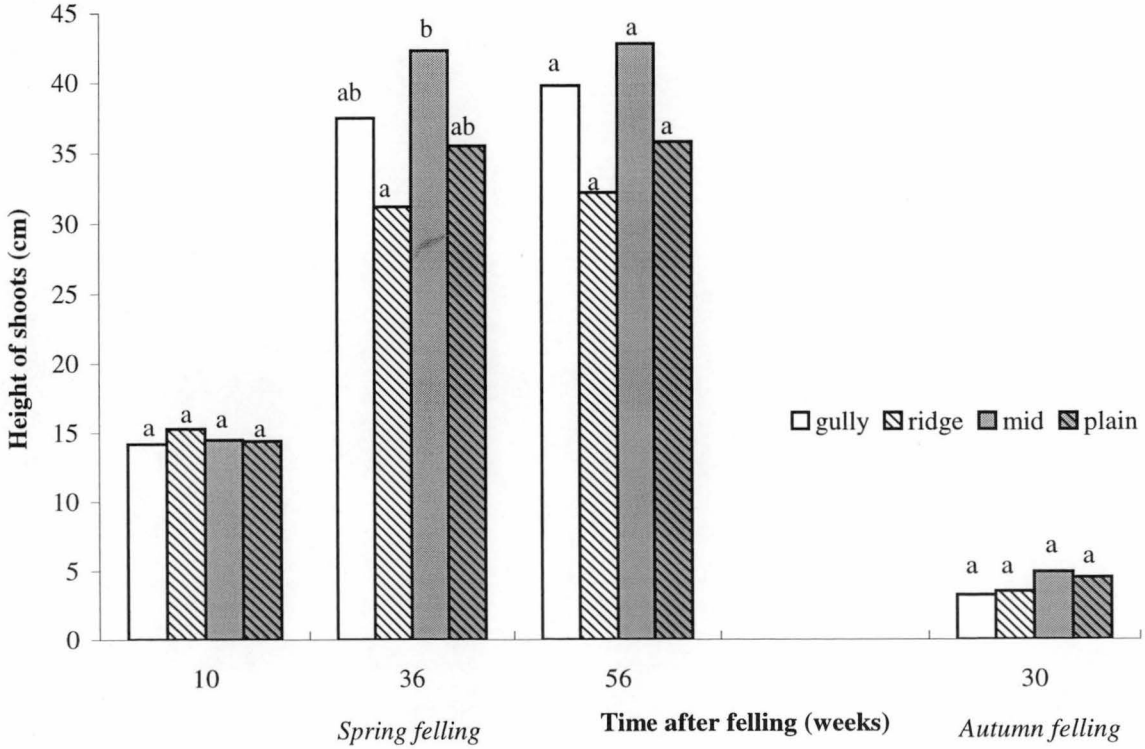


Figure 7.6 Differences between planting sites for the proportion of stumps with live coppice shoots at various periods after felling in spring and autumn across three planting sites. Identical letters indicate non-significant subsets for each time period and felling (p>0.05)

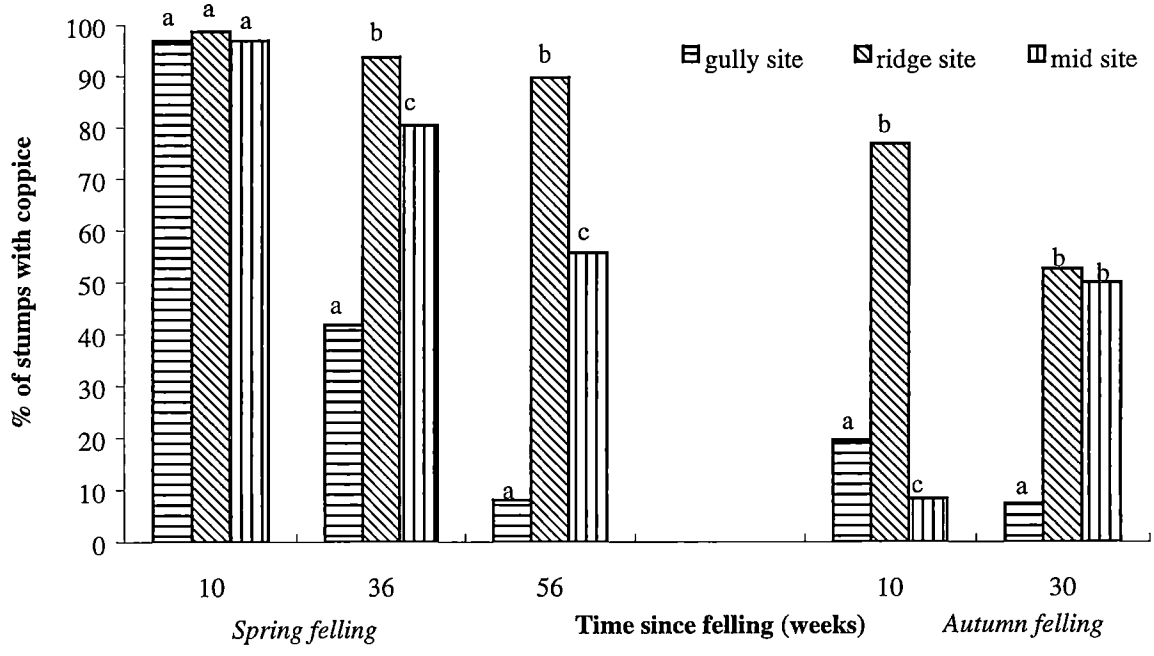
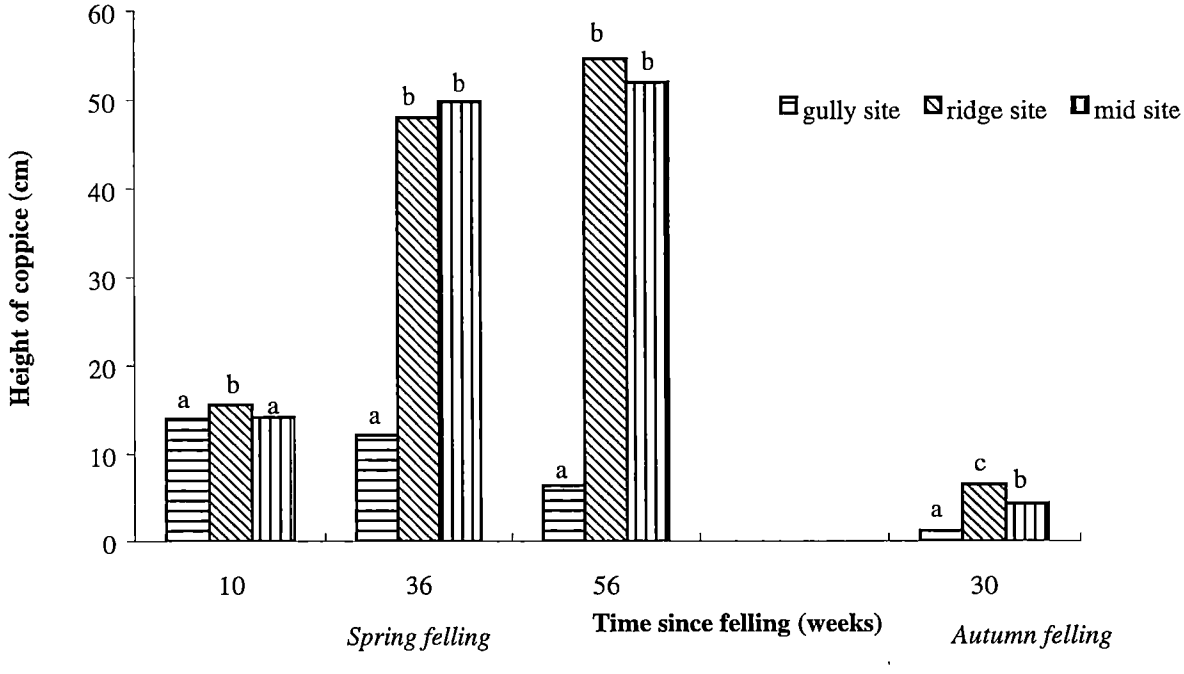


