The water relations of seed pretreatments and their effects on the germination of radiata pine seeds

by

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Abbreviations and Symbols

ABA	abscisic acid
ANOVA	analysis of variance
ATP	adenosine triphosphate
CP	control-pollinated
FD	day first germinant recorded
GA	gibberellic acid
GC	germination capacity
ISTA	International Seed Testing Association
LSD	least significant difference
MGT	mean time to germination
m	molality
OP	open- or wind-pollinated
STBA	Southern Tree Breeding Association
TMC	target moisture content

Ψ	water potential
Ψ_e	measured embryo water potential
Ψ_s	measured seed water potential
Ψ_{sol}	measured solution water potential
π	osmotic potential
τ	matrix potential
P	turgor pressure

Species names

Scientific name	Common name
Abies balsamea (L.) Miller	balsam fir
Abies procera Rehd.	noble fir
Apium graveolens L. var. *dulce* (Miller) Pers.	celery
Beta vulgaris L.	sugar beet
Brassica napus L.	canola
Capsicum annuum L.	pepper
Cucumis melo L.	muskmelon
Echinochloa crus-galli (L.) Beauv.	barnyard grass
Emmenanthe penduliflora Benth.	whispering bells
Fraxinus excelsior L.	European ash
Glycine max (L.) Merrill	soybean, soya bean
Lactuca sativa L.	lettuce
Larix decidua Miller	European larch
Larix gmelinii (Rupr.) Kuzen	Dahurian larch
Larix kaempferi (Lamb.) Carr.	Japanese larch
Larix laricina (DuRoi) K. Koch	tamarack
Larix occidentalis Nutt.	western larch
Lesquerella fendleri (Gray) Wats.	Fendler's bladderpod
Lycopersicon esculentum Mill.	tomato
Malus domestica Borkhausen	apple
Oryza sativa L.	common rice
Phaseolus vulgaris L.	bean, common bean, French bean
Picea abies (L.) Karst.	Norway spruce
Picea glauca (Moench) Voss	white spruce
Picea mariana (Mill.) BSP.	black spruce

Picea rubens Sarg.	red spruce
Picea sitchensis (Bongard) Carriere	sitka spruce
Pinus attenuata Lemmon	knobcone pine
Pinus banksiana Lamb.	jack pine
Pinus brutia Ten.	east Mediterranean pine
Pinus clausa var. *clausa* D.B. Ward	Ocala sand pine
Pinus contorta Dougl. ex Loud	lodgepole pine
Pinus echinata Mill.	shortleaf pine
Pinus edulis Engelm.	pinyon pine
Pinus elliottii Engelm.	slash pine
Pinus halepensis Mill.	aleppo pine
Pinus jeffreyi Grev. & Balf.	jeffrey pine
Pinus koraiensis Sieb & Zucc.	Korean pine
Pinus lambertiana Dougl.	sugar pine
Pinus monticola Dougl.	western white pine
Pinus muricata D. Don	bishop pine
Pinus nigra Arn.	Austrian pine
Pinus nigra var. *maritima* (Ait.) Melville	Corsican pine
Pinus palustris Mill.	longleaf pine
Pinus peuce Grisebach	Macedonian pine
Pinus pinaster Ait.	maritime pine
Pinus ponderosa Laws.	ponderosa pine
Pinus radiata D. Don	radiata pine, Monterey pine
Pinus resinosa Ait.	red pine
Pinus strobus L.	eastern white pine
Pinus sylvestris L.	scots pine
Pinus taeda L.	loblolly pine
Pinus thunbergii Parl.	Japanese black pine

Pinus virginiana Mill.	Virginia pine
Pisum sativum L.	garden pea, English pea, green pea, snap pea
Pseudotsuga menziesii (Mirb.) Franco	douglas-fir
Taxus baccata L.	English yew
Taxus brevifolia Nutt.	pacific yew
Trifolium pratense L.	red clover
Triticum aestivum L.	wheat
Typha latifolia L.	cattail
Vicia faba L.	faba bean
Zea mays L.	maize

Table of Contents

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Summary

Seeds sown in *Pinus radiata* D. Don (radiata pine, Monterey pine) nurseries are generally the culmination of generations of breeding and selection. Seed germination characteristics have no generally been of high significance to the breeding programme and seedling emergence from valuable seeds is often low and variable. Seed pretreatments are sometimes used in an attempt to overcome variation in seed quality and to achieve best possible germination.

The present study investigated commonly used seed pretreatments and their effect on the germination of *Pinus radiata* seeds in laboratory, glasshouse and field conditions.

Current nursery practice is to soak seeds at or near room temperature prior to sowing. In this study it was found that soaking decreased the time to emergence in the laboratory and field but damage occurred to some seedlots when soaked. Dissolved oxygen in soak water was depleted rapidly during soaking and damage was prevented when soak water was aerated throughout soaking.

Stratification (moist chilling) was once widely used to pretreat radiata pine seeds in Australia, but use of the practice has declined recently. Twelve weeks of stratification at 5 ° C decreased the time to emergence in the laboratory by up to 12 days. This effect decreased with shorter duration of stratification. After 12 weeks of treatment seeds began to germinate at low temperature and were susceptible to damage and desiccation during sowing.

A third pretreatment not presently in commercial use examined the effect of stratifying while controlling seed water potential. A treatment was developed that enabled seeds to attain the benefits of stratification without risking damage. This was achieved by

limiting the moisture available during treatment, by controlling the relative humidity of the environment in which the seeds were chilled. The treatment was as effective as conventional stratification in overcoming barriers to rapid germination. The seeds were fully hydrated by 4 weeks of treatment, but extending the treatment to 12 weeks further improved germination. This suggests that processes occur in fully hydrated seeds held at low temperature which overcome barriers to germination.

In view of the interrelationship between metabolic activity and water uptake, aspects of water relations and gas exchange were investigated in more detail. An examination of seed water relations during imbibition at 20 ° C revealed that the embryo was apparently fully hydrated within 24 hours. Water potential of intact seeds rose to near -8 MPa within 24 hours. Final water potential achieved 6 days later before splitting and germination was also -8 MPa. After initial water uptake there appears to have been a readjustment of both water content and seed water potential over the next 4 days, as both declined following the rapid initial rise. The decline was also associated with increasing respiration, which suggests there may be active movement of water out of the seed after imbibition. After this period of readjustment potential increased rapidly up until embryo expansion and radicle emergence. During the period of readjustment of seed water potential there remained a potential difference of 7 - 8 MPa between the extracted embryos and the remainder of the seed, in spite of available free water.

Results are discussed in terms of the physiology of water uptake and seedling emergence and practical implications of commercial management of high value *P. radiata* seeds.

1. General Introduction

Demand for pulp and timber has led to the development of large areas of plantation forest across Australia. The North American species *Pinus radiata* (radiata pine) is grown in plantations to meet these demands. Plantations are established from nursery transplants usually produced from seed. Breeding programs for improved growth form, timber qualities and disease resistance have been developed. The seed is generally produced in open- or wind-pollinated (OP) seed orchards. However, control-pollinated (CP) seed, with known parentage, is increasingly being used.

There is growing interest in using vegetative propagation to produce nursery transplants, but at present and into the immediate future seed remains the most widespread method of propagating improved material.

Control-pollinated seed is expensive to produce due to the labour-intensive nature of the process. There are concerns that the genetic gain, in terms of wood production, achieved through breeding may be obtained at the expense of quality germination characteristics. In addition to CP seedlots, some open-pollinated seedlots have shown low and variable germination in field nurseries. Generally, laboratory germination tests have indicated that seedlots in current use in nurseries exhibit high germination capacity. However, as the species is a temperate conifer, there is the distinct possibility that dormancy will be exhibited by some seedlots.

The traditional approach to overcome dormancy and seedlot germination deficiencies in temperate conifer species is to apply a pretreatment such as stratification to the seed prior to sowing in the nursery. Pretreatments are generally applied to improve germination percent, rate and uniformity of a seedlot in nursery conditions. Stratification has been applied to radiata pine seeds in nurseries in Australia in the past.

However, due to difficulties in applying the treatment and concerns regarding effectiveness, the practice of presoaking has become more prevalent in preference to stratification. At present the most commonly used seed pretreatment is to soak seed for a short period (48 hours or less) prior to sowing.

Good nursery management results in 60 to 70 % of the seed sown producing usable trees in the nursery (Boomsma, pers. comm.). There exists, therefore, the potential to substantially improve production. This is particularly a concern with increasing seed cost. The application of pretreatments to seed may potentially improve production by improving seedlot quality which affects not only germination but also the ability of the seedlot to perform in less than ideal conditions.

There is currently a lack of scientific knowledge about how pretreatments influence the success of germination and early growth in the field nursery. The objectives of this study were to investigate pretreatment effects on the germination of radiata pine seeds and to examine seed physiology during pretreatment and germination, particularly in terms of water relations.

2. Literature Review

2.1 Introduction

Radiata pine

Pinus radiata D. Don (radiata pine, Monterey pine) is an evergreen tree belonging to the family Pinaceae of the Gymnosperms. The family consists of 9 genera and about 200 species (Curtis and Morris 1975). The species belongs to a group known as the California closed-cone pines, along with bishop pine (*Pinus muricata* D.Don) and knobcone pine (*Pinus attenuata* Lemmon) (Fielding 1961).

Radiata pine has a natural distribution of about 6700 hectares in coastal central California in the U.S.A. and about 200 hectares on Guadalupe and Cedros Islands off the coast of Mexico. As it is a rapidly growing species it is useful for shelter belts and as a source of softwood for construction and pulpwood. The timber is used for construction and pulpwood. The species has been introduced and is now grown extensively in Australia, New Zealand, Chile and South Africa. Breeding programs for improved growth and wood properties have been developed in these countries (Fielding 1961; Lee 1994).

Industry situation

Radiata pine plantations in Australia presently extend over more than 700 000 hectares. Nursery transplants for plantations are raised from seeds or vegetatively propagated from cuttings. The STBA supplies genetically improved radiata pine seeds to member companies as control-pollinated (CP) or open-pollinated (OP) seedlots produced in seed orchards throughout southern Australia. The seeds are used to produce seedlings which will either be transplanted in plantations, or used as stock plants for vegetative propagation. The use of CP seedlots is expected to become more prevalent, as

controlling pollination allows better capture of genetic improvement. Seeds from OP orchards often fail to reach the potential genetic gain available from the clones present in the orchard (Arnold 1990). Crossing of elite material to produce CP seeds has increased the cost of the seed to the grower. Consequently, although use of OP seeds is still widespread, industry practice is tending towards raising seedlings from control-pollinated seeds, and further multiplying the stock using vegetative cuttings. Since propagation from cuttings becomes increasingly difficult with increasing mother plant age, stock plants are generally replaced every three years. This allows current genetically improved stock to be used.

Demand for seeds to produce plantation stock is expected to remain high; seeds allow improved material generated by breeding programmes to be incorporated in plantations. However, due to the expense of the seeds, industry considers that it is essential that seedlots fulfil their potential for germination and growth.

The performance of a seedlot is determined by the interaction of environmental factors with seedlot quality. Seed quality is a complex issue, as it is determined and influenced by a range of factors including: genetic background and its effect on such things as dormancy; seed production and handling, including pretreatment application; and conditions during germination. This literature review discusses some of the factors that may influence radiata pine seedlot quality and the measures used to overcome poor quality.

2.2 Conifer seed development and structure

Seed structure

A mature conifer seed consists of tissue of three different genetic types. The seedcoat and remnants of the nucellus are derived from maternal tissue (Figure 2.1). The megagametophyte differs from the endosperm of angiosperms, although it performs the same nutritional function. The endosperm is triploid and arises when a sperm unites with the two polar nuclei of the embryo sac, whereas the megagametophyte is haploid and develops from the megaspore (the maternal gamete). The maternal contribution to the seed anatomy (2n seedcoat, 1n megagametophyte, 1n embryo) is thus more dominant compared with the male contribution (1n embryo). This explains the strong genetic control on germination parameters (Edwards and El-Kassaby 1996), particularly considering the role of the maternal tissues in dormancy (Barnett 1996). This is discussed in more detail in Chapter 2.3.

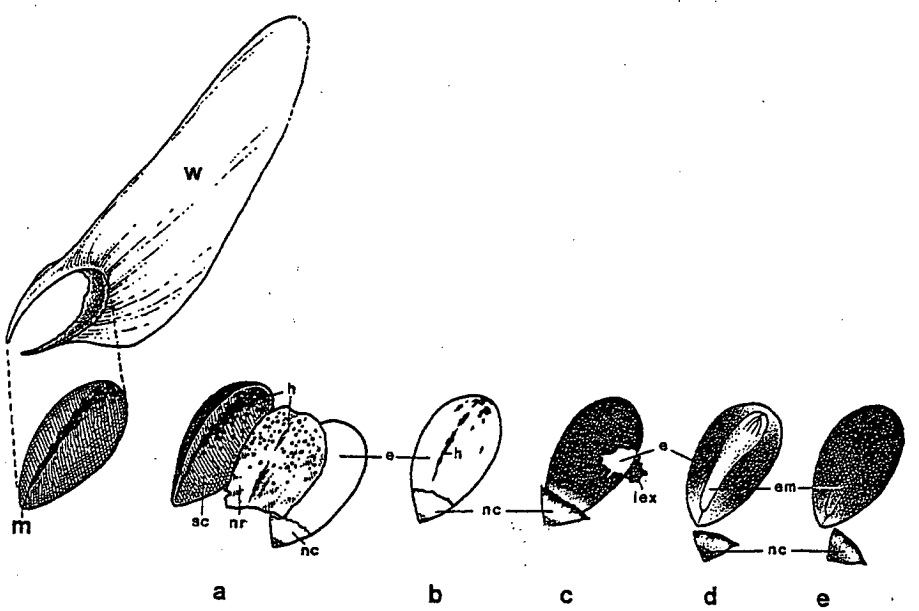

Figure 2.1 Structure of Scots pine (*Pinus sylvestris*) seed. *m* = micropyle, *w* = wing, *sc* = seedcoat, *nr* = nucellar remnants, *nc* = nucellar cap, *e* = megagametophyte, *h* = ridge, *iex* = inner exine, *em* = embryo (Tillman-Sutela and Kauppi 1995a).

The majority of the reserve foods of pine seeds are stored in the megagametophyte, although small quantities are present in the embryo. The reserves of the Pinaceae are primarily lipids stored in lipid bodies, and proteins stored in protein vacuoles (Stone and Gifford 1997). King and Gifford (1997) identified arginine as the most abundant amino acid in the megagametophyte of *Pinus taeda* L. (loblolly pine) seeds. The embryo, consisting of cotyledons, epicotyl anlage, hypocotyl, root meristem, root cap and suspensor, lies inside a cavity in the megagametophyte (Berlyn 1972). The embryo is thus dependent on the megagametophyte for food and water, as absorbed moisture passes through the megagametophyte to the embryo (Baron 1978). The seedcoat, nucellar layers and megagametophyte play a role in regulating water uptake by the embryo and hence germination rate. Tillman-Sutela and Kauppi (1995a, 1995b) reported that water uptake by Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst) embryos was regulated by the nucellar layers between the seedcoat and the megagametophyte.

Stone and Gifford (1997) identified three tissue layers in the megagametophyte of loblolly pine seeds. The central region, which comprised the majority, contained large cells separated by intercellular spaces. The outer region consisted of a single layer of epidermal-type cells. This differs substantially from the endosperm of cereal grains which consists of two tissue types, the aleurone layer consisting of living cells, and the starchy endosperm cells which are dead at maturity and contain starch and protein storage compounds (Dominguez and Cejudo 1999). The inner region of the megagametophyte of loblolly pine seeds was 1 - 3 cells thick, with a carbohydrate-containing layer which projected into the corrosion cavity.

Seed and cone development

Cone development

Radiata pine trees have a juvenile phase of about nine years before reproductive structures are produced (Fielding 1960). Species of the Pinaceae are monoecious. Following the onset of maturity, female cones develop in the place of lateral shoots; male cones replace short shoots. Most *Pinus* species display a three year reproductive cycle. Male and female cones are initiated in the first year when apical meristems irreversibly develop into strobili (cone) initials (Owens and Blake 1985). Sweet and Bollmann (1970) reported that radiata pine development from strobili initiation to cone maturity takes about 30 months in New Zealand. Development ceases over the winter period then resumes with the onset of spring (Owens and Molder 1984). Seasonal growth appears to coincide with vegetative growth rather than aspects of seed development (Sweet and Bollmann 1970). However, Cremer (1992) reported that most of the growth of male radiata pine cones in Canberra occurred between July and September at a time when vegetative growth was slow due to low temperatures. In the same study, female radiata pine cones were found to grow mainly between October and January, more than a year later.

Owens and Blake (1985) suggested that the relatively long reproductive cycle of conifers results in an increased susceptibility to a reduction in seedlot quality. Environmental conditions and seed orchard cultural practices may adversely affect the quality of seeds developing on the tree over this period.