Figure 7.7 Differences between planting sites for the height of live coppice shoots at various periods after felling in spring and autumn across three planting sites. Identical letters indicate non-significant subsets for each time period and felling (p>0.05)



4. Discussion

Morphological characters such as leaf size generally show considerable phenotypic plasticity in response to different environmental conditions (Potts 1985). In contrast, characters such as leaf shape exhibit very little environmental modification and are therefore key indicators for taxonomic differentiation between species and subspecies (Potts 1985)

The results from the current studies do not provide any evidence of genetic differences in leaf morphology between the four populations. Leaves from four year old progeny within each population were not significantly different for any of the measured leaf characters. It is therefore likely that environmental factors were primarily responsible for the phenotypic differences recorded between the adult leaves from the original maternal trees. Ladiges and Ashton (1974) found that phenotypic differences in the leaf dimensions of *E. viminalis* were correlated with the mean annual rainfall of the mother trees. They found that leaves from drier sites were smaller, shorter and narrower than leaves from more mesic sites. Leaf shape was correlated with annual rainfall, but also appeared to be influenced by other environmental factors such as soil nutrient status. These results are consistent with some of the trends noted in the current studies. For example, saplings from the mesic Gully planting site had a significantly larger leaf area than saplings from the drier Ridge planting site. However, the planting site had no effect on leaf shape. In contrast, maternal trees from the Forestier Ridge site had leaves with a significantly lower length:width ratio than the mother trees at the Forestier Gully site. Overall, the phenotypic differences in the adult leaf characters of the mother trees were not marked and the importance of variation in the lamina length:width ratio should not be over emphasised given the greater inherent statistical error associated with such ratios (Ladiges and Ashton 1974).

Capsule morphology is strongly inherited in eucalypts at the species level (Pryor 1976). Grose (1963) also considered capsule size at the intra-specific level (within *E. delegatensis*) to be determined mainly by heredity, with little variation due to environmental factors. In contrast, Ladiges and Ashton (1974) found that capsule size in populations of *E. viminalis* was significantly correlated with the local rainfall. Capsule size was shown to be positively correlated with seed size (r^2=66%, $p<0.001$) within a collection of 22 provenances from the Australia-wide range of *E. obliqua* (Green 1971). Green also found that seed size was positively correlated with seedling height for at least the first two months after germination (r^2=16%, $p<0.05$). Similar correlations have been demonstrated for other species (Grose and

Zimmer 1958, Ladiges 1974, Boland and Dunn 1981). Eucalypt seeds are non-endospermic and germination is epigeal, thus seed size is likely to influence initial growth because it determines the photosynthetic area of the cotyledons (Grose and Zimmer 1958, Boland and Dunn 1981). Large seeds will thus have a faster absolute growth rate than small seeds, even though the relative growth rate may be equivalent for all seeds (Grose and Zimmer 1958, Shipley and Peters 1990). The present study confirmed the positive correlation between seed size and cotyledon area. Differences in cotyledon area between populations is therefore largely related to the differences in seed size detailed in chapter 4. However, there were also differences in cotyledon area within each of the two larger seed size classes, suggesting that the production of large cotyledons may also be influenced by other factors.

Laboratory studies have indicated that the growth advantage of large seeds may occur for up to 10 to 16 weeks (Grose and Zimmer 1958, Green 1971) but that beyond this time there is likely to be no correlation between seed size and growth rate (Turnbull and Doran 1987). However, differences in competitive ability between large and small seeds may be more pronounced under competitive, rather than non-competitive, ground covers (Gross 1984). Rapid early establishment may be important for eucalypt seedlings on wet forest sites, where the seedbed is rapidly re-colonised by competing vegetation. The production of large cotyledons could therefore be of adaptive significance. Among the Lune populations, there were significant differences between the Mid and Plain populations for both cotyledon size and the growth to age five years of seedlings (chapter 3), suggesting that differential selection pressures for growth rate may occur at a number of levels between these two populations. In contrast, the apparent lack of differentiation between the Gully and Ridge populations for cotyledon size indicates that this factor may not be subjected to strong selection pressures at the Forestier sites. However, the Gully population had a higher germination energy than the Ridge population (chapter 4), suggesting that more than one mechanism may be important for ensuring the early establishment of seedlings on wet forest sites.

Differences in the development of lignotubers between the populations support previous studies which indicated that populations from harsh sites are more likely to develop lignotubers than those from mild, wet forest sites (Ladiges and Ashton 1974, Ladiges 1974a, Clucas and Ladiges 1979, Potts 1985). These results are not unexpected, given that the main function of the lignotuber in *E. obliqua* is to provide a reservoir of protected buds (Carrodus and Blake 1970) which allows the seedling to recover from defoliation brought about by extreme environmental conditions. Drought is the factor most likely to result in the loss of

foliage, and the risk of desiccation to the seedling is more pronounced during the first summer when the seedling roots may not have developed sufficiently to withstand the drying out of the upper soil profile (Cremer *et al.* 1978). The significantly higher production of lignotubers by the Ridge seedlings compared to the Gully seedlings, particularly during the first year of growth, suggests that differential selection pressures for this attribute may have resulted in genetic differentiation over the comparatively short distance separating the two populations.

Coppicing ability in species such as *E. obliqua* and *E. regnans* has been shown to vary with the season of cutting, with maximal sprouting reported to occur when young saplings are cut in winter or early spring (Blake 1983, Cremer 1973, Wilkinson and Neilsen 1995). Cremer (1973) also observed that the recovery of *E. obliqua* and *E. regnans* seedlings was better on sites with low growth rates than on sites with high growth rates. The current results concur with these findings. Differences between sites may be attributed to variation in the degree of shading by the retained vegetation, and to impacts such as browsing. Despite differences in the persistence of coppice there appeared to be no differences in the growth rate to 56 weeks of surviving coppice shoots across the sites (excluding the Gully site where browsing had a major effect on growth). This result is in contrast to the substantial variation in seedling growth across these sites (chapter 3) but is in agreement with Abbott and Loneragan (1982) who found no difference in the growth rate over a 50 year period of *Eucalyptus marginata* coppice in forests with different site qualities, as defined by annual rainfall, site fertility and height of mature trees.

The results from the current study indicate that the potential to initiate coppice shoots does not significantly vary between the four populations. However, there are differences in the persistence of coppice, with the Plain population demonstrating the highest level of persistence. Differences in persistence were most pronounced at the Gully planting site, where heavy browsing by native mammals reduced the proportion of stumps containing live coppice shoots from 95% (10 weeks after the spring felling), to less than 8% (after 56 weeks). However, the higher persistence of coppice by the Plain population was maintained across the Ridge and Mid planting sites, where the loss of shoots due to browsing appeared to be low. These results suggest that there may be some other inherent physiological differences between the populations in addition to those related to differential browsing. The ability for vegetative recovery following the partial or total loss of the main crown could be an advantage for open forest populations such as the Plain, where conditions for seedling

establishment are harsh, and successful development to maturity depends upon the ability of the individual to survive periodic stresses such as fire and severe frost.

5. Conclusions

There was no evidence of genetic differentiation between populations for characters related to leaf morphology. However, there was evidence of differentiation for characters such as cotyledon size, development of lignotubers and the persistence of coppice. These characters have significant adaptive value and differences between adjoining populations provide an indication of differential selection forces acting upon populations at the site level.

CHAPTER 8 DISCUSSION AND CONCLUSIONS

Genetic differentiation between sites may arise as a result of geographic isolation or differential selection forces (Barber and Jackson 1957, Moran 1992). The natural distribution of *E. obliqua* within Australia can be regarded as widespread, with several disjunct populations. Within south-eastern Tasmania the distribution pattern is relatively continuous over extensive distances. Isozyme studies suggest that disjunct populations are likely to be associated with high levels of genetic variation between populations, whilst the continuous populations are likely to have high levels of both total genetic diversity and variation within populations, but low levels of variation between populations (Moran 1992). This pattern appeared to be confirmed by previous studies of *E. obliqua* which have demonstrated clear differences in the allelic frequencies of the major 'disjunct' populations in NSW, Victoria, Tasmania and South Australia (Brown *et al.* 1975), and little difference between populations within Tasmania (Brown *et al.* 1976).

The distribution pattern between the Forestier and Lune provenances in the current study is relatively continuous over a land distance of about 130km, although there are some discontinuities on drier sites of up to 10km where *E. obliqua* is displaced by other species. Eucalypt seed has a dispersal distance restricted to about 60-90m (Cremer 1966) and the majority of gene flow is likely to result from pollen dispersal, which generally occurs over short distances of 50-300m (Barber 1965, Potts and Reid 1983) but may extend to over 1 km (Ashton 1981). However, genetic divergence is more likely to be related to evolutionary time since separation rather than to current geographic isolation between populations (Coates and Sokolowski 1989) and the minor discontinuities over the geographical distance between these two provenances are therefore unlikely to have provided major barriers to gene flow. Within the study sites the two sets of adjoining populations formed a continuous distribution, with the distance between the nearest and most distant maternal trees from each population being 80-920m at Forestier and 180-620m at Lune. It could therefore be assumed that very active gene flow would occur within and between the populations on the adjacent sites at Forestier and Lune.

The ecological gradients at both sites were characterised by substantial differences in forest type and phenotypic variation within *E. obliqua*. The phenotypic variation was mainly expressed as differences in the height and form of the mature trees, with taller, straighter and larger trees in the wet forest types than in the open forest types. Only minor phenotypic

differences were noted for maternal leaf characters, and subsequent analysis of progeny leaf characters indicated no basis for genetic differences in leaf morphology. However, significant differences for a range of other attributes were recorded, providing evidence of genetic differentiation between populations at the site level. The lack of geographical isolation between the adjoining sites implies that differentiation must be a function of different selection forces operating within each site.

Population differentiation for growth rate was clearly evident between the Lune sites but not between the Forestier sites. Rapid early growth on wet forest sites allows seedlings to gain site occupancy and establish an advantage over the competing vegetation. On less favourable sites, selection may be more related to tolerance of environmental stresses such as frost, drought and low soil fertility (Pederick 1976). There were significant differences in the growth of seedlings at the Forestier Gully and Ridge planting sites, with the mean height of seedlings at the Gully site 2.5 times greater than at the Ridge site and the total volume production 14 times greater at age four years. Nevertheless, the lack of differentiation in growth between the Gully and Ridge populations implies that similar selection forces for growth operate at these two sites, at least for seedlings up to the age of five years. In contrast, the highly significant difference between the Lune Mid and Plain populations implies differential selection for growth between these two sites. Rapid early growth would be of substantial adaptive value on the Mid site, where prolific regeneration of the sedge *Gahnia grandis* provides intense competition to young eucalypt seedlings. In contrast, slow growth during the seedling stage on the Plain site may have adaptive value in minimising the risk of damage to soft new growth by harsh environmental factors such as frost and summer drought. Slow growth would need to be balanced against the need to compete with the regeneration of sedges such as *Gymnoshoenus sphaerocephalus*. This understorey forms a very dense, but relatively low stratum 0.5 to 2.0m in height (Marsden-Smedley 1993) and the eucalypts are likely to be suppressed unless their growing tips remain above the general height of the sward.

Differences between populations in factors such as the germination energy of seeds and cotyledon size could also be associated with selection for early establishment. Ladiges (1974) found that populations of *E. viminalis* from dry forest sites had a slower germination rate than populations from wet forests. Similar results were obtained in the current studies, with populations from the open forest populations having significantly lower germination energy than the adjoining wet forest populations. Rapid and uniform germination would be

of adaptive value on wet forest sites, where seedlings must become established before the seedbed is covered by bryophytes and other vegetation. In open forests such as the Ridge and Plain, the seedbed remains receptive for longer, but is more susceptible to adverse conditions such as the intermittent drying out of the surface soil (Ridge) or very cold temperatures (Plain). On such sites, a spread of germination over time may be important to maximise the opportunities for successful germination and establishment.