Cones are considered ripe once they have become brown and desiccated. In the natural range, seed is shed in winter. However, it is not uncommon for cones to remain closed for months or even years (Krugman and Jenkinson 1974).

Pollination, fertilisation and early development

The female cone consists of spirally-arranged bract scales, each of which subtends an ovuliferous scale bearing two ovules. Each ovule consists of a megasporangium enclosed by the integument. The megasporangium gives rise to a megaspore which becomes the megagametophyte after pollination. The megasporangium wall becomes the nucellus, and the integument grows around the nucellus leaving a hole, the micropyle, at the apex (Sporne 1965). About four weeks after pollination, mitosis begins in the megagametophyte. Free nuclear division proceeds for about a year, by which time the megagametophyte contains from several hundred to 2000 free nuclei (Sporne 1965; Owens and Blake 1985; Raven et al. 1992). Archegonia are formed at the micropyle end of the megagametophyte. *Pinus* seeds generally contain three archegonia, however, radiata pine ovules contain only one (Berlyn 1972). This decreases the likelihood of seeds containing more than one embryo. However, seeds containing more than one embryo can develop after fertilisation, when the zygote divides to form four embryos. Generally, however, three of these embryos do not develop any further.

Maturation drying and the onset of germinability

Following a period of embryo growth and reserve synthesis, the seeds become desiccated which is generally considered to mark the onset of germinability. Before desiccation, immature radiata pine embryos were found to be incapable of germination and did not respond to stratification (Rimbawanto et al. 1989). Radiata pine embryos ceased growth by late April, but germination was poor at this stage. Over the following month seed moisture content fell to about 20% (FW) and germinability increased to 30%. One month later germinability had increased to 90%, and continued to improve over the next three months.

Kong et al. (1997) suggested that germinability of white spruce (*Picea glauca* (Moench) Voss) is acquired with desiccation, but germination itself is suppressed by ABA. McCarty (1995) reported that maternal ABA prevents germination of *Arabidopsis thaliana* seeds on the parent plant and allows the development of desiccation tolerance. As tissue sensitivity to ABA declines, seed germination becomes controlled by environmental factors such as water availability. It has been suggested that *Lycopersicon esculentum* Mill. (tomato) seeds contained within the fully hydrated environment of the fruit cannot germinate because the osmotic potential prevents uptake of sufficient water (Bewley 1995). However, it is difficult to distinguish between cause and effect at this stage due to the simultaneous timing of events including seed desiccation, abscission from the mother plant, increased ABA content, and the transition from the development to germination (Hilhorst and Toorop 1997).

2.3 Dormancy

The definition and classification of dormancy

Seed dormancy is characterised by the absence of germination in conditions that would usually be suitable for such a process. Vleeshouwers et al. (1995) defined seed dormancy as a *"seed characteristic, the degree of which defines what conditions should be met to make the seed germinate"*. Dormancy is an adaptive mechanism designed to ensure germination takes place under conditions suitable for subsequent seedling growth. Seeds with a chilling requirement to overcome dormancy will germinate in spring after exposure to cold, moist conditions during winter. This decreases the likelihood of germination during unseasonably warm periods in autumn or winter.

Northern hemisphere temperate zone conifer seeds typically exhibit some degree of dormancy, which is generally overcome by stratification (or chilling). There are mixed reports about the dormancy of radiata pine seeds and their response to stratification. Grose (1958) reported that radiata pine seeds were dormant. Stratification has been recommended as a presowing treatment for radiata pine seeds in the USA (Krugman and Jenkinson 1974) and Australia (Minko and Craig 1976).

The definition of dormancy is an area of much debate (Lang et al. 1985 and 1987; and Vleeshouwers et al. 1995). The fundamental difficulty is that the dormancy status of an individual seed is not known until it fails to germinate. Except in the case of impermeable seedcoats, a dormant seed is unable to germinate as the radicle is unable to expand and break through the surrounding tissues, even though apparently fully imbibed. The two conditions that could lead to this situation are resistance of the tissues surrounding the embryo to penetration, or inability of the embryo to develop sufficient expansive force. Tissue resistance contributes to the force required.

Recently, interest has developed in describing the dormancy behaviour of seed populations rather than individual seeds (Ni and Bradford 1993; Bradford 1995, 1996). Dormant seeds will not germinate until dormancy breaking treatment exceeds the threshold for the seed. There is evidence that the threshold variation within a population has a normal distribution (Bradford 1996). The greater the difference between the threshold and the level of treatment, the more rapidly the seed is able to germinate. More information about the level of dormancy in a population can therefore be gained from germination rates rather than germination percentages. Even when 100% of the seedlot is able to germinate following the application of dormancy breaking treatment, the rate of germination can usually be increased further by increasing the level of treatment (Gordon 1973; Bradford 1996).

Bradford (1996) suggested that because dormancy prevents radicle elongation and this relates to the water potential of the embryo, a "hydrotime" model of seed germination rates could be used to describe the dormancy and germination time course of a seedlot, although it does not indicate the mechanism involved in dormancy. Treating a seedlot to overcome dormancy shifts the distribution of water potential thresholds up and reduces the standard deviation. More seeds are able to germinate in water, and there is a greater difference between the base water potential threshold for individual seeds and that of the solution, thereby increasing the germination rate. This accounts for the increased germination percentage, improved rate and uniformity of germination, and the ability to germinate over a greater range of conditions commonly seen in a population following the application of a dormancy breaking treatment. The mechanisms involved in imposing dormancy are still not properly understood. It has been suggested that to further our knowledge, studies should be carried out either on dormant or non-dormant seeds, but it is difficult to establish the dormancy state of a seed until germination has been tested (Bradford 1996).

Although the mechanisms involved in imposing dormancy are not yet clearly understood, various systems for classifying seed dormancy have been developed (Nikolaeva 1977; Lang et al. 1985, 1987). Perhaps the simplest and most logical is the division of dormancy into physiological dormancy and coat-imposed dormancy. A dormant seed is said to exhibit physiological dormancy when the isolated embryo cannot germinate normally, and coat imposed dormancy when the isolated embryo germinates normally (Bewley and Black 1994). However, the type of dormancy can vary within a genus and both types of dormancy may be present in any one seed (Kelly et al. 1992).

Physiological dormancy

It has been suggested that the variety of conditions that are able to break dormancy indicate that there is probably more than one system involved (Dennis 1994). However, there can be little doubt of the role of the growth regulators, ABA in particular, in the regulation of dormancy of some species. The evidence supporting a role for ABA in dormancy was reviewed by Hilhorst and Karssen (1992).

Dormancy of *Malus domestica* Borkhausen (apple) and *Taxus baccata* L. (English yew) seeds was relieved when ABA was leached out of the embryo (Bewley and Black 1994). Hilhorst and Karssen (1992) noted that in *Triticum aestivum* L. (wheat) seeds that more dormant seeds were inhibited by concentrations of ABA that did not affect less dormant seeds, even though similar amounts of ABA were found in dormant and non-dormant seeds. This evidence suggests that it is either tissue sensitivity to ABA, the amount of ABA delivered to meristematic cells, or the ratio of growth regulators within the seed that determines the degree of dormancy rather than the concentration of ABA.

Much research into dormancy has been conducted using ABA-deficient *Arabidopsis thaliana* mutants. Seeds of ABA- and GA-deficient mutants are able to germinate normally, which suggests that the ratio of growth regulators, rather than absolute levels, may control germination (McCarty 1995). Studies of the progeny of these types have shown that only the ABA synthesised by the embryo is capable of inducing dormancy. ABA in the maternal seed tissues or applied to the seeds does not induce dormancy (Karssen et al. 1983). Hilhorst and Karssen (1992) suggested that ABA may regulate dormancy via gene expression. ABA-responsive genes are expressed more strongly in dormant seeds.

Noland and Murphy (1986) suggested that increased protein synthesis in the embryo and megagametophyte of imbibing dormant sugar pine seeds at 25 ° C might indicate the production of a germination inhibitor. Protein synthesis has been found to increase in the embryo and megagametophyte of loblolly pine (Schneider and Gifford 1994) and *Pinus lambertiana* Dougl. (sugar pine) (Barnett et al. 1974) seeds during stratification. However, the synthesis of specific proteins decreased as germinability increased. Schneider and Gifford (1994) suggested these proteins may be involved in inhibiting gemination. Alternatively, they may represent mRNAs or proteins associated with seed development and conserved in the seed at desiccation.

The growth regulators GA and ethylene also play a role in the transition from dormancy to germinability, although it is not clear whether or not they are associated with releasing the seed from dormancy or promoting germination (Karssen et al. 1989b; Hilhorst and Karssen 1992). Applying GA or ethylene to dormant seeds induces germination in some species, for example *Lesquerella fendleri* (Gray) Wats. (Fendler's bladderpod) (Evans et al. 1996; Kepczynski and Kepczynska 1997). GA concentration increased in Scots pine and *Picea sitchensis* (Bongard) Carriere (sitka spruce) seeds given a dormancy-breaking light treatment. It should be noted that

applying GA to seeds can have a lasting effect on seedling morphology as seen in *L. fendleri* seedlings where GA-treated seeds produced taller seedlings with fewer but longer leaves (Evans et al. 1996).

Although the mechanism of ethylene action is poorly understood it has been suggested that it may promote cell expansion in the hypocotyl of lettuce (*Lactuca sativa* L.) seeds by causing the accumulation of solutes to increase the osmotic potential, and increasing respiration (Kepczynski and Kepczynska 1997).

Coat-imposed dormancy

Seedcoats and other tissues surrounding the embryo may impose dormancy by acting as impermeable barriers or by physically restricting embryo expansion. In the extreme case, the seed is unable to imbibe until the integrity of these tissues is compromised in some way.

Seed coat impermeability

Impermeability to water or oxygen is believed to be the cause of dormancy in a range of angiosperm and gymnosperm species. Impermeability in some legume species is caused by shrinkage during desiccation, which results in the compression of the outermost cell layers. Hydrophobic substances present in these cell layers interfere with water uptake and gaseous exchange, and may also prevent leaching of inhibitors from the embryo (Kelly et al. 1992). Dormancy in *Emmenanthe penduliflora* Benth. (whispering bells) was found to be regulated by an impermeable waxy cuticular layer situated between the seedcoat and the endosperm (Egerton-Warburton 1998). This species germinates following fire. As little as five minutes exposure to smoke increased the number and size of permeating channels in the cuticle. This enhanced water uptake, and the seeds germinated.

Removing the pericarp and seedcoat stimulated the germination of an indica rice (*Oryza sativa* L.) variety, but inhibited the germination of a japonica rice variety. Inhibition was overcome by exposing the decoated seed to anaerobic conditions. This suggests that japonica rice germinates under anaerobic conditions as decoating the seed exposed the embryo to oxygen (Miyoshi and Sato 1997). Dormancy in *Leucospermum* seeds is also regulated by impermeability of the seedcoat to oxygen (Brits et al. 1995). It has been suggested that coat impermeability to oxygen may play a role in seed dormancy in some pine species including radiata pine (Rimbawanto et al. 1989). Higher respiration rates have been recorded in decoated radiata (Rimbawanto et al. 1989), loblolly (Barnett 1972), and eastern white (*Pinus strobus* L.) (Kozlowski and Gentile 1959) pine seeds compared with intact seeds. Kozlowski and Gentile (1959) also found that seeds had increased oxygen uptake in high oxygen environments and suggested that this evidence indicated that the seedcoat inhibits germination by restricting oxygen and water uptake. Barnett (1972) suggested that oxygen uptake is not a controlling factor in loblolly seed germination. Stratified or clipped seeds had similar uptake rates as intact and unstratified seeds. The seedcoat and probably also the dense megagametophyte present barriers to oxygen diffusion, but from the evidence it is difficult to say to what extent this controls pine seed germination.

Tillman-Sutela and Kauppi (1995a, 1995b) found that the seedcoats of Scots pine and Norway spruce were highly permeable to moisture, but papery tissue layers between the megagametophyte and the seedcoat represented a major barrier to water uptake. The micropylar end of the seed was particularly well protected by the nucellar cap. The papery layer has been found to be an important factor in restricting the water uptake in loblolly, sugar and western white (*Pinus monticola* Dougl.) pine seeds (Barnett 1976; Baron 1978; Hoff 1987).

Removing or clipping the seedcoat, which also disrupts the papery layer, improved the germination of loblolly (Barnett 1972, 1976; Carpita et al. 1983), longleaf (*Pinus palustris* Mill.), slash (*Pinus elliottii* Engelm.), shortleaf (*Pinus echinata* Mill.) (Barnett 1976), sugar (Baron 1978), eastern white (Kozlowski and Gentile 1959) and western white pine (Hoff 1987) and white spruce (Downie and Bewley 1996) seeds. This suggests that dormancy in seeds of some members of the Pinaceae is regulated by the seedcoat and other tissues surrounding the embryo. In some species, germination of decoated or clipped seed was further improved by a period of stratification (Downie and Bewley 1996). Decoating and stratifying white spruce seeds gave the same germination capacity as excising the embryo which suggests that the megagametophyte and nucellus undergo further weakening during stratification to eliminate the barrier to germination (Downie and Bewley 1996). This was supported by a reduction in the force needed to puncture the megagametophyte and nucellus and increased activity of endo-β-mannanase in the megagametophyte and nucellar tissue during stratification of dormant seed (Downie et al. 1997b).

Seedcoat constraints

The tissues surrounding the embryo can offer considerable resistance to the emergence of the radicle. Seeds will be dormant if the embryo cannot generate sufficient force to break through these tissues. The endosperm of lettuce seeds and the perisperm of muskmelon (*Cucumis melo* L.) seeds have been found to represent a barrier to radicle elongation (Welbaum et al. 1995). Two factors involved in this mechanism are tissue resistance and the turgor pressure of cells of the radicle. There is evidence that before dormancy-breaking treatment the embryos of some species cannot generate sufficient force to penetrate surrounding tissues, but following treatment either this force increases or resistance is reduced (Welbaum et al. 1995). It has been suggested that seedcoat constraints are likely to be the cause of dormancy in some pine seeds. Barnett (1972, 1976, 1996, 1997) and Schneider and Gifford (1994) suggested that water

uptake is limited by the inability of the megagametophyte and embryo to swell when contained within the seedcoat. Phase I of imbibition proceeded rapidly, but phase II was slow in seeds with intact seedcoats. Deeply dormant loblolly pine seeds showed much slower water uptake during this phase than shallowly dormant longleaf pine seeds. Longleaf pine seeds also showed a higher proportional increase in size (greater swelling) during imbibition (Barnett 1976). Kozlowski and Gentile (1959) attributed dormancy of eastern white pine seeds to seedcoat impermeability to oxygen, but also noted that punctured seeds had a higher moisture content in the plateau phase than their intact counterparts. Baron (1978) reported that phase II was eliminated altogether in sugar pine seeds with their coats removed, moisture content increased steadily until the embryos germinated. However, it is difficult to say if restricted swelling is a consequence of limited water uptake, or if water uptake is restricted by constraints to swelling.

Barnett (1972, 1976, 1996, 1997) found that the ratio of seedcoat weight to total seed weight gave an estimate of the relative dormancy of seeds of the southern pine species. Thicker and heavier seedcoats were more restrictive than thinner, lighter coats. Variation in seedcoat weight with latitude and longitude explained the dormancy distribution within the range of loblolly pine. Variation with longitude and elevation explained the distribution of dormancy within the ponderosa pine (*Pinus ponderosa* Laws.) population (Barnett 1997). Dormancy of loblolly pine seeds varies between parent trees but was found to be constant for individual trees over 4 years of seed production (McLemore and Barnett 1966a). Germination of Virginia pine (*Pinus virginiana* Mill.) seeds has also been found to be significantly influenced by maternal effects. Given that the seedcoats of pine seeds are of the same genotype as the parent plant, maternal effects on germination may therefore be a consequence of dormancy imposed by the seedcoat or other maternal tissues.

In contrast to the species mentioned above, Ocala sand pine (*Pinus clausa* var. *clausa* D. B. Ward) seed germination rate was not improved by removing the seedcoat prior to sowing in soil (Outcalt 1991).

2.4 Seed pretreatment to improve germination

Seed pretreatments are applied to seeds prior to sowing to improve germination and seedling growth. In some species improvement may be due to overcoming dormancy. A wide range of treatments can be applied including priming, presoaking, hardening, humidifying, chitting, aeration, irradiation, growth regulator application, stratification, thermal shock, scarification, and coating with pesticides, nutrients or microbes (Khan 1992). Presoaking, stratification, and priming are treatments commonly applied to conifer seeds prior to sowing. Presoaking and stratification treatments have previously been used to pretreat nursery sown radiata pine seeds (Krugman and Jenkinson 1974; Minko and Craig 1976). These treatments are discussed in more detail below.