Differences between populations for seed/cotyledon size may also be a function of differential adaptation to site. Seed size appears to be under both genetic and maternal/environmental influences (Wulff 1986, Gutterman 1992, Probert 1992) and within the eucalypts, large seeds produce large cotyledons which have a faster absolute growth rate than small seeds (Grose and Zimmer 1958, Boland and Dunn 1981). Large seeds also have a higher germination energy than small seeds (Grose and Zimmer 1958, Aktar 1973). This growth advantage has been recorded to last for up to 10 to 16 weeks under glasshouse conditions (Grose and Zimmer 1958, Green 1971). However, a more pronounced effect may be expected under conditions of competition in the field (Gross 1984). The production of large cotyledons by the Mid population could therefore be regarded as an additional mechanism for ensuring early germination and rapid establishment within an environment characterised by intense inter- and intra-specific competition.

Overall, the results from the current studies indicate that several mechanisms, including germination energy, cotyledon size and seedling vigour, may be associated with adaptation for establishment and early growth on adjoining sites. Once established, factors other than inherent vigour may determine the relative adaptation of individual seedlings to specific sites. For example, the rapid development of lignotubers is regarded as an adaptation that allows seedlings to tolerate harsh environmental conditions such as drought, defoliation by animals or fire (Jacobs 1955) and previous studies have indicated that populations from harsh sites are more likely to develop lignotubers than those from mild sites (Ladiges and Ashton 1974, Ladiges 1974a, Clucas and Ladiges 1979, Potts 1985). The current studies indicate that differential selection for lignotuber development may occur over very short distances between populations. The significantly higher development of lignotubers by the Ridge population may be an adaptive response to the higher fire frequency of the dry forest environment, or to the higher risk of drought during the early stages of seedling development. Competition for moisture would have been minimised in the current trials because of an absence of overstorey trees from the planting sites. More pronounced competition would be

expected under the natural system of regeneration, in which the retained overstorey trees partially suppress the growth of seedling regeneration and exacerbate competition for soil moisture (Battaglia and Wilson 1990). Greater lignotuber development by the Ridge population may also indicate an adaptive response to browsing by native mammals, since this population was subjected to significantly higher browsing pressure than the adjoining Gully population (chapter 6). Differences in lignotuber development between the Mid and Plain populations were not evident. However, the persistence of coppice shoots was higher for the Plain than for the Mid population. This form of vegetative recovery may be an important attribute within the Plain environment where there is a high risk of damage to the crown as a result of frequent fires and severe frosts.

Leaf disease may be a primary cause of poor health and growth loss in eucalypts (Marks *et al.* 1982) and is also regarded as a secondary symptom of other stress, such as that associated with plantings of species or provenances on unsuitable sites (Wardlaw 1990). Population variation in disease susceptibility has been reported in eucalypts at the provenance level (Harris *et al.* 1985, Purnell and Lundquist 1986) and the current study has found that differentiation can occur at the site level, with significant differences between progeny from adjoining populations. Conditions conducive to high infection levels are most likely to occur within the humid environment of dense stands on wet sites, where the lower susceptibility of the wet forest populations would be important for the maintenance of vigorous growth.

Differences in susceptibility to browsing by native mammals could result from direct selection, or may indicate parallel or unrelated selection for differences in leaf characteristics (Gill 1992, Haukioja *et al.* 1991). Browsing pressure is highly variable from one forest area to another (Wilkinson and Neilsen 1995). The current study provided evidence of substantial variability in browsing pressure over the distance of 500m that separated the planting trials at the Forestier Gully and Ridge sites, with the heaviest browsing pressure at the Gully site. Severe browsing can result in very high mortality and growth losses, and these effects are exacerbated by vigorous competition from understorey vegetation on wet forest sites (Wilkinson and Neilsen 1995). Accordingly, the reduced susceptibility of the Gully population to browsing may indicate direct selection for this attribute. In contrast, the Ridge population may have an alternative strategy for tolerating browsing pressure, such as a higher capacity for recovery from lignotuberous shoots.

The majority of genetic variation within eucalypt species has been reported to occur within, rather than between populations (Moran 1992). Previous studies of population variation within eucalypt species have generally focussed on populations at the 'provenance' level. Provenances are often broad areas, with geographical separation over the scale of tens or hundreds of kilometres (e.g. previous studies within *E. obliqua* by Green (1971) and Brown *et al.* (1976)). Clinal variation may occur over much shorter distances of 0.8 to 1.6 km (Barber and Jackson 1957). Ecotypic variation has also been reported to occur on highly contrasting sites that are separated by distances of about 30 km (Anderson and Ladiges 1978, 1982, Anderson 1982a, 1982b). The current study indicates that population differentiation may occur at the local site level, involving populations that may adjoin each other over a geographic scale of only a few hundred metres. This form of population variation may partly account for the high intra-provenance variation often reported in genecological studies within eucalypts. Differences between provenances have been reported to be much greater if the provenance collection comprises a large number of relatively small geographical units (c. 10 km apart) rather than a smaller number that cover a broader geographical range (Tibbits and Reid 1987b).

Variation between populations at the site level has important implications for the maintenance of genetic diversity within native forests. In forests being managed for wood production, the genetic diversity of artificially regenerated stands may be substantially lower than that of virgin stands, probably as a result of the restricted number of maternal trees that contribute to the sowing mix (Gömöry 1992). The maintenance of high levels of diversity is fundamental to the maintenance of an adaptive response to environmental change. This may be particularly important for species that occupy a broad ecological range. Native forest sites often comprise a mosaic of heterogeneous sites that are associated with distinctive patterns of niche differentiation at the species level. Clearfelling followed by artificial sowing with seed from a mixture of species generally leads to vigorous natural selection and the maintenance of the original species patterns (Elliott *et al.* 1991). The omission of specific species from the sowing mix can lead to a loss of species diversity and occurrence of poorly adapted species on specific sites (Elliott *et al.* 1991).

The differentiation of populations in the current study indicates that similar selection forces may also operate at the intra-specific level. The potential narrowing of the genetic diversity within seed mixtures used for regeneration purposes could therefore result in long term effects on adaptation and growth. For example, the use of seed from the Plain population

could result in substantially lower productivity on highly productive sites compared to seed from the Mid population. In contrast, the Plain population may have a higher probability of long term survival and growth on harsh sites.

Variation at the site level therefore has important implications for the management of seed collection and sowing programmes in native forest regeneration areas. Forest management units are mapped by photographic interpretation (PI) to a scale of about 3ha (G. Dowl, Forestry Tasmania, pers. comm.). PI units are mapped on the basis of forest type characteristics, such as mean tree height, tree density and age structure. Harvesting coupes are generally about 30 to 70ha in size, and can therefore comprise a mosaic of many different patches. Ecological gradients, such as the topographic sequences used in the current study, commonly occur within coupes, particularly within steep country where there can be pronounced differences between the ridge top forest and the gully forest. In such situations the collection of seed from the lower gully position is often very difficult for two reasons. Firstly, the gully sites are generally occupied by wet forests, which tend to produce lower and less frequent seed crops than the large open-crowned trees of the dry forests on the ridges (Edwards 1995). Secondly, the roading system is generally constructed along ridge tops and physical access to the seed crops in the gully forest is often very difficult. In the past, phenotypic variation within a coupe has been largely attributed to environmental factors, and although seed collection guidelines sought to collect from as many trees as possible across the coupe (Forestry Commission 1991), there was no specific provision for the recognition of variation at the site level.