Presoaking

Presoaking may be carried out as part of a treatment such as stratification (Tanaka and Edwards 1986; Mason et al. 1995), or as a seed pretreatment in itself (Rudolf 1950; Barnett 1971; Wilcox and Firth 1980; Kyauk et al. 1995). Although they reported no soak effects, Wilcox and Firth (1980) presoaked radiata pine seeds for 24 hours in cold water prior to sowing, in a nursery in an experiment comparing cone-ripening treatments. Rudolf (1950) found soaking at 5 ° C for 40 days to be as effective as stratification when applied to eastern white pine, white spruce, black spruce (*Picea mariana* (Mill.) BSP), balsam fir (*Abies balsamea* (L.) Miller) and tamarack (*Larix laricina* (DuRoi) K. Koch). Soaking for this period of time reduced the time taken to treat and germinate the seeds by 25 to 60 % compared with stratification. Barnett (1971) also found soaking loblolly pine seeds to be as effective as stratification in overcoming dormancy. When seeds were soaked at temperatures less than 25 ° C, soaked seedlots had a higher germination capacity than those given an equivalent period of stratification (7, 14, 21 or 28 days). Where soaks were carried out at temperatures above 25 ° C, soaked seeds had a lower germination capacity than

stratified seeds, and this was attributed to insufficient aeration of the water at this temperature.

Soak injury: limiting oxygen

Barnett (1971) attributed poor germination of loblolly pine seeds following soaking at 25 ° C to insufficient aeration. The supplied air was only able to maintain dissolved oxygen at about half the saturation level. The solubility of oxygen in water decreases as temperature rises (Cole 1979) and oxygen-consuming respiration increases with increasing temperature. Both factors contribute to the development of hypoxic conditions when seeds are soaked at elevated temperatures.

The link between reduced oxygen levels and poor subsequent germination suggests that the seeds of some species are susceptible to soak injury as a consequence of hypoxic effects on respiration. Few studies have investigated seed anaerobiosis. Root metabolism under oxygen deficiency was reviewed by Drew (1997). Like seeds, root apical zones are very active and have few intercellular spaces to conduct oxygen, however it is difficult to make direct comparison as roots are less tolerant of anoxia than seeds (Cobb et al. 1995).

Plant cells are thought to contain relatively low stores of ATP. In order to survive anoxia for any length of time, anaerobic metabolism must take place. Glycolysis is able to operate under aerobic and anaerobic conditions to produce pyruvate, but under conditions of limiting oxygen, further oxidation of pyruvate and NADH is unable to take place. Consequently these substances begin to accumulate. Under these conditions fermentation begins. NADH becomes abundant enough to act as a reductant, and pyruvate is reduced to either ethanol or lactate (Salisbury and Ross 1992; Bewley and Black 1994). Fermentation yields two molecules ATP per molecule of glucose and is thus less efficient than oxidative phosphorylation which yields about 36.

Seed injury under anoxic or hypoxic conditions has variously been attributed to reduced ATP production, the accumulation of toxic end-products, and lack of substrates for respiration (Drew 1997). Ethanol accumulation was initially believed to be responsible for injury (Crawford 1977). *Echinochloa crus-galli* (L.) Beauv. (barnyard grass) is a weed common to rice paddies that is able to germinate in the absence of oxygen. Rumpho and Kennedy (1981) found that 85% of the ethanol produced by seeds of this species diffused into the surrounding solution, but the seeds under anoxic conditions still contained 90 times more ethanol than an aerated control. It is not known if this is a damaging level of accumulation (Kennedy et al. 1992).

Attention has increasingly been focused on acidification of the cytoplasm as a cause of injury. In maize (*Zea mays* L.) roots, acidification occurs during fermentation due to the production of lactate. As the pH drops, ethanol is produced in the place of lactate and the pH steadies (Kennedy et al. 1992). Fan et al. (1997) suggested that nitrate metabolism in germinating rice seedlings provides a sink for potentially damaging protons and NADH produced during fermentation.

Seedlings given a hypoxic pretreatment by exposure to low-oxygen conditions before experiencing anoxic conditions, are often able to survive subsequent anoxia. Hypoxic pretreatment is thought to allow acclimation to low oxygen conditions. Protein synthesis is inhibited under anoxia but enzymes involved in glycolysis, ethanol fermentation, and lactate transport systems are produced (Kennedy et al. 1992; Cobb et al. 1995; Drew 1997; Ellis et al. 1999). ABA application can be substituted for hypoxic pretreatment in arabidopsis roots. Ellis et al. (1999) suggested that ABA induced synthesis of proteins associated with tolerance to anoxia.

Soak injury: loss of solutes

Soak or imbibition injury has also been observed in the seeds of some leguminous species with large cotyledons but it is thought to be caused by physical injury rather than anoxia. Soaking decreased the germination of peas (*Pisum sativum* L.) (Rowland and Gusta 1977; Powell and Matthews 1978) and faba beans (*Vicia faba* L.) (Rowland and Gusta 1977). Seedling growth rates were also reduced. Soaking also reduced respiration rates and reserve mobilisation in peas (Powell and Matthews 1978).

Solute flux from soaked seeds has been observed (Spaeth 1987). Cell contents are lost rapidly for the first few minutes of soaking before the flux becomes slow and constant (Simon and Raja Harun 1972). Matthews and Powell (1986) reported that pea seedlots with damaged seedcoats were more severely damaged by imbibition than those with uncompromised coats, as a result of more rapid imbibition. Seedlots with low initial moisture content were also more susceptible to solute loss (Sivritepe and Dourado 1995). Spaeth (1987) found that blisters formed on the cotyledons of bean (*Phaseolus vulgaris* L.) seeds when the outer cell layers expanded away from the underlying tissue. When the blisters burst, cell contents were forced out of the tissue.

It is uncertain whether or not the loss of solutes from imbibing seed is due to the progressive hydration of cell layers as imbibition proceeds or if it is due to loss of semi-permeability of cellular membranes. Simon and Mills (1983) suggested that loss of solutes could be explained by structural changes in cell membranes. They hypothesised that membranes form a hexagonal phase at low moisture content, and solute leakage occurs with the transition from the hexagonal phase to the normal semi-permeable bilayer organisation. A hexagonal phase has been described in low-moisture-content phospholipid extract from the human brain (Luzzati and Hudson 1962, cited in Simon and Mills 1983). Chabot and Leopold (1982) found irregularities and disorganisation in dry soybean (*Glycine max* (L.) Merrill) seed membranes.

However, McKersie and Senaratna (1983) found that phospholipid-water preparations from cattail (*Typha latifolia* L.) seeds were characteristic of a lamellar structure and NMR measurements could not detect a hexagonal phase. Priestly (1986) and Hoekstra et al. (1989) reviewed the evidence regarding membrane organisation in dry seeds and concluded that although considerable stress is placed on the membranes during desiccation, there is no compelling evidence to suggest that the bilayer organisation is lost, particularly as membranes are likely to be protected by stabilising carbohydrates. Hoekstra et al. (1989) suggested that loss of solutes may result from the presence of bulk water during the transition of the membrane phospholipids from the gel phase to a liquid crystalline state.

Shabala et al. (2000) studied K^+, Ca^{2+} and H^+ flux profiles around the surface of germinating wheat (*Triticum aestivum* L. cv. Machete) seeds and found that functionally different seed zones showed different flux profiles, which changed during imbibition. A large initial K^+ efflux was observed, which was followed by continued efflux at a reduced rate for up to 24 hours. The use of metabolic inhibitors suggested that the efflux was at least partly caused by plasma membrane transporters. This evidence suggests that ion efflux was not related to the loss of membrane integrity.

Priming

Priming involves incubating seeds in a solution of known osmotic potential at a specific temperature for a given period of time (Heydecker et al. 1973). The solution osmotic potential is usually between - 1.0 and - 1.5 MPa. Water potentials (Ψ) in this range are generally lower than the water potential at which the radicle begins to elongate. Holding seeds in solutions of this Ψ prolongs phase II of imbibition and prevents the embryo taking up sufficient water for radicle protrusion (Karssen et al. 1989a). When primed seeds are transferred to conditions suitable for germination, phase II of imbibition may be reduced.

Haridi (1985) reported that the germination rate of slash pine seeds was improved by priming at -1.5 MPa and 25 ° C for five days. Bourgeois and Malek (1991) reported that jack pine (*Pinus banksiana* Lamb.) seeds primed at -1.7 MPa for six days germinated two days earlier than unprimed seeds. Hallgren (1989) reported that loblolly and shortleaf pine germination rate was increased by priming. Huang (1989) reported that germination capacity of lodgepole pine (*Pinus contorta* Dougl. ex Loud.) and white spruce was reduced by priming but uniformity of germination was increased and the number of abnormal seedlings was reduced. In contrast, Hallgren (1989) found that the germination capacity of loblolly pine and some slash pine seeds was increased by priming.

Some species, including lettuce (Valdes and Bradford 1987), lodgepole pine, white spruce (Huang 1989), loblolly, shortleaf and slash pine (Hallgren 1989) germinated over a wider range of temperature following priming. Huang (1989) also found that primed lodgepole pine and white spruce seeds were less affected by deterioration than untreated seeds, and also that germination of deteriorated seed could be improved by priming.

There is some uncertainty about the mechanisms by which priming acts. Karssen et al. (1989a) reported that the endosperm of celery (*Apium graveolens* L. var. *dulce* (Miller) Pers.), tomato and lettuce seeds was hydrolysed at the micropylar end of the seed, opposite the radicle tip, during priming. This enabled the radicle to expand rapidly when imbibition began. It was found that celery seeds would not germinate until the embryo attained a critical length, which was achieved by cell division during priming (Karssen et al. 1989a). Nuclear replication and increased embryo length during priming have also been reported in pepper (*Capsicum annuum* L.) (Lanteri et al. 1996) and Scots pine (Sahlen and Wiklund 1995) seeds, respectively. Despite increased

embryo size in Scots pine seeds after priming, only immature embryos showed improved germination (Sahlen and Wiklund 1995). Huang and Zou (1989) suggested that priming may improve membrane integrity in *Pinus sylvestris* var. *mongolica* and *Larix gmelinii* (Rupr.) Kuzen (Dahurian larch) seeds. Primed seeds lost fewer cell contents to the imbibition solution than control seeds.

Stratification

Stratification has long been used to pretreat seeds of temperate conifer species to overcome dormancy. Barton (1928) reported that loblolly pine seed germination rate could be increased by stratification. The treatment aims to replicate the conditions the seeds experience during winter in their natural environment. Many of these species shed their seed in autumn or early winter (Krugman and Jenkinson 1974). Once shed, the seeds are able to take up water but cold winter temperatures and dormancy prevent germination. Over time dormancy is overcome, and as temperatures rise in spring the seeds germinate. The dormancy status of radiata pine seeds is not known, but Grose (1958) found that radiata pine seeds do not germinate readily and concluded that a degree of dormancy was present. Krugman and Jenkinson (1974) recommended stratifying radiata pine seeds for 35 to 45 days and Minko and Craig (1976) reported that stratification was the standard pre-sowing treatment for radiata pine seeds in north-eastern Victoria (Australia) at the time.

Stratification most commonly improves germination capacity and rate, and has been reported to improve germination under less than ideal conditions. Such conditions include dry soils (Outcalt 1991), the absence of light (Li et al. 1994) and temperatures outside the optimal range (Allen 1962b; Gosling 1988; Jones and Gosling 1994). Skordilis and Thanos (1995) reported that three provenances of East Mediterranean pine with varying degrees of dormancy responded differently to treatment. After treatment, one provenance had an increased germination rate, the second had a

broadened temperature range for germination, and the third provenance had an absolute requirement for stratification.

Stratification has also been reported to improve the diameter and height of loblolly pine seedlings at harvest, as a result of increased rate of germination (Barnett and McLemore 1984; Boyer et al. 1985). Gosling and Peace (1990) reported that the only seedlots that would not benefit from stratification were those whose germination capacity was reduced by the treatment. Stratification was found to increase germination rate and capacity, decrease the time to achieve seedlings of a given size, and widen the range of temperatures over which radiata pine seeds germinated (Grose 1958; Minko and Craig 1976). In contrast, Rimbawanto et al. (1988, 1989) found that stratification did not significantly affect the germination of radiata pine seeds, but it should be noted that these authors stratified for only one week.

The requirements for successful stratification are sufficient moisture, low temperature, and adequate ventilation (Bonner et al 1974). Stratification traditionally involved burying seed in layers of moist sand (Figure 2.2), hence the name. The seeds were exposed to winter temperatures. In more recent times this has been replaced by a method sometimes called naked stratification or moist chilling. Seeds are either soaked for a short period of time, drained and chilled in plastic bags or containers, or placed on moist filter papers and held at low temperature (Bonner et al. 1974; Jones and Gosling 1990; Aldhous and Mason 1994).

There is variation in the method applied to seeds of different species. Some seedlots may be damaged by stratification. Barnett and McGilvray (1971) reported that germination of 2 out of 16 shortleaf pine seedlots was decreased by stratification, regardless of the duration of treatment. Mason et al (1995) found that Macedonian pine (*Pinus peuce* Grisebach) seeds cannot achieve reasonable germination following

conventional stratification. The seeds germinated well following 14 weeks or more of alternating warm (20 ° C) and cold (5 ° C) stratification. This species has an unusually high proportion of immature embryos in shed seed. Alternating temperature may allow embryo development and dormancy-breakage to proceed simultaneously.

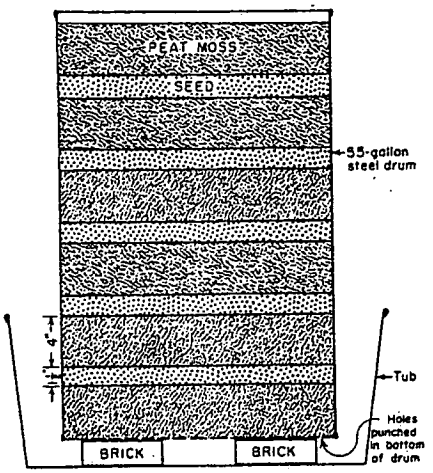

Figure 2.2 Desired arrangement for stratification in a large barrel (Bonner et al. 1974).

The treatment factors: duration of stratification; the amount of moisture available to the seed during treatment; and the post-stratification treatment vary substantially between studies.

Length of stratification

Generally, increasing the duration of stratification increases the effects on germination (Allen 1962b; Barnett and McGilvray 1971; Baron 1978; Jones and Gosling 1994; Mason et al. 1995). The duration of stratification required to achieve maximum germination can vary between seedlots of a species (Hoff 1987). The rate and capacity of germination of radiata pine (Grose 1958) and sugar pine (Stanley 1958; Baron 1978)seeds were correlated with the length of the stratification period. Fifty percent of sugar pine seeds germinated after a month of stratification, but nearly 90% germinated following 3 months of stratification (Baron 1978).

It appears that treatment has a greater effect on germination rate than germination capacity. For sitka spruce, Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and lodgepole pine seeds it was found that the shortest length of prechilling to achieve the fastest germination rate was 36 weeks, which is longer than the prechill required to achieve the maximum germination percent (Jones and Gosling 1994).

After a certain length of time, seeds begin to germinate at the stratification temperature (Grose 1958; Stanley 1958). Aldhous and Mason (1994) recommend stratifying for no longer than 10 weeks to prevent seed germination during treatment. Although no mechanism was reported, Edwards (1986) reported that extending the stratification duration is detrimental to the germination of slash pine seeds.

Moisture content during stratification

Despite the benefit obtained from longer stratification, restrictions are often placed on the duration of treatment to prevent seeds germinating during treatment. Germinated seeds can be damaged during sowing (Gosling and Rigg 1990) and the seeds are susceptible to desiccation once removed from treatment. Limiting the amount of water available to the embryo during stratification theoretically allows the seeds to benefit from the treatment without imbibing enough water to allow the radicle to elongate sufficiently to germinate.

A number of studies have examined the effect of stratification with limited water on species including eastern white pine (Downie and Bergsten 1991), sitka spruce (Gosling and Rigg 1990; Jones and Gosling 1994; Jinks et al. 1994), Douglas-fir, lodgepole pine (Jones and Gosling 1994), noble fir (*Abies procera* Rehd.) (Tanaka and Edwards 1986), Japanese larch (*Larix kaempferi* (Lamb.) Carr.) (Jinks et al 1994) and European ash (*Fraxinus excelsior* L.) (Van de Walle 1987). Some studies allowed

seeds to take up enough water to reach a given moisture content, sometimes called a target moisture content (TMC). TMCs were set at about 30 % fresh seed weight. TMCs 2 % below the maximum moisture content achieved by soaking seed for 48 hours were used by Jones and Gosling (1994) and Jinks et al (1994). Jinks et al (1994) recommended that TMC stratification not be used on Scots pine, Corsican pine (*Pinus nigra* var *maritima* (Ait.) Melville) and noble fir, but did not indicate the reason. In contrast, Tanaka and Edwards (1986) found nursery emergence of noble fir was improved by stratifying for 4 weeks, drying to 25 - 30 % moisture content and continuing the stratification for a further 8 weeks.