The maintenance of genetic diversity across heterogeneous sites in wood production forests could be provided in a number of ways. Firstly, natural regeneration systems can be used by the implementation of partial logging systems in appropriate forest types. Such systems are currently applied to the majority of dry forest types in Tasmania, and their extension into suitable wet forest types is continuing to be reviewed (Wilkinson 1992). Natural regeneration systems have the advantage of utilising *in situ* seed sources at the local site level. However, consideration needs to be given to the level of retention and degree of relatedness of potential seed sources as low retention levels can lead to a loss of genetic diversity (Gömöry 1992) and the creation of patches of related individuals that are more extensive than those found within natural forests (Richmond 1971).

In forest types not currently suitable for natural regeneration systems, the maintenance of genetic diversity could be achieved by the delineation and treatment of heterogeneous patches, or by sowing diverse seed mixtures across all sites. The first option would be appropriate where it is possible to clearly delineate major changes in forest type. Collections of seed could be undertaken from within these patches and then applied to the specific sites using ground sowing techniques (Forestry Commission 1991) or by using the new technology in aerial application which allows areas as small as 0.25ha to be individually sown (B.S. Hodgson, Forestry Tasmania, pers. comm.). The second option across highly heterogeneous sites would be to ensure that seed is collected from across the full range of sites for mixing and application across all sites. This system relies upon the processes of natural selection to maintain patterns of genetic diversity.

The delineation of specific sites for special seed collection programmes will be determined by the likelihood of genetic variation, as indicated by the phenotypic variability of the stands and the environmental heterogeneity of the sites. The current results provide evidence of significant differentiation between adjoining populations for a range of attributes that may be associated with long term adaptation and growth. Collection of seed across the range of sites within heterogeneous management units may therefore be important for the long term maintenance of genetic diversity within native forests.

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APPENDIX 1 LIST OF SPECIES PRESENT WITHIN TASFORHAB PLOTS AT MATERNAL TREES WITHIN EACH POPULATION

Forestier Gully

Maternal tree no. 1

Acacia melanoxylon
Acacia verticillata
Beyeria viscosa
Bryophyte spp
Clematis aristata
Coprosma quadrifida
Cyathodes glauca
Eucalyptus globulus
Eucalyptus obliqua
Geranium potentilloides
Juncus spp
Olearia argophylla
Olearia ramulosa
Olearia viscosa
Pimelea drupacea
Pittosporum bicolor
Polystichum proliferum
Pomaderris apetala
Viola hederacea
Zieria arborescens

Maternal tree no. 2

Acacia melanoxylon
Bryophyte spp
Clematis aristata
Coprosma quadrifida
Cyathodes glauca
Dicksonia antarctica
Eucalyptus globulus
Eucalyptus obliqua
Gahnia grandis
Geranium potentilloides
Goodenia ovata
Olearia ramulosa
Pittosporum bicolor
Polystichum proliferum
Pomaderris apetala
Viola hederacea
Zieria arborescens

Maternal tree no. 3

Acacia melanoxylon
Beyeria viscosa
Blechnum nudum
Blechnum wattsii
Bryophyte spp
Clematis aristata
Coprosma quadrifida
Dicksonia antarctica
Eucalyptus obliqua
Geranium potentilloides
Olearia argophylla
Polystichum proliferum
Pomaderris apetala
Pteridium esculentum
Senecio spp
Tasmannia lanceolata
Viola hederacea

Maternal tree no. 12

Acacia verticillata
Beyeria viscosa
Bryophyte spp
Chiloglottis gunnii
Cyathodes glauca
Eucalyptus obliqua
Hibbertia empetrifolia
Lepidosperma elatius
Pomaderris apetala
Pteridium esculentum
Viola hederacea
Zieria arborescens

Maternal tree no. 13

Acacia verticillata
Bedfordia salicina
Beyeria viscosa
Chiloglottis gunnii
Ctenopteris heterophylla
Cyathodes glauca
Eucalyptus obliqua
Hymenophyllum cuppressiforme
Lepidosperma elatius
Pomaderris apetala
Pterostylis elatius
Viola hederacea

Maternal tree no. 14

Acacia verticillata
Atherosperma moschatum
Bedfordia salicina
Beyeria viscosa
Bryophyte spp
Ctenopteris heterophylla
Cyathodes glauca
Drymophila cyanocarpa
Eucalyptus obliqua
Eucalyptus regnans
Exocarpos cupressiformis
Histiopteris incisa
Hymenophyllum cuppressiforme
Lepidosperma elatius
Monotoca glauca
Pomaderris apetala
Tasmannia lanceolata
Viola hederacea

Maternal tree no. 15

Acacia verticillata
Atherosperma moschatum
Beyeria viscosa
Bryophyte spp
Chiloglottis gunnii
Coprosma quadrifida
Ctenopteris heterophylla
Cyathodes glauca
Dianella tasmanica
Dicksonia antarctica
Drymophila cyanocarpa
Eucalyptus obliqua
Eucalyptus regnans
Hymenophyllum cuppressiforme
Monotoca glauca
Notelaea ligustrina
Olearia argophylla
Polystichum proliferum
Pomaderris apetala
Tasmannia lanceolata
Viola hederacea
Zieria arborescens

Maternal tree no. 16

Acacia verticillata
Beyeria viscosa
Bryophyte spp
Calendula spp
Chiloglottis gunnii
Correa reflexa
Cyathodes glauca
Cyathodes juniperina
Eucalyptus obliqua
Gonocarpus humilis
Lepidosperma elatius
Monotoca glauca
Notelaea ligustrina
Pimelea drupacea
Pomaderris apetala
Pultenaea juniperina
Viola hederacea
Zieria arborescens

Maternal tree no. 17

Acacia verticillata
Bedfordia salicina
Beyeria viscosa
Bryophyte spp
Cyathodes glauca
Eucalyptus obliqua
Exocarpos cupressiformis
Gahnia grandis
Grammitis billardierei
Hymenophyllum cuppressiforme
Lepidosperma elatius
Monotoca glauca
Pomaderris apetala
Pteridium esculentum
Pterostylis elatius
Viola hederacea
Zieria arborescens