Target moisture contents are usually achieved either by restricting the amount of water available to the seed from the beginning of the chilling treatment (Gosling and Rigg 1990; Downie and Bergsten 1991; Jones and Gosling 1994), or partially stratifying the seed, drying to obtain a particular moisture content and continuing chilling (Tanaka and Edwards 1986; Van de Walle 1987). To inhibit germination of deeply dormant European ash seeds during stratification, Van de Walle (1987) interrupted stratification after 3 months, and continued stratifying at low water potential (-1.5 MPa) for another 4 months. Jinks et al. (1994) reported a technique called soak and spin dry which involved soaking the seed, then spin drying, and repeating until the seeds reach the desired moisture content.

Jones and Gosling (1994) and Downie and Bergsten (1991) used the following formula to calculate the amount of water to be added to fresh seeds to reach TMC:

$$x = [(a-ab)/(1-c)] - a$$

where x is the amount of water to be added (g), a is the initial weight of the seed (g), b is the initial seed moisture content as a percentage of fresh weight expressed as a decimal, and c is the desired moisture content as a decimal.

Post-stratification treatment

There has been much interest in drying seeds after treatment. If seeds can be dried back without losing the benefits of stratification they may be stored if conditions are unsuitable for sowing immediately after treatment. Allen (1962b) and Danielson and Tanaka (1978) reported that post-stratification drying of Douglas-fir seeds reduced the effect of stratification when seed moisture content dropped below about 40 %. However, the effects of stratification were not completely lost, even when dried back to 10 % moisture content and stored for a year at 0 - 2 ° C (Allen 1962b). Douglas-fir seeds stratified at high moisture content (70%) were less affected by post-stratification storage than those stratified at low moisture content (40%) (Allen 1962a).

In contrast, Danielson and Tanaka (1978) reported that ponderosa pine seeds could be held at 26 % moisture content for up to 9 months without any reduction of germination capacity. Jones and Gosling (1990) reported that sitka spruce seeds can be dried back to their original moisture content (about 7 %) following stratification without reducing germination capacity. However, drying to increasingly lower moisture contents progressively reduced the germination rate. Stratified Ocala sand pine seeds also retained their germination capacity when dried back to moisture contents less than 10%, but it was not reported whether or not the treatment affected the rate of germination (Outcalt 1991).

Seed physiology during stratification

Although a number of studies have examined imbibition and reserve mobilisation in conifer seeds during stratification, there is little understanding of the mechanisms by

which stratification acts to improve germination. In sugar pine and loblolly pine, seedcoat removal is as effective in improving germination capacity as stratification (Baron 1978; Carpita et al. 1983). It appears likely that during stratification the barrier to germination represented by the seedcoat is compromised in some way. Downie and Bewley (1996) and Downie et al (1997b) reported that the force required to puncture the megagametophyte and nucellus of white spruce seeds declined during stratification. The tissues may be weakened by mannanase, the activity of which increased during stratification (Downie et al. 1997b). However, when the seedcoat of western white pine seed was removed, only 50 % of the seeds germinated (Hoff 1987) and the germination rate of uncoated loblolly and western white pine seeds was further improved by stratification (Carpita et al 1983; Hoff 1987). This suggests that in at least some pine species the seedcoat barrier is not wholly responsible for slow germination.

Stanley (1958) related the interval between the completion of stratification and germination of sugar pine seeds in part to the moisture content of the embryo at the end of treatment. The time to germination was reduced when seeds reached a higher moisture content during stratification. Schneider and Gifford (1994) found that increased germinability of loblolly pine seeds also correlated well with increased water content during stratification. Baron (1978) reported that seeds of this species show much the same pattern of imbibition when held at 5 ° C as at 20 ° C, but the phases were lengthened under stratification. Stanley (1958) found that after 90 days of stratification the seeds had taken up sufficient water to germinate. Schneider and Gifford (1994) reported that improved germination of loblolly pine seeds following stratification also correlated well with the moisture content at the end of stratification. These results suggest that the level of seed imbibition achieved during stratification determines the time taken to germinate. Longer periods of stratification allow the seed to reach a higher moisture content. The seed is able to germinate more rapidly as there is a small difference between the seed moisture content and the threshold moisture

content it must reach for the radicle to expand. However, it is still unclear if releasing seeds from dormancy during stratification controls seed imbibition.

Baron (1978) suggested that the accumulation of sugars in the embryo during stratification might explain rapid seedling growth rates following stratification. There is evidence that reserves are mobilised during stratification. Murphy and Hammer (1993) summarised the metabolic activities in pine seeds during stratification. The systems induced by stratification include carbohydrate metabolism in the embryos and lipid metabolism in the megagametophyte. Stone and Gifford (1997) observed that the innermost layer of the megagametophyte of loblolly pine seeds became mucilaginous and malleable and the carbohydrate layer expanded to fill much of the corrosion cavity during stratification. Ching (1963a, 1966) found that during stratification carbohydrates accumulated in both the embryo and megagametophyte of Douglas-fir seeds. Lipid concentration in the megagametophyte was reduced and the activity of lipases increased during stratification (Ching 1968). Ching and Ching (1972) reported increased ATP in the embryo and megagametophyte of ponderosa pine seeds after stratification. In contrast, Schneider and Gifford (1994) reported that the total lipid content of the loblolly pine megagametophyte did not change during stratification, and Carpita et al. (1983) found that the effect of stratification on radicle elongation was not altered if seeds were stratified without the megagametophyte. The extent to which radicle elongation depends on reserves from the megagametophyte is therefore difficult to determine from this evidence.

Schneider and Gifford (1994) found the synthesis of a set of proteins in the megagametophyte and embryo of loblolly pine seeds decreased after 14 days of stratification. It was suggested these proteins may be associated with the imposition and maintenance of dormancy, as their decline coincided with the greatest increase in germinability. At about this time the synthesis of a different set of proteins increased in

the megagametophyte. The authors suggested these may be associated with removal of the constraint to germination. Synthesis of new proteins in the embryo only increased when stratification was completed (Groome et al. 1991).

2.5 Germination

Defining germination

Bewley and Black (1994) define germination in the following way: *"Germination begins with water uptake by the seed (imbibition) and ends with the start of elongation by the embryonic axis, usually the radicle"*. The quiescent seed has low moisture content and low metabolic activity, and may be stored in the soil for a long period of time. Germination occurs when the seed hydrates under conditions suitable for metabolism, such as a suitable temperature regime and the presence of oxygen, which depend to an extent on the species involved. According to the above definition germination may not include seedling growth, and processes such as storage reserve mobilisation are post-germination events. However, a discussion of reserve mobilisation is included in this section. During germination the seed begins taking up water, the cells become rehydrated and begin to metabolise, the embryonic axis elongates and the seed coat splits allowing the radicle to emerge.

In the absence of biochemical markers, germination is generally measured by the emergence of the radicle from the seed tissues. Germination curves consisting of the proportion of germinated seed for a population over time are commonly used to describe the timing of germination. The germination curve of a population is characterised by the capacity of the population to germinate (maximum percentage germination), the uniformity or synchrony of germination, and the rate of germination.

Germination capacity is determined by factors including the degree of dormancy in the seedlot, the proportion of non-viable seeds, and the overlap of the germination conditions with the environmental requirements for germination. Germination rate is the reciprocal of the time it takes for the germination process to be completed. Variability of rates within a population indicates the uniformity or synchronicity of

germination (Bewley and Black 1994). Rapid germination allows seedlings to become established before the seedbed deteriorates. If all seeds in a bed germinate at about the same time, individual seeds are not at a competitive disadvantage (Bramlett et al. 1983).

Germination parameters of conifer species seedlots are influenced by both genetic control and environmental conditions. Germination varies between cones from an individual tree in some species and maternal factors such as seed coat properties can overwhelm the influence of genotype on germination and growth (Adams and Kunze 1996; Barnett 1996; Edwards and El-Kassaby 1996).

The regulation of germination

Despite much recent work, hormonal regulation of germination is still poorly understood, and there are few papers which establish specific relationships between the source and level of growth regulators and response. Much of the recent literature deals with the germination of hormone mutants of arabidopsis and tomato, reviewed by Hilhorst and Toorop (1997). It appears that GAs are involved in the metabolic processes that lead to embryo growth and radicle expansion such as the mobilisation of food reserves and the weakening of the tissues physically preventing radicle expansion (Thomas 1992). Brits et al. (1995) found that in certain Proteaceae seeds exhibiting secondary dormancy, increased GA levels occurred within the embryo once it was exposed to dormancy-breaking conditions. Increased GA levels induced germination. The pattern of endogenous cytokinin levels suggest that cytokinins may control early mobilisation of storage reserves, cotyledon expansion and seedcoat splitting, and the growth of the radicle (Brits et al. 1995).

ABA, jasmonic acid, ethylene, and cytokinins have been found to affect the germination of various species. The growth regulators interact and may have widely

differing effects in different species. The inhibitory effect of ABA on germination has received much attention. The role of ABA in dormancy was discussed in Chapter 2.3. ABA inhibited the elongation of embryos of *Brassica napus* L. (canola) seeds by increasing the rigidity of the cell walls, thereby preventing water uptake (Hilhorst and Toorop 1997). Groot et al. (1988) suggested GA from tomato embryos induced endo-β-mannanase activity in the endosperm which resulted in weakening. However, although germination was inhibited by ABA, enzyme activity was not affected. This may indicate that although endo-β-mannanase activity contributed to endosperm weakening it did not play a key role (Dahal et al. 1997).

Respiration and water relations during germination

Patterns of water uptake and oxygen consumption during imbibition

The uptake of water by dry seeds as a percentage of seed weight is generally described as a triphasic curve (Figure 2.3). During phase I water is rapidly imbibed, uptake then slows as moisture content plateaus during phase II. The transition to phase III is marked by a further rapid increase in moisture content (Bewley and Black 1994).

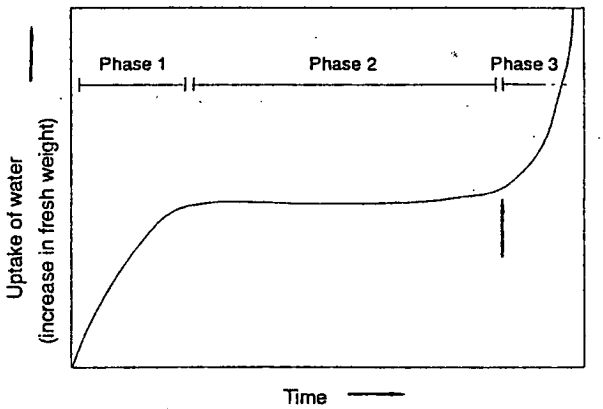

Figure 2.3 Triphasic water uptake by germinating seeds. Radicle protrusion occurs at the arrow (Bewley and Black 1994).

Oxygen consumption is commonly used to measure seed respiration. Respiration increases with increasing hydration of the seed (Bewley and Black 1994) and follows a similarly shaped curve. Oxygen consumption by the entire seed increases rapidly with emergence of the radicle. As the storage reserves are mobilised oxygen consumption by the megagametophyte decreases (Botha et al. 1992). Imbibition and oxygen consumption by sugar pine seeds during germination show the usual triphasic curves (Stanley 1958). The features of the curves will be discussed using sugar pine seeds as an example.

Rapid water uptake and oxygen consumption occurs for up to 12 hours when seeds are introduced to moisture at 30 ° C. During this phase gas is released from colloidal adsorption (Bewley and Black 1994). From 12 to 60 hours of imbibition further water uptake is not evident in sugar pine seeds, and a low rate of oxygen consumption occurs. The seeds are in the plateau phase of imbibition (Stanley 1958). The length of this phase is critical to the timing of germination (Karssen et al. 1989b) as it ends as the radicle penetrates the seed coat. Anaerobiosis may be experienced during the plateau phase if oxygen diffusion is hampered by impermeable barriers or dense tissue (Botha et al. 1992).

After about 60 hours rapid water uptake resumes, oxygen consumption increases, and the radicle emerges (Stanley 1958). The quantity of carbon dioxide produced during germination is usually close to the quantity of oxygen consumed unless the seed is rich in fats or oils rather than starch. Oxidisation of fats and oils requires a large amount of oxygen relative to the carbon dioxide produced (Salisbury and Ross 1992). The substrate for respiration during pine seed germination is not known, but the sucrose and reducing sugar levels in sugar pine seeds decrease during germination (Murphy and Hammer 1988).

Water relations of imbibing seed

Although it has been recognised that seed water relations are central to germination, there is still a limited comprehension of the water relations of seed germination. Haigh and Barlow (1987) and Bradford (1995) briefly reviewed some of the research concerning water relations during germination. Hallgren and Wu (1995) speculated about some of the water relations mechanisms that may be involved in pine seed germination based primarily on evidence from angiosperm seeds.

"Dry" seeds are in equilibrium with the water potential of the atmosphere in the storage environment, and generally have a low water potential (Ψ_s) relative to free water. Atmospheric Ψ varies according to the temperature and relative humidity:

$$\Psi = (RT/V_w) ln(\%\ relative\ humidity/100) \quad (1)$$

where Ψ is in bars, R is the gas constant (8.3143 joules/mole), T temperature in ° K, V_w the partial molal volume of water (cm^3/mole) (Nobel 1974).

The differential between seed and solution Ψ drives water uptake when the seed comes into contact with a solution. Water potential in hydrated plant tissue is governed by the relationship:

$$\Psi = \pi + P + \tau \quad (2)$$

where τ is the matric potential, π is osmotic potential, and P turgor pressure (Tyree and Jarvis 1982).

Matric potential is determined by the interfacial forces between solid and liquid phases. The forces are short range and contribute very little to the Ψ of hydrated tissues. In the

usual application of this relationship (transpiring leaves) τ is often close to zero and in practical terms is usually disregarded (Tyree and Jarvis 1982). However, actively growing tissue rarely reaches the low Ψ experienced by seeds during storage and early imbibition. In contrast in "dry" seeds, τ is the dominant factor (Bewley and Black 1994) and generally regarded as driving the initial phase of rapid water uptake when seeds are introduced to water.

Vertucci and Leopold (1983, 1984) identified two components of early imbibition in soybean embryos. The first component was identified as a wetting stage and the second as flow through seed tissue (Vertucci and Leopold 1983). Further investigations into the wetting stage using sorption isotherms identified three levels of water affinity. Below 8% moisture content water was strongly bound. Between 8 and 24% water was weakly bound and respiration was first detected. Respiration rates increased rapidly and water was very loosely bound as the moisture content rose above 24% (Vertucci and Leopold 1984).

Recently however, transport of water into seeds has been described using Darcy's law (equation 3) for liquid flux, and Fick's law for the movement of water in the vapour phase (equation 4) for tissue at a given water content (Schneider and Renault 1997):

$$q_l = - K(\omega)\, \delta\psi/\delta r \qquad (3)$$

$$q_v = - D(\omega)\, [(M_w/\rho_w)\, (1/RT)]\, \delta P_v/\delta r \qquad (4)$$

where q_l and q_v are liquid and vapour fluxes of water, respectively (m s^{-1}), K is the hydraulic conductivity (m^2 Pa^{-1} s^{-1}), D is the vapour diffusion coefficient (m^2 s^{-1}), ψ is the water potential (Pa), r is the radius (m), ω is the water content (kg kg^{-1}), M_w is the

molar mass of water (kg mol^{-1}), ρ_w is the bulk density of liquid water, R is the gas constant (8.315 J mol^{-1} $^{\circ}K^{-1}$), T is the temperature (K), and P_v is the water vapour pressure (Pa).

The complex structure of the seed and changes in conductivity and diffusion coefficient during hydration limit analysis of the imbibition process in terms of Fick's and Darcy's Laws. The conductance of liquid or vapour through the tissues surrounding the embryo and the water potential gradient between the embryo and the environment determine the rate of imbibition. If K and D are constant, flux is directly proportional to the water potential gradient. However, hydraulic conductance may vary between the tissue layers. Many species have impermeable tissue layers that operate as barriers to water uptake, in particular, tissues rich in waxes in association with matrices of suberin or cutin, or with lignin-impregnated cell walls (Egley 1989). Tillman-Sutela and Kauppi (1995a, 1995b) reported that water uptake by Scots pine and Norway spruce was regulated by the megaspore membranes between the seed coat and megagametophyte. Movement of liquid into the seed occurred firstly at the chalazal end away from the micropyle, and through the ridge joining the two halves of the seed. There is also evidence that conductance varies as tissue water content changes (Vertucci and Leopold 1983, 1984; Priestly 1986). At low moisture contents water is strongly bound and uptake occurs as a wetting action. It is not until moisture content has risen above a critical level that water uptake occurs as flow through a porous matrix (Vertucci and Leopold 1984).