Maternal tree no. 18

Beyeria viscosa
Bryophyte spp
Cyathodes glauca
Drymophila cyanocarpa
Eucalyptus obliqua
Gahnia grandis
Lepidosperma elatius
Monotoca glauca
Pomaderris apetala
Prostanthera lasianthos
Viola hederacea
Zieria arborescens

Maternal tree no. 22

Acacia verticillata
Bedfordia salicina
Beyeria viscosa
Calendula spp
Chiloglottis gunnii
Correa reflexa
Cyathodes glauca
Dianella tasmanica
Drymophila cyanocarpa
Eucalyptus obliqua
Exocarpos cupressiformis
Lepidosperma elatius
Monotoca glauca
Notelaea ligustrina
Pomaderris apetala
Tasmannia lanceolata
Viola hederacea
Zieria arborescens

Maternal tree no. 23

Acacia verticillata
Atherosperma moschatum
Beyeria viscosa
Blechnum wattsii
Bryophyte spp
Calendula spp
Chiloglottis gunnii
Ctenopteris heterophylla
Cyathodes glauca
Eucalyptus obliqua
Eucalyptus regnans
Hymenophyllum cuppressiforme
Lepidosperma elatius
Leptospermum scoparium
Monotoca glauca
Olearia lirata
Pomaderris apetala
Prostanthera lasianthos
Tasmannia lanceolata
Viola hederacea

Forestier Ridge

Maternal tree no. 4

Acacia melanoxylon
Acacia verticillata
Acaena novae-zelandiae
Bedfordia salicina
Bryophyte spp
Calendula spp
Chiloglottis gunnii
Comesperma volubile
Coprosma quadrifida
Cyathodes juniperina
Dianella tasmanica
Eucalyptus globulus
Eucalyptus obliqua
Exocarpos cupressiformis
Geranium potentilloides
Gonocarpus humilis
Goodenia ovata
Lomandra elatius
Lomatia tinctoria
Olearia lirata
Olearia viscosa
Pimelea drupacea
Pimelea nivea
Poaceae spp
Pteridium esculentum
Pultenaea daphnoides
Viola hederacea

Maternal tree no. 5

Acacia mucronata
Acacia verticillata
Bryophyte spp
Calendula spp
Comesperma volubile
Eucalyptus delegatensis
Eucalyptus globulus
Eucalyptus obliqua
Exocarpos cupressiformis
Gonocarpus humilis
Goodenia ovata
Lomandra elatius
Lomatia tinctoria
Olearia archeri
Pimelea nivea
Poaceae spp
Pomaderris apetala
Pteridium esculentum
Pultenaea daphnoides
Pultenaea juniperina
Senecio spp
Viola hederacea

Maternal tree no. 6

Acacia genistifolia
Acacia mucronata
Acaena novae-zelandiae
Banksia marginata
Bryophyte spp
Chiloglottis gunnii
Coprosma hirtella
Correa reflexa
Cyathodes juniperina
Davesia ulicifolia
Dianella tasmanica
Eucalyptus obliqua
Eucalyptus pulchella
Gonocarpus humilis
Lomandra elatius
Lomatia tinctoria
Pimelea nivea
Poaceae spp
Poranthera microphylla
Pultenaea juniperina
Veronica formosa
Viola hederacea

Maternal tree no. 7

Acacia melanoxylon
Acaena novae-zelandiae
Banksia marginata
Bryophyte spp
Chiloglottis gunnii
Coprosma quadrifida
Correa reflexa
Cyathodes juniperina
Dianella tasmanica
Epacris impressa
Eucalyptus obliqua
Eucalyptus viminalis
Exocarpos cupressiformis
Gonocarpus humilis
Goodenia ovata
Lomatia tinctoria
Olearia archeri
Olearia lirata
Pimelea nivea
Poaceae spp
Poranthera microphylla
Pteridium esculentum
Viola hederacea

Maternal tree no. 8

Acacia melanoxylon
Banksia marginata
Comesperma volubile
Coprosma quadrifida
Cyathodes juniperina
Drymophila cyanocarpa
Eucalyptus delegatensis
Eucalyptus obliqua
Eucalyptus pulchella
Exocarpos cupressiformis
Gonocarpus humilis
Goodenia ovata
Lepidosperma elatius
Lomatia tinctoria
Olearia lirata
Olearia viscosa
Pimelea nivea
Poaceae spp
Pteridium esculentum
Pultenaea daphnoides
Pultenaea juniperina
Viola hederacea

Maternal tree no. 9

Acacia genistifolia
Acacia melanoxylon
Bedfordia salicina
Bryophyte spp
Coprosma quadrifida
Cyathodes glauca
Cyathodes juniperina
Drymophila cyanocarpa
Eucalyptus delegatensis
Eucalyptus obliqua
Eucalyptus viminalis
Exocarpos cupressiformis
Geranium potentilloides
Gonocarpus humilis
Goodenia ovata
Lomandra elatius
Lomatia tinctoria
Olearia viscosa
Pimelea drupacea
Pimelea nivea
Poaceae spp
Pteridium esculentum
Pultenaea daphnoides
Pultenaea juniperina
Viola hederacea
Zieria arborescens

Maternal tree no. 10

Acacia verticillata
Bedfordia salicina
Bryophyte spp
Calendula spp
Chiloglottis gunnii
Coprosma quadrifida
Dianella tasmanica
Drymophila cyanocarpa
Eucalyptus obliqua
Exocarpos cupressiformis
Geranium potentilloides
Gonocarpus humilis
Goodenia ovata
Lepidosperma elatius
Lomatia tinctoria
Olearia lirata
Pimelea drupacea
Pimelea nivea
Poaceae spp
Pomaderris apetala
Pteridium esculentum
Pultenaea juniperina
Viola hederacea

Maternal tree no. 11

Acacia genistifolia
Bedfordia salicina
Calendula spp
Chiloglottis gunnii
Coprosma hirtella
Coprosma quadrifida
Correa reflexa
Cyathodes juniperina
Davesia ulicifolia
Eucalyptus amygdalina
Eucalyptus globulus
Eucalyptus obliqua
Eucalyptus pulchella
Eucalyptus viminalis
Exocarpos cupressiformis
Gonocarpus humilis
Goodenia ovata
Lepidosperma elatius
Lomatia tinctoria
Olearia lirata
Pimelea drupacea
Pimelea nivea
Poaceae spp
Pultenaea juniperina
Viola hederacea

Maternal tree no. 19

Acacia genistifolia
Banksia marginata
Bryophyte spp
Calendula spp
Comesperma volubile
Coprosma hirtella
Correa reflexa
Cyathodes juniperina
Eucalyptus amygdalina
Eucalyptus obliqua
Eucalyptus pulchella
Eucalyptus viminalis
Exocarpos cupressiformis
Gonocarpus humilis
Goodenia ovata
Leptospermum scoparium
Lomandra elatius
Lomatia tinctoria
Pimelea nivea
Poaceae spp
Pteridium esculentum
Pultenaea daphnoides
Pultenaea juniperina