Models developed to describe imbibition using Fick's law make assumptions regarding the factors mentioned above, the geometry of the seed which is generally assumed to be spherical, the absence of any preferential pathways for water movement, and the absence of swelling which affects the water potential gradient by changing the radial distance (Schneider and Renault 1997).

Water relations of the elongating embryo

As tissue hydrates both P and π approach maximum values, Ψ_s approaches zero and water movement into the seed slows or ceases, indicating the transition from phase I to phase II of imbibition. However, the Ψ gradient between the embryo and the environment must be maintained to ensure continued water uptake, embryo elongation and germination. Assuming water and solute flux are not limiting, the parameters limiting the rate of radicle expansion as described by Cosgrove (1993) and Pritchard (1994) are π, P and the properties of the cell wall. The rate of cell expansion is determined predominantly by the cell wall properties, according to the equation:

$$r = \phi (P - Y)$$

where r is the expansion rate, ϕ is the wall extensibility, P is the turgor pressure, and Y is the wall yield threshold (Cosgrove 1993; Pritchard 1994).

When the turgor pressure exceeds the wall yield threshold the cell expands, dissipating P thereby reducing Ψ_s which drives further water uptake (Cosgrove 1993). Passioura (1994) noted that for a step change in turgor, the rate of expansion changes only transiently, and then returns close to its original value, and described a molecular model of the expanding wall to explain this. As microfibrils in the cell wall become separated during expansion, an increased number of the hemicellulose molecules which tether the microfibrils become load-bearing. The load is then shared over a greater number of these molecules and separation ceases. If load-bearing molecules are sheared by enzymes, separation continues. The rate of expansion is determined by the frequency of loosening of the load-bearing molecules multiplied by the amount of expansion allowed before another molecule becomes load-bearing.

Solute accumulation has also been suggested as a possible mechanism of radicle emergence. Before the seedling is able to photosynthesise, osmotic potential is presumably controlled by sugar solubilised from starch reserves, balanced by the metabolic activity using the sugars. Bradford (1986) attributed uptake of water by lettuce seeds during the lag phase primarily to the generation of solutes. It was suggested that variation in lag periods at different Ψ may represent the time required to generate sufficient solutes to lower the osmotic potential to the level required to allow radicle expansion. Hallgren and Wu (1995) suggested that degradation of loblolly pine megagametophytic proteins during germination may have provided osmotically active substances to increase turgor and cell expansion. In spite of this evidence, it is difficult to accept that solute accumulation is a primary driving force for water uptake, when K^+ efflux is a characteristic of early stages of germination in many species (Shabala et al. 2000).

More recent work by Bradford (1990) found that endosperm weakening rather than solute accumulation was responsible for driving embryo expansion in lettuce seeds. Cytological and morphological changes in the endosperm cells adjacent to the radicle tip were observed. Lettuce endosperm cells became highly vacuolated and storage reserves mobilised just prior to radicle elongation (Karssen et al. 1989b). Bradford (1990) suggested that the length of phase II is a consequence not of slow accumulation of solutes, but of the rate of weakening of the endosperm. Radicle protrusion in celery and tomato seeds has also been found to depend on hydrolysis of the endosperm cells opposite the radicle tip. Mannans are the main cell wall polysaccharides in tomato endosperm cells. During imbibition endo-β-mannanase activity increases coincident with a decline in endosperm resistance to penetration (Karssen et al. 1989b).

In seeds where the tissues surrounding the embryo prevent expansion, the resistance of these tissues becomes the yield threshold that needs to be exceeded for radicle

expansion. Welbaum and Bradford (1990) found that muskmelon seeds showed no evidence of solute accumulation prior to germination, but isolated embryonic axes reached the plateau phase of imbibition at a higher moisture content than embryonic axes imbibed in intact seeds. Haigh and Barlow (1987) also found that tomato seed embryos were able to absorb an additional 20% moisture when imbibed once excised from surrounding tissues. This result was interpreted as indicating that the tissues surrounding the embryo prevent full imbibition. Embryonic axes during phase II were at a lower Ψ than the other tissues, any constraint on the embryo would increase the turgor pressure, bringing the embryo into equilibrium with the rest of the seed. During imbibition cracks developed in the perisperm tissue surrounding the embryo and the force required to penetrate this tissue decreased, which indicated that the yield threshold decreased (Welbaum et al. 1995).

In summary, embryo tissue expansion is a similar process to other plant tissue expansion and seeds will not germinate until the P of the embryo cells exceeds the cell wall yield threshold, allowing the cells to elongate. An increase in P results from a decrease in Ψ of the embryo and may be regulated by solute accumulation or weakening of the tissues surrounding the embryo. In the complex seed structure maternal tissue around the embryo may restrict expansion, superimposing external control on Ψ, P and water uptake by the embryo.

The hydrotime model of the germination of a seedlot

As applying cell growth models to seedlot germination has been difficult, Bradford (1990) suggested an alternative approach to describing seedlot germination rates. This has become known as the hydrotime model, and is based on the timing of radicle emergence at different external Ψ. It is an extension of the model described by Gummerson (1986) who proposed that the time to germination is related to the difference between the Ψ of the seed or environment and the Ψ for radicle expansion

(the base or threshold Ψ). The base water potential of late germinating seeds is higher than that of earlier germinating seeds, suggesting that it is the variation in base water potential in a seedlot which determines the shape of the germination curve (Bradford 1990).

The hydrotime model proposes that although radicle emergence varies between seeds of a population and seeds differ in their base Ψ, the hydrotime (MPa days) to radicle emergence is constant (Bradford 1990). The hydrotime model shows that factors including GA and ABA (Ni and Bradford 1993) affect germination by influencing base Ψ, thereby determining rate of water uptake and consequently, rate of germination (Hilhorst and Toorop 1997).

The base water potential and the standard deviation of this value for a population have been used to accurately predict germination time courses of lettuce seedlots at a given external water potential (Bradford 1990). In tomato seeds, however, the base water potential was found to vary with the external water potential (Dahal and Bradford 1990). The slow rate of germination of seeds at low water potentials was attributed by Bradford (1990) not to slow rates of imbibition or changes in osmotic potential, but to the time required for the yield threshold to fall below the turgor pressure. The time required was determined primarily by the rate of change of yield threshold of the tissues surrounding the embryo.

Mobilisation of storage reserves

Reserve mobilisation is generally regarded as a post-germination event which provides the seedling with the nutrients required to maintain growth. However, Wyman et al. (1996) observed cell cycle activity in the megagametophyte of jack pine seeds before it began in the embryo and suggested it may be related to reserve mobilisation. Gifford et

al. (1989) reported that hydrolysis of megagametophyte proteins of lodgepole pine seed began prior to radicle emergence.

Krutovskii et al. (1997) reported that the Douglas-fir megagametophyte has sufficient storage reserves only to allow the seedling to reach the stage of coat-shedding, at which time the seedling becomes dependent on photosynthesis.

In the seeds of many starchy species such as legumes, reserve carbohydrates are deposited as hemicellulose in the cell walls and are mobilised by enzymatic hydrolysis (Hilhorst and Toorop 1997). McKersie and Senaratna (1983) reported that mobilisation of reserves in the cotyledons of bean seeds was accompanied by an increased proportion of the membrane lipid found in a gel phase, which was thought to indicate increased membrane permeability, the release of enzymes involved in hydrolysis, or the inactivation of membrane-bound enzymes, all of which may play a role in the mobilisation of reserves. Dominguez and Cejudo (1999) reported that reserves in the starchy endosperm of wheat grains are hydrolysed by enzymes produced by the aleurone layer. The enzymes were regulated by GAs that diffused from the embryo. GA-regulated acidification of the endosperm during imbibition enhanced the activity of hydrolases and transport of the products of hydrolysis - sugars, peptides and amino acids, to the growing seedling.

The major storage reserves of many conifer species are proteins and lipids and the vast majority of these are found in the megagametophyte (Ching 1963a, 1963b; Groome et al. 1991). A small quantity of starch was found in the embryo of Douglas-fir seeds but starch grains were not present in the megagametophyte (Ching 1963b). Murphy and Hammer (1994) reported that starch accounted for only 3% of pinyon pine (*Pinus edulis* Engelm.) embryo dry weight. The loblolly pine embryo survived on the small amount of reserves it contained until the radicle emerged (Groome et al. 1991).

However, further embryo growth required a food source. Carpita et al. (1983) reported that isolated loblolly pine embryos were able to grow when incubated on a media supplemented with sucrose.

Ching (1966) reported that carbohydrates accumulated in both the embryo and megagametophyte of Douglas-fir seeds during the earliest stages of germination. As the radicle emerged, carbohydrates accumulated rapidly. At this time they began a gradual decline in the megagametophyte tissue. Murphy and Hammer (1994) reported that starch accumulation in pinyon pine seedlings accounted for 50% of the sucrose transported from the megagametophyte after germination. It is not clear at this stage what mechanisms are involved in transporting the products of reserve breakdown from the megagametophyte to the embryo.

The initiation of reserve mobilisation depends on the synthesis or activation of a range of enzymes. A change in enzymatic activity with the transition to reserve consumption has been observed in sugar pine (Noland and Murphy 1984) and loblolly pine (Groome et al. 1991) seeds following radicle emergence.

During the early stages of germination of Douglas-fir seeds there is an increase in the carbohydrate concentration in the embryo and megagametophyte, and a decrease in the number and weight of lipid bodies and protein vacuoles (Ching 1963a, 1966). Protein vacuoles contain insoluble proteins conjugated with lipids (Ching 1966). Ching (1968) reported that the protein vacuoles of the Douglas-fir megagametophyte tissue became granulated and fragmented following germination. This was thought to indicate hydrolysis. Hallgren and Wu (1995) reported that the size and number of protein bodies in the megagametophyte of loblolly pine decreased during germination. Stone and Gifford (1997) identified two waves of protein vacuole change in the megagametophytes of loblolly pine seeds. The first wave began in the inner layer after

about 5 days of imbibition at 30 ° C and progressed into the central region. The second wave began in the outer layer the following day and progressed inwards. King and Gifford (1997) examined the mobilisation and utilisation of storage proteins in loblolly pine seeds and found that protein depletion occurred primarily after the radicle emerged and it corresponded with an increase in amino acids present in the seedling.

During germination, lipid bodies acquired an outer membrane indicating in situ lipolysis, and the lipids contained within were converted to sugars via the glyoxylate pathway (Ching 1966, 1968). Despite little change in fresh weight, a 50% reduction in dry weight of the megagametophyte of Douglas-fir seeds was observed during germination (Ching 1966). In the megagametophyte lipid weight decreased from 13.1 to 12.1 mg per seed during germination, a reduction from 36% of seed weight to 12% Ching (1963b).

Seed quality

Germination tests indicate the ability of a seedlot to achieve the first stages of growth, generally under ideal conditions. It has been recognised that germination tests under such conditions rarely predict the emergence and growth of a seedlot under field conditions. Germination tests give little indication of the ability of the seedlot to germinate and grow under less than ideal conditions. Seed quality or vigour describes the ability of a seedlot to germinate and seedlings to emerge under a range of conditions. A high quality seedlot demonstrates rapid, uniform emergence and the development of a normal seedling under a range of environmental conditions (McDonald 1980). A low quality seedlot has reduced viability, reduced germination and emergence rates, poor stress tolerance, a narrow range of temperatures over which maximum germination occurs, and reduced growth rates (Finch-Savage 1995).

Seed quality is determined partly by genetics. Dickson (1980) reviewed studies of genetics of various characteristics influencing seed quality, including solute leakage, hardseededness, seed size and protein content, and found that many were under polygenic control. Quality also depends on the conditions experienced by the seed during development and maturation on the parent plant, harvesting and handling, and post-harvest storage. The post-maturation pre-harvest environment while the seed is still held on the parent plant can also affect seed quality. High humidity and temperature, or weathering, once the seed has attained physiological maturity can reduce quality (Coolbear 1995). Stoehr et al. (1998) reported that the environment in which the seeds were produced affected the germination of some white spruce seedlots.

The relationship of seed quality to crop yield was reviewed by TeKrony and Egli (1991). In general poor seed quality affects the ability of the seedling to accumulate dry matter. Differences between high and low quality seedlots are most apparent in early seedling growth, and are primarily due to effects on rate and level of establishment.

Seed quality is tested using a range of techniques, the ideal method depends on the species involved. Most studies have been directed towards establishing a relationship between germination in the laboratory or glasshouse and performance in the field. Coolbear (1995) indicated that the quality of many legume seeds has been correlated with conductivity of the solution in which the seeds are soaked. Increased conductivity arose when the contents of damaged cells were lost, with increased solute availability, and where membranes were compromised. Finch-Savage (1995) reported that the germination of seeds under conditions of limiting oxygen indicated seed quality. Wang et al. (1994) found that field performance of red clover (*Trifolium pratense* L.) seed was best indicated by germination in laboratory conditions following controlled deterioration treatment. Treatment involved holding seed at 16 - 18% moisture content

at 45 ° C for 24 hours. Controlled deterioration was an effective vigour test for coniferous forest tree seeds (Edwards and El-Kassaby 1996). Wang et al. (1992) reported that germination tests after controlled deterioration of loblolly pine seeds at 40 ° C and 98 % RH for up to 21 days, accurately indicated field performance. Pepper seed emergence is best predicted by seedling growth rates and laboratory germination at cool temperatures (Trawatha et al. 1990). Sugar beet (*Beta vulgaris* L.) field emergence correlated better with germination under flooding than with cool temperature germination (Lovato and Cagalli 1992). Germination tests on prechilled southern pine seedlots were found to be good predictors of tree yield in the nursery (Barnett 1996).

2.6 Effects of conifer seed orchard management and handling on seed quality and quantity

Seed orchard management

Factors influencing seed quality have been covered in the previous section. However, given that the present study is related primarily to seed orchard produced seed, various management factors influencing quality in commercial seed production have been briefly reviewed in this chapter. Studies examining the effects of seed orchard management on cone production rarely include a discussion of seed quality. However, Barnett (1996) reviewed the influence of cultural practices in southern pine orchards on seed performance.

The application of fertilisers and insecticides in combination with cultural practices including mowing, subsoiling, pruning and thinning, increased cone production in a loblolly pine seed orchard, and also substantially increased the number of viable seeds per cone (Fatzinger et al. 1985 cited in Barnett 1996). Fertiliser application has also been reported to increase the size of longleaf pine seeds (McLemore 1975b cited in Barnett 1996).

Cones within a seed orchard ripen at different rates. Hence it could be expected that, at a given harvest date, maturity will vary within a cone crop. Wakely (1954) reported that the germination of southern pines was directly related to cone maturity at the time of seed extraction. However, harvest and storage of immature cones appears not to adversely affect the germination of radiata pine seeds. Wilcox and Firth (1980) harvested two successive cone crops from 15 radiata pine clones. At harvest, the older crop had ripened on the tree but the younger crop was green and immature. The green cones were stored at 20 to 24 ° C for 10 weeks. Germination and growth rates were the same for all seeds.

Cones harvested before fully ripened need to be kilned to extract the seed. Many conifer species have an optimal temperature range and duration of kilning above which seed quality is reduced, and below which cones will not open. The germination of longleaf pine seeds was reduced at kiln temperatures above 46 ° C (Barnett 1996) while loblolly pine cones kilned at about 38 ° C for 48 hours showed improved germination (McLemore and Barnett 1966a). Wang et al. (1992) found that drying lodgepole pine cones was least harmful to germination if seeds were able to escape from the kiln once the cones had opened. They suggested that increased heat exposure could result in a loss of membrane integrity.

Following extraction, seeds are dewinged, dead seeds are removed and the seedlot dried to storage moisture content. Injury caused by de-winging is a common cause of poor seed quality in the southern pine species (Barnett 1996). Seedlots are usually graded by size to improve the uniformity of germination and remove the smallest seeds. However, seed size varies among pine clones, and particular clones may be lost from a seedlot if the seeds from an orchard are bulked and the smallest seeds discarded (Edwards and El-Kassaby 1996). Loss of genetic diversity from a seedlot may also occur if bulk seedlots are sown and thinned. Bramlett et al. (1983) demonstrated that the time to germination of Virginia pine seeds is under genetic control. Late-germinating clones may be lost from a crop if they are smaller at the time of thinning due to later emergence.

Seed size and number

Studies on seed size usually report correlations between seed size and germination capacity, growth rate, and resistance to stress. Reich et al. (1994) found that among 24 Scots pine populations, seed weight was not related to growth rate but was positively related to hypocotyl height and cotyledon number. However, the effect of seed weight

had become less apparent or disappeared after five to seven years of growth. Ching (1963a) reported that heavier Douglas-fir seeds contained more nitrogenous compounds and lipids and had a higher rate of lipid reserve breakdown and a faster growth rate than lighter seeds. Smith and Camper (1970) (cited in Delouche 1980) reported that soybean seeds sized into three classes produced higher yields than the mixed lot, which suggests that sowing seeds that are more uniform in size and therefore likely to be uniform in germination characteristics reduced the effects of competition on yield.

Seed size varies between seeds produced by a single parent plant, and between seeds produced by different parent plants. Seed size variation in coniferous species has been attributed to the effect of the position of the seed in the cone, the height of the cone on the parent tree, and the aspect of the cone in the crown (Edwards and El-Kassaby 1996). Adams and Kunze (1996) reported a positive correlation between the number of white spruce cones per tree and the number of seeds. White spruce and black spruce trees that produced more cones tended to have smaller seeds than those with fewer cones.

The effect of environmental conditions on seed size and composition was reviewed by Delouche (1980), Fenner (1992) and Hilhorst and Toorop (1997). Generally, water availability, temperature, soil nutrients, photoperiod and light quality influence seed mass (Hilhorst and Toorop 1997). The size and composition of seeds from a given plant varies annually. This may be due to variation in environmental conditions or variation in embryo genotype. Seed size may decline throughout the season as resources become unavailable and the plant ages (Fenner 1992). Moisture stress during flowering reduces the number of seeds produced but once seeds are set, moisture stress reduces the weight of the seeds produced and increases the concentration of storage proteins (Delouche 1980; Fenner 1992). High temperatures reduce seed size by

increasing the rate of ripening so that there is less time for the assimilation of photosynthates. The protein content is increased and the relative proportions of fatty acids changes (Fenner 1992).

There is some indication that growth regulators may be involved in seed size determination. Fenner (1992) suggested that variation in seed size may be a consequence of differences in the amount, translocation or activity of growth regulators including ABA. ABA injected into developing wheat grains increased the grain size as more photosynthates were assimilated.

Post-harvest deterioration

Many seeds begin deteriorating or aging immediately after maturation. Severe deterioration leads to loss of viability. Coolbear (1995) listed the effects of less severe deterioration as reduced vigour, increased abnormal seedlings, reduced pollen viability, an altered ratio of male to female flowers, changes in the appearance of the seed tissues and dormancy induction.

Species, storage temperature and relative humidity are the major factors that determine the degree of deterioration during storage. Seeds are generally stored at low moisture content and low temperature. Seed content may affect storability, which might explain the different storage characteristics of different species. Pacific yew (*Taxus bervifolia* Nutt.) seeds which have high lipid and reducing sugar content have poor storage characteristics (Walters-Vertucci et al. 1996). Pine species including radiata pine deteriorate very slowly under storage conditions (Donald and Jacobs 1990; Barnett 1996).

At low seed moisture content metabolic activity is very low but some potentially damaging activity continues. Certain hydrolytic enzymes are active, membranes may

undergo phase transitions, and free radicals may be produced (Hilhorst and Toorop 1997). Free radicals can damage membranes by lipid peroxidation, leading to reduced respiratory activity due to mitochondrial membrane damage, loss of membrane-based organisation, changes in plasmalemma permeability, and reduced sensitivity to growth regulators where receptors are situated in the membrane (Roos 1980; Coolbear 1995). Free radicals can also damage other biomolecules including enzymes and DNA. Seeds that can be stored for long periods of time with minimal deterioration may contain detoxification enzymes.

Hilhorst and Toorop (1997) hypothesised that dormant seeds may be better able to withstand storage than non-dormant seeds as a consequence of lower unsaturated fatty acid concentration than dormant seeds. Unsaturated fatty acids may accumulate during stratification. It has been suggested that the slow germination rate of deteriorated seed is a consequence of the time taken for self-repair when imbibition begins (Coolbear 1995). Repair processes may be able to proceed under treatments such as priming. Where deterioration has proceeded beyond affecting vigour, and viability is reduced, there may be damage to repair mechanisms.

2.7 Summary and conclusions

This literature review has identified areas of present radiata pine seed orchard management and nursery seedling production that may contribute to the variable germination observed in nurseries, and may lead to future seed quality problems. The standard nursery treatment in Australia at present is to presoak seeds for a short period before sowing. The literature suggests that this treatment may have the potential to damage seeds. Poor germination following soaking has been observed in a range of species, including conifer species, and is believed to be caused by either rapid imbibition or anaerobic metabolism.

Since the species is a conifer with a natural range within the northern hemisphere temperate zone, the expression of dormancy appears likely. Many coniferous species exhibit complete or relative dormancy. The use of nursery pretreatments at present and in the past, including presoaking and stratification, suggests that relative dormancy may well be present. Consequently there is probably scope to improve nursery performance by applying treatments. Recent developments in stratification methodology and an improved understanding of the processes involved in moisture uptake by seeds during germination include limiting the amount of moisture available during the treatment, and measurement of seed water potential during the three phases of water uptake. These developments may offer a means of making the treatment easier to apply and more flexible in its duration.

In summary, radiata pine seeds presently grown in nurseries may be exhibiting variable germination due to poor seed quality, which may be caused by a range of factors. Nursery pretreatments currently in use may also reduce seed quality. The application of pretreatments such as stratification has the potential to improve nursery germination for both low and high quality seedlots.

3. General Materials and Methods

This chapter covers major aspects of materials and methods used throughout the study. Where materials and methods differed from those detailed below, or where they were specific to a single experiment, the information is given in the appropriate chapter. The experimental work is not necessarily presented in chrononlogical order, so in some cases the experimental design of later trials does not take into account the results of earlier trials.

Seedlots

Table 3.1 Seedlots used in this study. (* refers to the year the seed was collected).

Seedlot	Date Supplied	Description
W & F old and S.O. 2, 3, 4	March 1995	OP. 3 sizes < 4 mm, 4 - 5 mm, > 5 mm
*STBA Level 1 1995**	October 1996	OP. 4 - 5 mm
*STBA Level 1 1996**	June 1998	OP. Heat affected. 4 - 5 mm
*STBA Level 3.1 1994**	June 1998	OP. > 5 mm
51246 x 51201	June 1998	CP. Ungraded
51231 x 51235	June 1998	CP. Ungraded
Unknown	June 1998	OP. 4 - 5 mm

The seedlots used in this study were provided by the Southern Tree Breeding Association (STBA), at different stages during the study (Table 1). The STBA collects open pollinated seeds from a number of seed orchards throughout southern Australia. The seeds are classed according to breeding value. Seeds from Level 1 are expected to produce 10 to 15 % increase in growth over the unimproved land race, whereas seeds from Level 3 will produce a 20 to 25 % increase. Seeds from orchards of a given class are bulked into a single lot. The seedlot *W & F old and S.O. 2, 3, 4* originated from three open-pollinated seed orchards. Initial work was carried out using seedlot *W & F*

old and S.O. 2, 3, 4, however, this seedlot became unavailable during the course of this study and the commercial seedlot *STBA Level 1 1995 heavy 4 - 5 mm* was used for subsequent work.

All seedlots had been cleaned and were substantially free of litter. The seeds were stored in the dark at 5 ° C in airtight bags. The seeds for each experiment were sampled from the lot by quartering. The quantity of seeds needed for each experimental unit was counted using a Contador® seed counter and sorted to remove any cracked seeds.

Germination studies

The seeds were germinated in a Contherm - G.P.M. Cooled Incubator. The incubator was illuminated by two 15W fluorescent tubes located at the rear. A temperature gradient of 0.5 to 1 ° C was found between the front and rear of the incubator when set at 20 ° C. Germination conditions in the incubator corresponded to those recommended by the International Seed Testing Authority (ISTA 1999) for *P. radiata*. The incubator was set at 20 ° C with continuous light. The seeds were germinated with standard petri dishes serving as plots. Each petri dish contained approximately 50 seeds, 3 filter papers and 5 ml distilled water.

During all experiments germination counts were carried out at the same time each day for 28 days. As biochemical markers of germination are not available, radicle emergence, which is usually taken as the indication of the completion of germination was used as a marker of germination (Bewley and Black 1994). Seeds were considered as germinated once the radicle was greater than 5 mm in length, as emerged radicles become recognisable once they have attained this length. Downie and co-workers (1991 and 1993) considered conifer seeds germinated once the emerged radicle was the length of the seed. Jones and Gosling (1990, 1994) considered a germinated conifer seed to have a radicle three times the length of the seed. However,

as radicle emergence is commonly interpreted as the completion of germination, and emerged radicles rarely ceased expanding once they had begun, it was decided to use 5 mm as the critical length. When counted as germinated, the seeds were removed from the petri dish.

Water loss by evaporation was measured by weighing, and where necessary water was added to return to the original weight. In later experiments petri dishes were sealed with plastic film between counts to minimise evaporative water loss, reduce variation in water stress between petri dishes, and prevent the introduction of contaminants during watering.

At the completion of each experiment any ungerminated seeds were dissected to determine the proportion that were dead, empty or ungerminated. Seeds were considered dead if the tissues were soft and discoloured and showed no sign of seedling development (ISTA 1999). Ungerminated seeds were considered healthy if a healthy embryo and megagametophyte were present. The number of seeds that were empty, containing no megagametophyte or embryo, was also recorded.

A fungicide treatment is not recommended by the ISTA (1999). No surface sterilisation procedures were used and no fungicide was added to the water in the petri dishes. A small amount of fungal contamination occurred in some replicates but inhibition of growth appeared minimal and a control was considered unnecessary.

Soaking conditions

Except where detailed otherwise, soaking was carried out at 20 ° C in darkness, with a ratio of 0.4 ml distilled water per seed in glass, screw topped 20 ml capacity vials. Soak water was held at the required temperature for 24 hours prior to the addition of seeds to ensure the water was at the desired temperature. Once seeds were added the

vials were wrapped in foil and returned to the treatment temperature. At the end of the soak period, water was drained and the seeds removed using tweezers. Evaporation from vials was recorded initially but measured losses were found to be so low that it was considered unnecessary to monitor water loss for short-term experiments.

Determination of dry or imbibed seed weight

Before determining the moisture content of imbibed seeds it was necessary to remove adherent surface moisture. Seeds were blotted on six fresh sheets of paper towel, and then allowed to air dry for 30 minutes on a further fresh piece of paper towel on the laboratory bench (Donovan 1996).

To determine seed water content of air dried seeds or following various imbibition treatments, the low temperature oven method of moisture content determination was used, as recommended for *P. radiata* by the ISTA (1999). After removal of surface moisture as described above, the seeds were placed in a glass vial, weighed, and placed in a drying oven at 103 ° C. The vials were removed from the oven, sealed, allowed to cool and reweighed at 24 hourly intervals until they maintained constant weight. Generally this was achieved within 24 hours. The moisture content prior to oven drying was calculated by expressing the weight lost during drying as a percentage of either the fresh (initial) or dry weight of the seeds. Expression as a percentage of fresh or initial unimbibed seed weight is preferable for measurements taken over extended periods of imbibition, as the dry weight is likely to decrease as respiration begins. However, seeds were randomly sampled after imbibition in Chapter 6, consequently fresh weight was not known and moisture content was expressed as a percentage of dry weight, which was determined by drying the imbibed seeds.

Water potential measurement using the psychrometer

Ψ_s, the measured water potential of a sample of surface dried seeds, and Ψ_e, the measured water potential of a sample of excised embryos, were obtained using a Decagon Devices thermocouple psychrometer, model SC-10A and a NT-3 Nanovoltmeter. The method of surface drying (or air drying) imbibed seeds prior to water potential measurement is described above in "Determination of seed dry or imbibed weight". Samples of 15 seeds were placed in stainless steel cups of 2.25 ml capacity. It was found that 2 hours was a sufficient length of time to allow the seeds to equilibrate with the head space.

The psychrometer was calibrated according to the manufacturer's instructions using NaCl solutions having known water potentials. A calibration curve was obtained by plotting the microvolt reading against the known water potential for each solution. This was used to convert the microvolt reading into the water potential of a sample. Calibration was within the range - 0.232 and - 4.558 MPa. Water potentials for calibration supplied by the manufacturer correspond with the table published by Lang (1967).

Some readings on relatively dry seed were well below these values. Decagon Devices claim that the instrument is capable of accurately measuring down to - 100 MPa (Wacker, pers. comm.) but no information on linearity at these lower potentials was supplied.

Osmotic pressure is a colligative property of a solution, which indicates it changes linearly in proportion to the concentration of solute particles. A 4.381 molal *(m)* solution of NaCl has a potential of -27.9 MPa according to extrapolation of Lang's (1967) table. Using the instrument calibrated within the range 0 to -4.558 the reading

for this NaCl concentration was -26.5 MPa, suggesting that the microvolt/MPa relationship remains essentially linear at least to this point.

Where the measurement temperature differed from the calibration temperature a correction was made using the equation:

$$\Psi_{cor} = \Psi_m\{1 - 0.025(T_m - T_c)\}$$

where Ψ_{cor} is the corrected water potential measurement, Ψ_m the measured value, T_m the psychrometer temperature during measurement, and T_c the psychrometer temperature during calibration.

Data analysis and presentation

Results were analysed using ANOVA. The analysis was carried out using general linear models in the FASTAT package (Systat Corp.). Means were compared using Least Significant Difference (LSD) (Steele and Torrie 1980).

Petri dishes were arranged within the incubator in a randomised complete block design. Blocks were arranged parallel to the rear wall due to the presence of an apparent light and measured temperature gradient within the incubator. Petri dishes within each block were re-randomised daily.

The mathematical description of the germination of a seedlot is a complex issue not yet properly resolved and there are a range of descriptive measures currently in use. Various indices have been developed to quantify germination, with varying degrees of success, as noted by Brown and Mayer (1988a, 1988b). There are no conventional or standard methods published for *Pinus radiata*. From the generally accepted measures reviewed by Bewley and Black (1994), germination capacity and mean germination

time were chosen to quantify and compare germination. Germination capacity and mean germination time are frequently used conventional methods of describing germination and are less subject to interpretation or subjective judgement than other parameters.

Germination capacity (GC) is the mean cumulative germination at the end of the germination test. The ISTA (1999) recommended a 28 day germination test for radiata pine seeds. GC indicates the proportion of a seedlot that is capable of germination under the conditions of the germination test. The number of empty seeds was subtracted from the total number of seeds per petri dish to give the number of potential germinants. GC is expressed as a percentage of potential germinants.

The mean time taken by the seeds of a seedlot to germinate (MGT) gives an indication of the rate of germination of a seedlot. Seed samples often have similar final germination and uniformity, but differ in germination rate (Bewley and Black 1994). MGT is calculated using the following formula:

$$t = \quad \Sigma (t * n) / \Sigma n$$

where t is the time in days starting from the day of sowing (day 0) and n is the number of seeds completing germination on day t. The reciprocal of t, sometimes called the rate of germination is also used in some publications.

A third measure was also used. The first day of germination (FD) is the day on which the first seed of a sample germinated, with day 0 being the day of sowing (Smith 1995). This is a parameter that is not widely used in the literature, but during daily observations of germination experiments it became apparent that differences between seed samples should be quantified in this way.

As all of the above parameters are derived from the germination profiles the full plots have only been included in the results where the derived parameters did not show particular aspects of the germination pattern. To improve clarity, text figures of germination profiles show only treatment means, complete profiles with standard errors are given in the appendices for the experiments.

Germination was plotted as cumulative or daily germination. Cumulative germination is expressed daily as the number of germinated seeds as a percentage of the total number of seeds in the petri dish, minus the number of empty seeds. Means and standard errors were calculated for each treatment. The shape of a germination curve depends on the final germination and uniformity or synchrony of the seedlot. Steep curves reflect a highly uniform population, as most of the seeds germinate over a relatively short time (Bewley and Black 1994).

The percentage of seeds germinating per day, or daily germination, was plotted as a three day moving average, according to the following formula:

$$n = \quad (n_{x-1} + n_x + n_{x+1}) / 3$$

where x is the day in question. Daily germination was expressed as a percentage of the total number of seeds that germinated in a given petri dish. Averaging results over three days reduces much of the day to day variation, but also reduces the information that can be obtained from the profile. Germination curves are usually positively skewed, as more of the seeds germinate during the first half of the germination period (Bewley and Black 1994).

4. Presoaking experiments

4.1 Introduction

A common practice in radiata pine nurseries is to soak seeds for a short time before sowing in the nursery. This process, known as presoaking, is believed to increase the rate of seedling emergence. Treatments are usually carried out in large volumes of water for 24 to 48 hours at ambient temperature. The potential for anoxic conditions to develop is minimised by changing the water, or running water continuously through the seeds. Presoaking is preferred to the traditional stratification treatment, which involves supplying seeds with sufficient water for imbibition and holding at low temperature for a prolonged period, because it is quicker, easier and allows the nursery operator more flexibility in sowing date.