Maternal tree no. 20

Banksia marginata
Bryophyte spp
Coprosma hirtella
Correa reflexa
Cyathodes juniperina
Epacris impressa
Eucalyptus obliqua
Eucalyptus pulchella
Eucalyptus viminalis
Gonocarpus humilis
Goodenia ovata
Leptospermum scoparium
Lomandra elatius
Lomatia tinctoria
Pimelea nivea
Poaceae spp
Pultenaea juniperina
Veronica formosa
Viola hederacea

Maternal tree no. 21

Acacia melanoxylon
Bedfordia salicina
Chiloglottis gunnii
Coprosma hirtella
Coprosma quadrifida
Correa reflexa
Cyathodes glauca
Cyathodes juniperina
Davesia ulicifolia
Eucalyptus amygdalina
Eucalyptus obliqua
Eucalyptus pulchella
Eucalyptus viminalis
Exocarpos cupressiformis
Gonocarpus humilis
Goodenia ovata
Lomandra elatius
Lomatia tinctoria
Notelaea ligustrina
Olearia archeri
Pimelea nivea
Poaceae spp
Pultenaea juniperina
Veronica formosa
Viola hederacea

Maternal tree no. 24

Acacia verticillata
Acaena novae-zelandiae
Banksia marginata
Bedfordia salicina
Bryophyte spp
Coprosma quadrifida
Davesia ulicifolia
Eucalyptus amygdalina
Eucalyptus obliqua
Eucalyptus viminalis
Exocarpos cupressiformis
Gonocarpus humilis
Goodenia ovata
Lomandra elatius
Lomatia tinctoria
Notelaea ligustrina
Olearia lirata
Olearia viscosa
Pimelea drupacea
Pimelea nivea
Poaceae spp
Pomaderris apetala
Pteridium esculentum
Pultenaea juniperina
Veronica formosa

Lune Plain

Maternal tree no. 25

Acacia myrtifolia
Boronia pilosa
Dianella tasmanica
Diplarrena moraea
Eucalyptus obliqua
Exocarpos cupressiformis
Gahnia grandis
Gleichenia dicarpa
Gonocarpus humilis
Hakea epiglottis
Helichrysum scorpioides
Lepidosperma filiforme
Leptomeria drupacea
Leptospermum lanigerum
Leptospermum scoparium
Lindsaea linearis
Lomatia tinctoria
Melaleuca squarrosa
Poaceae spp
Selaginella uliginosa
Spengelia incarnata
Sphaerolobium minus

Maternal tree no. 26

Comesperma retusum
Eucalyptus obliqua
Gahnia grandis
Gleichenia dicarpa
Hakea epiglottis
Leptomeria drupacea
Leptospermum lanigerum
Leptospermum scoparium
Melaleuca squarrosa
Pimelea linifolia
Poaceae spp
Pteridium esculentum
Spengelia incarnata

Maternal tree no. 27

Acacia myrtifolia
Diplarrena moraea
Epacris lanuginosa
Eucalyptus obliqua
Gahnia grandis
Gleichenia dicarpa
Gymnoshoenus sphaerocephalus
Hakea epiglottis
Lepidosperma filiforme
Leptomeria drupacea
Leptospermum scoparium
Melaleuca squarrosa
Poaceae spp
Spengelia incarnata
Sphaerolobium minus

Maternal tree no. 28

Comesperma retusum
Epacris lanuginosa
Eucalyptus amygdalina
Eucalyptus obliqua
Eucalyptus ovata
Gahnia grandis
Gleichenia dicarpa
Gymnoshoenus sphaerocephalus
Hakea epiglottis
Lepidosperma filiforme
Leptomeria drupacea
Leptospermum lanigerum
Melaleuca squarrosa
Olearia ericoides
Poaceae spp
Selaginella uliginosa
Spengelia incarnata
Sphaerolobium minus
Thelymitra spp

Maternal tree no. 29

Boronia pilosa
Davesia ulicifolia
Diplarrena latifolia
Diplarrena moraea
Epacris impressa
Eucalyptus amygdalina
Eucalyptus obliqua
Exocarpos cupressiformis
Gahnia grandis
Gleichenia dicarpa
Gompholobium huegelii
Gonocarpus humilis
Lepidosperma filiforme
Leptomeria drupacea
Leptomeria drupacea
Leptospermum scoparium
Lindsaea linearis
Lycopodium deuterodensum
Poaceae spp
Pultenaea juniperina
Sphaerolobium minus
Thelymitra spp

Maternal tree no. 30

Blechnum wattsii
Eucalyptus amygdalina
Eucalyptus obliqua
Gahnia grandis
Gleichenia dicarpa
Gymnoshoenus sphaerocephalus
Leptomeria drupacea
Leptospermum lanigerum
Leptospermum scoparium
Melaleuca squarrosa
Poaceae spp
Pteridium esculentum
Pultenaea juniperina
Spengelia incarnata

Maternal tree no. 31

Eucalyptus obliqua
Gahnia grandis
Gleichenia dicarpa
Hakea epiglottis
Hibbertia procumbens
Leptomeria drupacea
Leptospermum lanigerum
Melaleuca squarrosa
Poaceae spp
Spengelia incarnata

Maternal tree no. 32

Acacia myrtifolia
Diplarrena moraea
Eucalyptus amygdalina
Eucalyptus obliqua
Gleichenia dicarpa
Gymnoshoenus sphaerocephalus
Hakea epiglottis
Lepidosperma filiforme
Leptomeria drupacea
Leptospermum scoparium
Melaleuca squarrosa
Patersonia fragilis
Poaceae spp
Pultenaea juniperina
Selaginella uliginosa
Spengelia incarnata
Sphaerolobium minus
Thelionema caespitosum
Thelymitra spp

Maternal tree no. 33

Acacia melanoxylon
Blechnum nudum
Boronia pilosa
Diplarrena latifolia
Diplarrena moraea
Eucalyptus amygdalina
Eucalyptus obliqua
Eucalyptus ovata
Gahnia grandis
Gleichenia dicarpa
Gymnoshoenus sphaerocephalus
Hakea epiglottis
Helichrysum scorpioides
Hibbertia empetrifolia
Lepidosperma filiforme
Leptospermum lanigerum
Leptospermum scoparium
Lindsaea linearis
Melaleuca squarrosa
Patersonia fragilis
Poaceae spp
Pultenaea juniperina
Sphaerolobium minus

Maternal tree no. 34

Bauera rubioides
Blechnum nudum
Blechnum wattsii
Comesperma retusum
Eucalyptus amygdalina
Eucalyptus obliqua
Eucalyptus ovata
Gahnia grandis
Gleichenia dicarpa
Helichrysum scorpioides
Lepidosperma elatius
Lepidosperma filiforme
Leptomeria drupacea
Leptospermum lanigerum
Melaleuca squarrosa
Olearia ericoides
Poaceae spp
Pteridium esculentum