There is, however, some evidence that soaking conifer seeds may be detrimental to germination. Earlier work by the present author indicated that soaking radiata pine seeds for 48 hours at room temperature was detrimental to germination (Donovan 1996). No other work has been reported for *P. radiata*, however, Barnett (1971) found when soaked at temperatures above 25 ° C loblolly pine seeds germinated poorly. The oxygen concentration in the soak water was found to be below saturation. The solubility of oxygen decreases as temperature rises (Cole 1979) and the respiration rate of seeds increases with increasing temperature. These two factors may have contributed to lower oxygen levels in soak water when seeds were soaked above 25 ° C. Soak injury has been reported in other species soaked at low temperature, particularly legumes with large cotyledons such as pea and faba bean (Sivritepe and Dourado 1995; Rowland and Gusta 1977). Loss of cotyledon cell contents into soak water has been observed (Spaeth 1987). The conductivity of the soak water following

imbibition has been correlated with low seed viability, low initial moisture content and poor vigour.

Since there is conflicting evidence as to the benefit of presoaking and the practice is in wide use in the forest nursery industry, it is important that the effect of presoaking on the subsequent germination of radiata pine seeds be clarified and the nature of any injury examined.

4.2 Varying the aeration and duration of a short presoak

Introduction

In a previous study using the seedlot *W & F old & S.O. 2, 3, 4,* Donovan (1996) found that germination suffered when the seeds were soaked for a short time at room temperature before germination. The objective of the present study was to determine the effect of varying the level of aeration of the soak water and the duration of the presoak on subsequent germination of this seedlot.

Materials and Methods

The experiment was a 3 x 2 x 3 factorial with 4 replicates, where the factors were: level of aeration, duration of soak and seed size, respectively. Seedlot *W & F old & S.O. 2, 3, 4* was commercially graded into three classes: < 4 mm (small); 4 - 5 mm (medium); and > 5 mm (large). The 4 - 5 mm class represented the bulk of the seedlot. The seeds were soaked with one of three levels of aeration (none, 50% of the time, and 100% of the time), for either 24 or 48 hours at 20 ° C. The water was aerated using two aquarium pumps. One operated on a cycle of three hours on followed by three hours off, to give the 50% aeration treatment. The other operated continuously. The seeds were soaked in twelve 250 ml conical flasks in cloth sachets, with 50 seeds per sachet. Each flask contained 120 ml distilled water and six sachets, two of each seed size. The initial volume of water per seed was 0.4 ml. Within each flask, 3 sachets were soaked for 24 hours (one of each seed size), and the remaining three for 48 hours. The seeds from each sachet were transferred to petri dishes at the end of soaking and germinated under standard conditions (see General Materials and Methods). Germination was compared using GC, MGT and FD (see General Materials and Methods).

Results

Seed size was the only factor to significantly affect MGT. Seeds of the small grade completed germination in a shorter mean time than medium or large seeds (Table 4.1). GC was significantly affected by seed size, level of aeration and duration of soak. A three-way interaction between these factors was found. Treatment means are given in Table 4.2. The method of soaking did not significantly affect the GC of small seeds. Significant differences in GC within the medium seed group were only observed between seeds soaked for 24 hours without aeration, and those soaked for 48 hours with 100% aeration. The latter treatment led to significantly higher germination. Within the large seed grade, seeds soaked for 48 hours without aeration had significantly lower GC than all other treatments. Large seeds soaked without aeration had significantly lower GC when soaked for 48 rather than 24 hours, and this was also the case when soaked with 50% aeration. When the soak water was aerated 100% of the time, however, GC of the large seeds was not significantly affected by the duration of the soak.

Table 4.1 Mean germination time (MGT) of small, medium and large seeds. Means for each seed size are taken across all treatments: 24 experimental units (three levels of aeration, two durations, four replicates) each of 50 seeds. LSD is 1.7 (α = 0.05).

Seed size	MGT (days)
Small	16.4
Medium	18.5
Large	18.7

Comparing GC for a given treatment across seed sizes, medium seeds soaked for 24 or 48 hours without aeration had significantly lower germination capacity than small

seeds given the same treatment. Large seeds soaked for 48 hours without aeration had significantly lower germination than small and medium seeds given the same treatment.

Table 4.2 Germination capacity (GC) and first germinant (FD) of samples of 50 small, medium and large seeds soaked for 24 or 48 hours with 0, 50% or 100% aeration.

Seed size	Aeration	Duration	GC (%)	FD (days)
Small	**0**	**24**	91.3	11.75
	50	**24**	85.2	11.25
	100	**24**	89.6	9.25
	0	**48**	92.5	10
	50	**48**	88.8	9
	100	**48**	93.2	8.75
Medium	**0**	**24**	76.0	12.25
	50	**24**	84.4	10.75
	100	**24**	82.8	11.5
	0	**48**	81.9	11.25
	50	**48**	84.9	12.25
	100	**48**	88.4	13
Large	**0**	**24**	83.5	12.25
	50	**24**	92.5	11.5
	100	**24**	83.2	12.5
	0	**48**	64.8	11
	50	**48**	78.7	12.25
	100	**48**	85.7	10
LSD (α = 0.05)			**10.4**	**1.92**

The day the first germinant was observed (FD) was significantly affected only by seed size, but a significant three-way interaction was present and comparisons were made between treatment means. Treatment means are given in Table 4.2. Within the large seed group, seeds soaked with 100% aeration germinated significantly earlier when soaked for 48 rather than 24 hours. The FD of seeds soaked for 24 hours without aeration, or 48 hours with 50% aeration was significantly later than those soaked for 48 hours with 100% aeration. Of the medium sized seeds, a significant difference was only observed between the seeds soaked for 24 hours with 50% aeration, and those soaked for 48 hours with 100% aeration. The latter treatment led to later germination. The FD of small seeds aerated 50% of the time was delayed if soaked for 24 rather than 48 hours. The FD of small seeds soaked for 24 hours with 100% aeration, 48 hours with 50 or 100% aeration was significantly earlier than those soaked for 24 hours with 0 or 50% aeration.

Comparing across seed sizes, small seeds soaked for 24 hours with 100% aeration or 48 hours with 50% aeration began germinating significantly earlier than medium or large seeds given the same treatment. Medium seeds soaked for 48 hours with 100% aeration began germinating significantly later than small or large seeds treated in the same way.

Discussion

While the germination capacity of small seeds was unaffected by soak method, there was some evidence that aerated soaks were beneficial to the germination of medium and large seeds, and soaking without aeration was detrimental. In particular, large seeds soaked for 48 hours without aeration had greatly reduced germination capacity compared with other seeds. Aeration may prevent or minimise seed injury in a number of ways. It is possible that if aeration is not supplied, the seeds are unable to respire normally during imbibition. Seed injury under anoxic or hypoxic conditions has

variously been attributed to reduced ATP production, the accumulation of toxic end-products of fermentation and lack of substrates for respiration (Crawford 1977; Rumpho and Kennedy 1981; Kennedy et al. 1992; Drew 1997).

As the seed samples were determined by the number of seeds and not by weight or volume of seeds, the large size seed samples had a greater volume than the small or medium seed samples. The volume of megagametophyte and embryo tissue per unit volume of soak water is therefore greater for a sample of large seeds than for small or medium seeds. This may increase susceptibility to damage in unaerated soaks as the tissue will have a greater demand for oxygen compared with seeds of a smaller size. Large seeds supplied with no aeration or aerated only 50% of the time during soaking had a lower germination capacity when soaked for 48 hours than when soaked for 24 hours. This may reflect a longer exposure to oxygen-limiting conditions in those soaked for 48 hours. In contrast, the length of the soak made no difference to the germination capacity of large seeds when aeration was supplied continuously during soaking. The volume of megagametophyte tissue in large seeds may slow diffusion of oxygen to the embryo and the diffusion of toxins away from it, due to the dense nature of the tissue. These two factors may also contribute to the greater effect of soaking on large compared with small seeds.

FD was reduced in large and small seeds when soaked for 48 hours with water that was aerated 100% of the time. As the soak water was aerated, dissolved oxygen depleted by respiration or lost to the atmosphere could be replenished, possibly maintaining oxygen close to saturation levels. Under such conditions the seeds may commence germination earlier as the requirements for germination have been met, the seeds have water and oxygen and are at a temperature at which germination will occur. That is, presumably the seeds imbibe and begin respiration and other processes

associated with germination while in the soak treatments. The seeds would thus be at an advanced stage of germination when transferred to the incubator.

It is evident from this trial that the seeds of the three different size classes had different germination characteristics. Small seeds germinated in a significantly shorter mean time than medium and large seeds. Small seeds generally had a higher germination capacity and a shorter time until the first emergent was observed, compared with medium and large seeds treated in the same way. The seeds also reacted differently to treatment, as indicated by the presence of interactions between the three factors in the analyses of GC and FD. Soaking generally had little effect on small and medium seeds, while the germination of large seeds was improved by long soaks when aeration was supplied and detrimentally affected by long soaks without aeration.

McDonald (1980) and Finch-Savage (1995) define vigorous or high quality seedlots as those that are able to germinate well under stressful conditions. Therefore, the results suggest that the large seeds are of poorer quality than the small and medium seeds. Lovato and Cagalli (1992) reported that presoaking can be used as a vigour test for sugar beet seeds and it is obvious from the present results that presoaking has the potential to be used as a vigour test for radiata pine seedlots.

Many studies have reported a positive association between seed size and seedlot characteristics including germination capacity and stress tolerance. Mogie et al. (1990) found a positive correlation between *Taraxacum hamatiforme* Dahlst. seed size and germination capacity and early seedling growth. Bonfil (1998) reported a positive association between seed size and seedling survival, growth and response to herbivory in two oak species (*Quercus rugosa* and *Q. laurina*). Dunlap and Barnett (1983) reported that larger loblolly pine seeds, despite having lower germination capacity, germinated more rapidly than smaller seeds and produced larger seedlings. As the

relationship between seed size and reserve tissue and embryo size in seeds is rarely reported, it is difficult to determine if the advantage of large seeds is due to initial embryo size or the availability of greater storage reserves for growth.

Barnett (1972, 1976, 1996, 1997) reported that the ratio of seedcoat weight to total seed weight indicates the degree of dormancy in the southern pines. The seeds with thicker and heavier coats are believed to be more dormant as megagametophyte and embryo swelling cannot progress. As the seedcoat develops from maternal tissue the degree of dormancy may vary between clones. Large seeds in the present study may have a large seedcoat weight relative to the total seed weight and therefore greater dormancy. Alternatively, the seeds of a given size group may develop at specific sites within the cone or in cones at different sites on the parent tree that vary in nutrition and time to maturation resulting in different germination characteristics.

Genotype has been found to affect seed size in Douglas-fir and sitka spruce. In these species the largest seeds produced by particular clones were smaller than the smallest seeds produced by other clones (Edwards and El-Kassaby 1996). The seedlot used in this study originated from three open-pollinated seed orchards, and would be expected to contain a range of genotypes due to the range of clones in these orchards. Seed size in radiata pine is also related to clonal identity (Boomsma, pers comm). The grades of seed may therefore represent specific genotypes, and the germination characteristics may be greatly influenced by the genotype of the seed.

4.3 Germination of presoaked seeds in the incubator and nursery

Introduction

The previous experiment demonstrated that presoaking affects the timing of the initiation of germination of a radiata pine seedlot, and depending on the nature of the presoak, has the potential to prevent some seeds of that seedlot from germinating. Germination capacity and rate affect the yield and seedling growth rate of pine seedlots planted in the nursery. Barnett and McLemore (1984) found that germination capacity was the best predictor of nursery yield of loblolly and slash pines, but for a given sowing date the rapidly germinating seeds produced the largest seedlings. These findings are supported by similar results for loblolly seedlings reported by Boyer and South (1988). Boyer et al. (1985, 1987) found that loblolly pine seedlots with rapid emergence produced seedlings with greater diameter at harvest, lower mortality and less variation in size than those produced by seedlots with slower emergence.

As presoaking has been found to affect germination in the laboratory an investigation was made into the effect of presoaking on the germination of a seedlot sown in the nursery. A commercial seedlot *STBA Level 1 1995 heavy 4 - 5 mm* was used in this trial (see General Materials and Methods).

Materials and Methods

The seeds were soaked for 0, 12, 24, 48, 72, and 96 hours at 20 ° C in darkness. Soaking was carried out on eight replicates of each treatment, each consisting of a plastic bag containing 350 seeds and 140 ml of distilled water, 0.4 ml per seed. At the completion of treatment the seeds were drained, blotted and thiram was applied by dusting. Approximately 1 g of thiram was placed into a bag containing 350 seeds and shaken to coat the seeds. Fifty seeds were randomly selected from each sample and placed in petri dishes in the germination cabinet in a randomised complete block design

with eight replicates in standard conditions (as described in General Materials and Methods). The remaining 300 seeds of each sample were sealed in individual plastic bags and transported to the nursery site in an insulated container.

The nursery was located on deep brown clay loam (Dimmock 1961) at Meadowbank in the Derwent Valley in southern Tasmania, Australia (42 ° 38 ' S, 146 ° 52 ' E) at an elevation of 50 m. The field site is shown in Plate 4.1. The trial was sown using a precision seed drill on December 1st, 1997, as a randomised complete block design with eight replicates. The trial was sown in a single raised bed, each plot consisted of two metres of bed containing six rows. Within each plot, fifty seeds were sown in each row. The seeds were sown 4 cm apart within the rows. Two metre buffer plots of untreated seeds were sown at the ends of the trial rows.

The number of emerged seedlings in the two inside rows was counted. A seedling was considered to have completed emergence once the seedcoat was raised above the soil surface (Danielson and Tanaka 1978). Counts were carried out daily after the first emerged seedlings were observed, but as emergence slowed they were made less frequently (every two to three days). Emergence counts were used to calculate FD, GC and MGT (see General Materials and Methods).

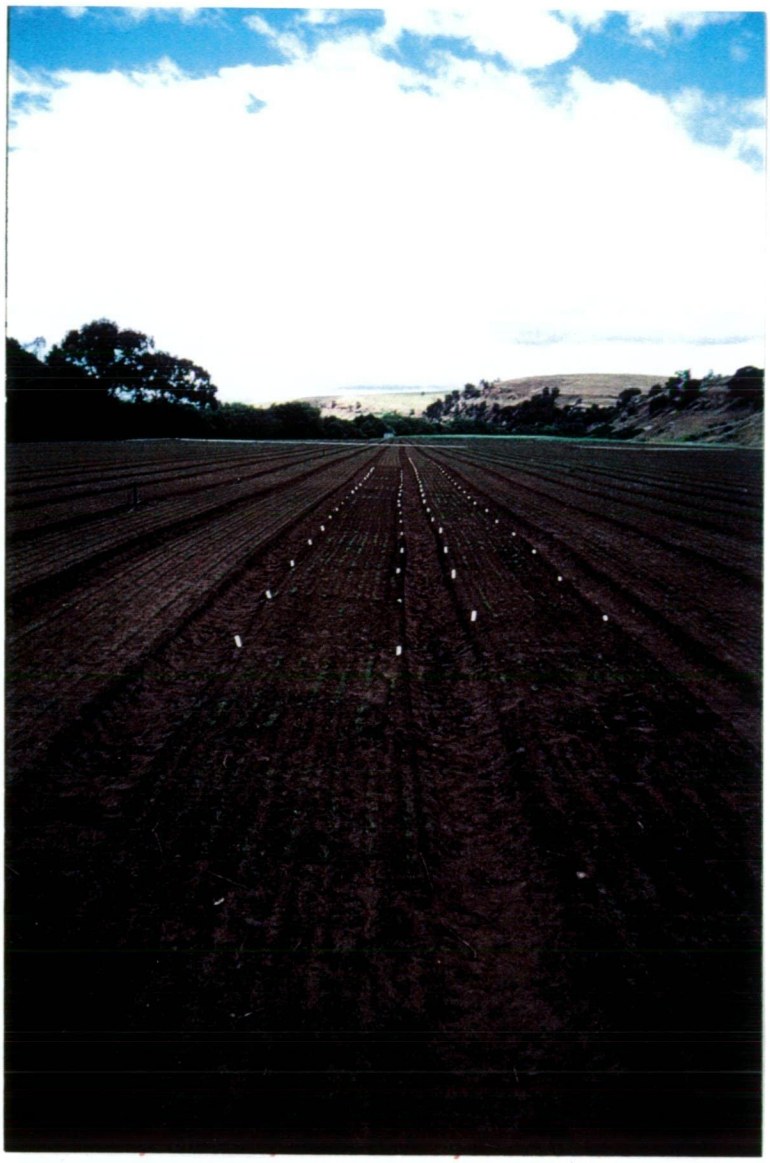

Plate 4.1 Radiata pine field nursery trial site

Results

Laboratory Germination: MGT, FD, and GC

Soaking significantly affected MGT. However, GC and FD were not significantly affected by treatment. Seeds presoaked for 24 hours had a significantly shorter MGT than unsoaked seeds or seeds soaked for 48 or 72 hours. Treatment means are given in Table 4.3.

Table 4.3 Treatment means (eight replicates of 50 seeds) for first germinant (FD), germination capacity (GC) and mean germination time (MGT) of seeds soaked for 0, 12, 24, 48, 72 or 96 hours and germinated in the laboratory. Only MGT was significantly affected by treatment LSD = 1.9 (α = 0.05).