Maternal tree no. 35

Banksia marginata
Bauera rubioides
Empodisma minus
Epacris impressa
Epacris lanuginosa
Eucalyptus amygdalina
Eucalyptus obliqua
Gleichenia dicarpa
Gymnoshoenus sphaerocephalus
Hibbertia procumbens
Leptomeria drupacea
Leptospermum scoparium
Lindsaea linearis
Melaleuca squarrosa
Poaceae spp
Selaginella uliginosa
Spengelia incarnata
Thelymitra spp

Maternal tree no. 36

Comesperma retusum
Diplarrena latifolia
Drosera auricula
Eucalyptus obliqua
Gahnia grandis
Gleichenia dicarpa
Gymnoshoenus sphaerocephalus
Leptospermum scoparium
Melaleuca squarrosa
Pimelea linifolia
Poaceae spp
Selaginella uliginosa
Spengelia incarnata
Sphaerolobium minus
Thelymitra spp

Lune Mid

Maternal tree no. 37

Acacia verticillata
Banksia marginata
Bryophyte spp
Daviesia ulicifolia
Dianella tasmanica
Drymophila cyanocarpa
Epacris impressa
Eucalyptus obliqua
Gonocarpus humilis
Goodenia ovata
Melaleuca squarrosa
Pomaderris ellipitica
Pteridium esculentum
Pultenaea juniperina
Zieria arborescens

Maternal tree no. 38

Acacia verticillata
Bedfordia salicina
Bryophyte spp
Coprosma quadrifida
Eucalyptus obliqua
Gahnia grandis
Geranium potentilloides
Gonocarpus humilis
Helichrysum dendroideum
Lepidosperma elatius
Olearia stellulata
Pteridium esculentum
Zieria arborescens

Maternal tree no. 39

Acacia verticillata
Banksia marginata
Bedfordia salicina
Bryophyte spp
Cuscuta tasmanica
Dianella tasmanica
Drosera auricula
Drymophila cyanocarpa
Epacris impressa
Eucalyptus obliqua
Gahnia grandis
Gonocarpus humilis
Goodenia ovata
Lepidosperma elatius
Leptospermum scoparium
Olearia stellulata
Poaceae spp
Pteridium esculentum
Pultenaea juniperina
Zieria arborescens

Maternal tree no. 40

Acacia verticillata
Bedfordia salicina
Bryophyte spp
Daviesia ulicifolia
Epacris impressa
Eucalyptus obliqua
Gompholobium huegelii
Gonocarpus humilis
Goodenia ovata
Hibbertia empetrifolia
Lepidosperma elatius
Leptospermum scoparium
Notelaea ligustrina
Olearia stellulata
Poaceae spp
Pteridium esculentum
Pultenaea juniperina
Zieria arborescens

Maternal tree no. 41

Acacia verticillata
Atherosperma moschatum
Banksia marginata
Bauera rubioides
Bedfordia salicina
Blechnum wattsii
Bryophyte spp
Davesia ulicifolia
Dicksonia antarctica
Drymophila cyanocarpa
Eucalyptus obliqua
Gahnia grandis
Gonocarpus humilis
Goodenia ovata
Lepidosperma elatius
Leptospermum scoparium
Melaleuca squarrosa
Olearia stellulata
Poaceae spp
Polystichum proliferum
Pteridium esculentum
Pultenaea juniperina
Rumohra adiantiformis
Zieria arborescens

Maternal tree no. 42

Acacia verticillata
Atherosperma moschatum
Bedfordia salicina
Blechnum wattsii
Bryophyte spp
Coprosma quadrifida
Dicksonia antarctica
Eucalyptus obliqua
Gahnia grandis
Geranium potentilloides
Gleichenia dicarpa
Gonocarpus humilis
Goodenia ovata
Helichrysum scorpioides
Histiopteris incisa
Lepidosperma elatius
Leptospermum scoparium
Lomatia tinctoria
Melaleuca squarrosa
Olearia stellulata
Poaceae spp
Pteridium esculentum
Rumohra adiantiformis
Zieria arborescens

Maternal tree no. 43

Acacia verticillata
Bedfordia salicina
Blechnum nudum
Blechnum wattsii
Bryophyte spp
Coprosma quadrifida
Eucalyptus obliqua
Gahnia grandis
Goodenia ovata
Lepidosperma elatius
Melaleuca squarrosa
Pteridium esculentum
Viola hederacea
Zieria arborescens

Maternal tree no. 44

Acacia verticillata
Acaena novae-zelandiae
Bedfordia salicina
Blechnum wattsii
Bryophyte spp
Coprosma quadrifida
Eucalyptus obliqua
Gahnia grandis
Geranium potentilloides
Gleichenia dicarpa
Goodenia ovata
Lepidosperma elatius
Melaleuca squarrosa
Olearia stellulata

Maternal tree no. 45

Acacia verticillata
Bauera rubioides
Bedfordia salicina
Blechnum wattsii
Eucalyptus obliqua
Gahnia grandis
Gonocarpus humilis
Goodenia ovata
Lepidosperma elatius
Leptospermum scoparium
Melaleuca squarrosa
Olearia stellulata
Polystichum proliferum
Pomaderris ellipitica
Pteridium esculentum
Rumohra adiantiformis

Maternal tree no. 46

Acacia verticillata
Bedfordia salicina
Bryophyte spp
Eucalyptus obliqua
Exocarpos cupressiformis
Gahnia grandis
Goodenia ovata
Lepidosperma elatius
Melaleuca squarrosa
Olearia stellulata
Pteridium esculentum
Pultenaea juniperina

Maternal tree no. 47

Acacia verticillata
Banksia marginata
Bedfordia salicina
Blechnum wattsii
Bryophyte spp
Coprosma quadrifida
Davesia ulicifolia
Dianella tasmanica
Dicksonia antarctica
Drosera auricula
Eucalyptus obliqua
Gonocarpus humilis
Lepidosperma elatius
Leptospermum scoparium
Olearia stellulata
Pteridium esculentum
Pultenaea juniperina
Zieria arborescens

Maternal tree no. 48

Acacia verticillata
Banksia marginata
Bauera rubioides
Cuscuta tasmanica
Davesia ulicifolia
Dianella tasmanica
Drosera auricula
Drymophila cyanocarpa
Epacris impressa
Eucalyptus obliqua
Gahnia grandis
Goodenia ovata
Helichrysum scorpioides
Lepidosperma elatius
Leptospermum scoparium
Lindsaea linearis
Melaleuca squarrosa
Notelaea ligustrina
Olearia stellulata
Poaceae spp
Pteridium esculentum