Soak (h)	GC (%)	MGT (days)	FD (days)
0	90.4	18.7	11.4
12	91.4	17.1	9.3
24	92.4	15.9	9.6
48	91.2	18.3	10.1
72	84.1	18.8	10.4
96	89.3	17.4	9.4

Nursery Germination: MGT, FD and GC

Treatment significantly affected MGT and FD of nursery-grown seedlings, but GC was unaffected. Means are given in Table 4.4. The unsoaked seeds had a significantly later FD than did the soaked seeds. MGT varied little between soak treatments, but unsoaked seeds were significantly slower to germinate than soaked seeds. The seeds soaked for 12 or 24 hours had a significantly longer MGT than seeds soaked for 48 or 72 hours. The seeds soaked for 24 hours also had a significantly longer MGT than seeds soaked for 96 hours.

Table 4.4 Mean first germinant (FD), germination capacity (GC) and mean germination time (MGT) of seeds soaked for 0, 12, 24, 48, 72 and 96 hours and sown in the nursery. Means are of eight replicates of 100 seeds. MGT and FD were significantly affected by treatment. LSDs are 1.0 and 0.52 (α = 0.05) for MGT and FD respectively.

Soak	GC (%)	MGT (days)	FD (days)
0	70.5	26.2	19.4
12	69.3	24.5	18.6
24	60.6	24.9	18.6
48	69.6	23.0	18.1
72	76.9	23.4	18.5
96	67.8	23.6	18.1

Discussion

In contrast to earlier work with a different seedlot (see previous section) the germination capacity of this seedlot, whether germinated in the laboratory or in the nursery, was unaffected by soaking. In the nursery, the seeds soaked for longer than 24 hours germinated earlier than the unsoaked seeds or seeds soaked for a shorter length of time. It appears that being at a more advanced state of imbibition is advantageous when seeds are sown in the less-than-ideal conditions of the nursery. However, prolonging the soak beyond 48 hours did not further reduce the time to germination (FD or MGT). This suggests that germination processes are unable to proceed beyond a certain point under soak conditions. The advantage due to soaking may simply be that phase I of imbibition, which is thought to occur without metabolic activity (Bewley and Black 1994), was completed before the seeds were sown. Phase I of uptake could reasonably be expected to be slower in the field than in the soak. The greater length of time taken by the unsoaked seeds to emerge may thus represent the time taken to complete phase I in the nursery.

Barnett and McLemore (1984) reported that earlier emerging loblolly and slash pine seedlings showed better growth in the nursery because they were at an advanced state compared with later emerging seedlings, as a consequence not of inherent vigour but simply because the seeds had been growing for longer. These results were supported by further work with loblolly pine by Boyer and co-workers (Boyer et al. 1985; Boyer et al. 1987; Boyer and South 1988) who found that seedlings that emerged earlier had a larger diameter and dry weight at lifting than those that emerged later, and such differences led to variation in volume at harvest. The earlier germination of presoaked radiata pine seeds may, therefore, lead to larger seedlings at harvest. In spite of in-vitro results with another seedlot showing reduced germination in response to soaking (see previous section), it appears that soaking may be beneficial to seedling production when the seedlot used in the present study is sown in the nursery.

4.4 The effect of thiram on germination of presoaked seeds in the incubator

Introduction

Thiram® was applied to seeds in the previous trial. Thiram coating of seeds is routinely used in radiata pine nurseries, primarily to repel birds. It has been standard procedure to apply thiram to forest tree seed in the U.S. since it was recommended by the Fish and Wildlife Service as a bird and rodent repellent in 1956 (Dobbs 1971). Mann (1968 cited in Demeritt and Hocker 1970) found that a hungry bird will eat only one or two thiram-coated seeds before it rejects more. While thiram is commonly used in radiata pine nurseries, there is some evidence that it is detrimental to germination. Donald (1968) found that the emergence of radiata pine seedlings sown in a nursery at low density was improved by thiram applied to the seedcoat, but in another nursery where the seeds were sown at a higher density, seedling emergence was reduced by the treatment.

Thiram (tetramethylthiuram disulfide - TMTD) belongs to the dithiocarbamate group of fungicides. Generally dithiocarbamates are not phytotoxic (Hewitt 1998). The effect of thiram on conifer seed germination varies considerably. However, it has been reported to reduce the germination capacity of seedlots of slash pine (Jones 1963; Runion et al. 1991), loblolly pine, shortleaf pine (Jones 1963), white spruce (Belcher and Carlson 1968; Dobbs 1971), red pine (*Pinus resinosa* Ait.) (Belcher and Carlson 1968), eastern white pine (Demeritt and Hocker 1970) and longleaf pine (Runion et al. 1991). Thiram application has been reported to slow the germination of slash pine (McLemore and Barnett 1966b; Runion et al. 1991), loblolly pine, shortleaf pine (McLemore and Barnett 1966b), white spruce, scots pine (Lamontagne 1974) and longleaf pine (Runion et al. 1991).

As there is conflicting evidence on the direct effect of thiram on germinating seeds, the objective of this study was to determine the effect of thiram on radiata pine germination in the laboratory.

Materials and Methods

Seedlot *STBA Level 1, 1995, 4-5mm,* was soaked for 24 hours under standard conditions before thiram was applied. Thiram was applied according to the treatments shown in Table 4.5. Treatments *a* - *e* were applied by adding the appropriate amount of thiram to 5 ml distilled water which was used to water the seeds when placed in the petri dishes. The weight of each petri dish was recorded so that water loss could be monitored and water replaced where necessary to maintain the concentration of thiram. Treatment *f* was carried out by placing a small quantity of thiram as a powder into the drained vial, and shaking to coat the seeds with powder. Excess powder was removed. The seeds were placed in petri dishes and 5 ml distilled water added. Each treatment was replicated 4 times in the incubator under standard conditions.

Table 4.5 Thiram treatments applied to seeds following 24 hours of soaking.

Treatment	g a.i. / kg seeds	% recommended rate of application	g thiram per 50 seeds
a	0	0	0
b	40	50	0.0865
c	80	100	0.1730
d	160	200	0.3460
e	320	400	0.6920
f	(dusted)	approx 90	approx 0.15

Results

Germination profiles

The application of thiram to radiata pine seeds had a substantial effect on the germination time course (Figure 4.1) and the proportion of seeds germinating daily (Figure 4.2). Plots with standard errors are given in Appendix I. Germination was delayed by the addition of thiram to the seeds (Figures 4.1 and 4.2). Figure 4.1 clearly illustrates the effect of increasing thiram concentration above the recommended level on the number of seeds able to complete germination.

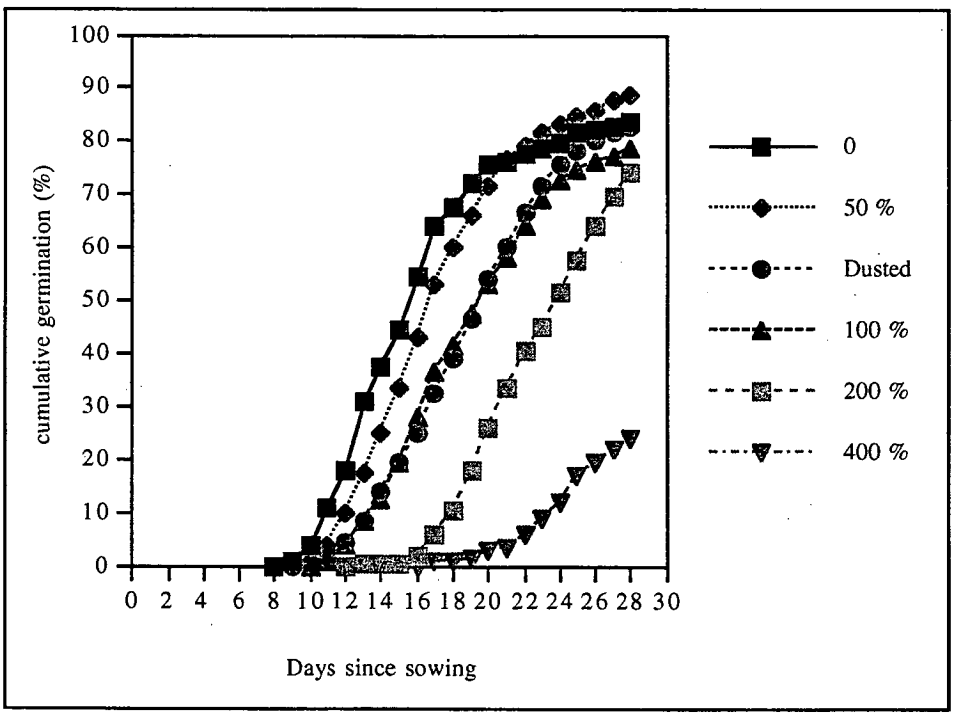

Figure 4.1 Cumulative germination profiles (as a percentage of the total number of seeds) of seeds treated with 0 (treatment a), 50% (treatment b), 100% (treatment c), 200% (treatment d), 400% (treatment e) the recommended level of thiram or dusted with thiram (treatment f). Each point is the mean of four replicates of 50 seeds.

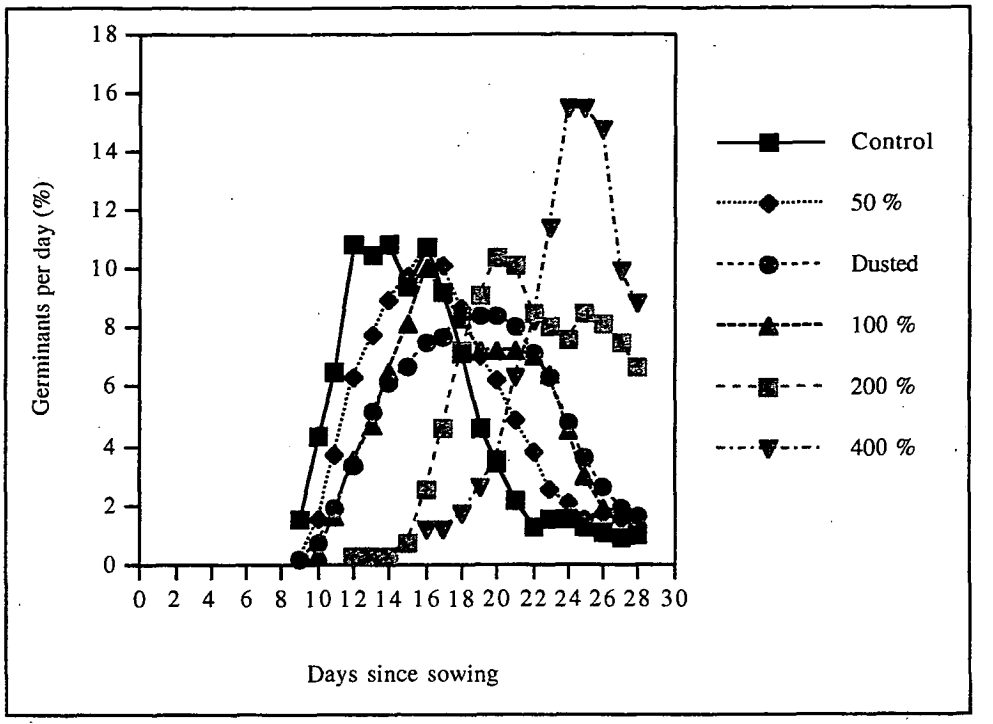

Figure 4.2 Daily germination profiles (as a percentage of the number of seeds that completed germination), of seeds treated with 0 (treatment a), 50% (treatment b), 100% (treatment c), 200% (treatment d), 400% (treatment e) the recommended level of thiram or dusted with thiram (treatment f). Germination is expressed as a three-day moving average. Each point is the mean of twelve replicates of 50 seeds.

MGT, FD and GC

Thiram significantly affected the MGT, GC and FD of this seedlot (Table 4.6). Applying thiram at the recommended rate or at higher rates significantly lengthened the time taken for the first radicle to emerge. The first germinated seed was observed significantly later in samples when twice the recommended rate of thiram was applied, compared with samples with lesser rates of application. The first germinant from samples treated with four times the recommended rate was significantly later than all other samples. Applying thiram at increasing rates progressively lengthened MGT. Seeds treated at four times the recommended rate of thiram had a significantly lower GC, while the seeds given twice the recommended level had significantly lower GC

than the seeds given 50% the recommended dose. The GC of seeds given the recommended level or less was unaffected.

Table 4.6 First germinant (FD), germination capacity (GC) and mean germination time (MGT) of seeds treated with 0 (treatment a), 50% (treatment b), 100% (treatment c), 200% (treatment d), 400% (treatment e) the recommended level of thiram or dusted with thiram (treatment f). Means are of four replicates of 50 seeds.

Treatment	GC (%)	MGT (days)	FD (days)
a	83.5	15.6	10.3
b	88.5	17.1	11.0
c	78.5	18.6	12.5
d	74.1	22.2	15.5
e	24.2	24.1	15.3
f	82.9	18.8	11.3
LSD (α = 0.05)	**12.5**	**1.3**	**1.8**

Discussion

The response of radiata pine seeds to thiram in this study confirms that thiram is toxic to the seeds, as is the case with other coniferous tree seeds (McLemore and Barnett 1966b; Demeritt and Hocker 1970; Dobbs 1971; Lamontagne 1974; Runion et al. 1991). Increasing thiram concentration lengthened the time taken for the first radicles to emerge (increased FD) and increased the average time taken by the seeds to germinate (increased MGT). Even at the recommended rate of application and with a dusting treatment which supplied the seeds with thiram at about 90% of the recommended level, thiram significantly increased the mean germination time. Such an effect could result from slowed imbibition due to coating the seed with thiram in the dusting treatment, but the effect was also noted when thiram was added to the water in

the petri dish. The germination capacity of the seedlot was not significantly decreased until the thiram concentration reached four times the recommended level of application. This contrasts with thiram applied to white spruce seeds reported by Dobbs (1971) where there was little delay in mean germination time unless germination capacity was substantially reduced.

The nature of the phytotoxic effect is not known. Hewitt (1998) suggested that because of the multi-site mode of action of the dithiocarbamate fungicides, uptake into higher plant tissue would probably cause damage. Donald (1968) found that thiram present in the seed bed was highly toxic to young radiata pine seedlings. Pawuk (1979) expressed uncertainty over the timing of damage, whether phytotoxicity of seed treatments resulted from injury to the seeds or to the emerging radicles. Jones (1963) found that abnormal seedlings were produced by loblolly, slash and shortleaf seeds if the radicle came into contact with thiram. The lower number of abnormalities produced in the field was thought to be because thiram is leached or weathered from the seed surface into the surrounding soil. As radiata pine seeds split prior to radicle emergence the growing embryo may have been exposed to the thiram before emergence, so it is impossible to determine if the phytotoxic effect was caused by injury to the seed before the seed splits, or as the radicle began to emerge.

The decreased uniformity and increased time to germination of seeds treated with thiram at, or below, the recommended level may be less of a concern to producers than the potential loss to birds and fungi, should the seeds be untreated.

4.5 Loss of oxygen from soak water

Introduction

Experiments previously reported in this chapter showed that germination of some radiata pine seedlots suffers if the seeds are presoaked. There have been suggestions in the literature that the degree of aeration of soak water plays an important role in determining the effect of presoaking on germination (Barnett 1971). The experiment reported in section 4.2 demonstrated that soak injury in large seeds of seedlot *W & F old & S.O. 2, 3, 4* could be alleviated by aerating the soak water. An investigation into the oxygen concentration of unaerated soak water was conducted to determine if seeds exhaust the oxygen supply during soaking, and if this occurs at a different rate in a seedlot susceptible to soak injury compared with one shown to be unaffected by soaking.

Materials and Methods

Seedlots previously shown to be unaffected by soaking (*STBA Level 1, 4 - 5mm, 1995*) and susceptible to soak injury (heat deteriorated *STBA Level 1, 4 - 5 mm, 1996)* were chosen for the trial. The seeds were soaked using the standard ratio of 0.4 ml distilled water per seed. The seeds were soaked under standard conditions (see General Materials and Methods) in conical flasks containing 400 ml of water and 1000 seeds. The dissolved oxygen was measured after 0, 12, 24, 48, 72 and 96 hours of soaking. Measurements were made using a Merck WTW Oxi 330 dissolved oxygen hand-held meter with a CellOx 325 dissolved oxygen probe. Trial design was a 2 seedlot, by 6 time factorial with 3 replicates.

Results and Discussion

The dissolved oxygen in the soak water was rapidly depleted during soaking (Figure 4.3). By 48 hours of soaking, the oxygen concentration was 25% of its original level,

and by 72 hours the concentration was close to zero. As shown in Chapter 7, radiata pine seeds begin respiring rapidly after imbibition begins, with oxygen demand in soaked seeds reaching about 0.05 µL / h/ mg dry weight after 12 hours soaking (Figure 7.2). In the present experiment seeds clearly experienced hypoxic conditions early in the soaking treatment.

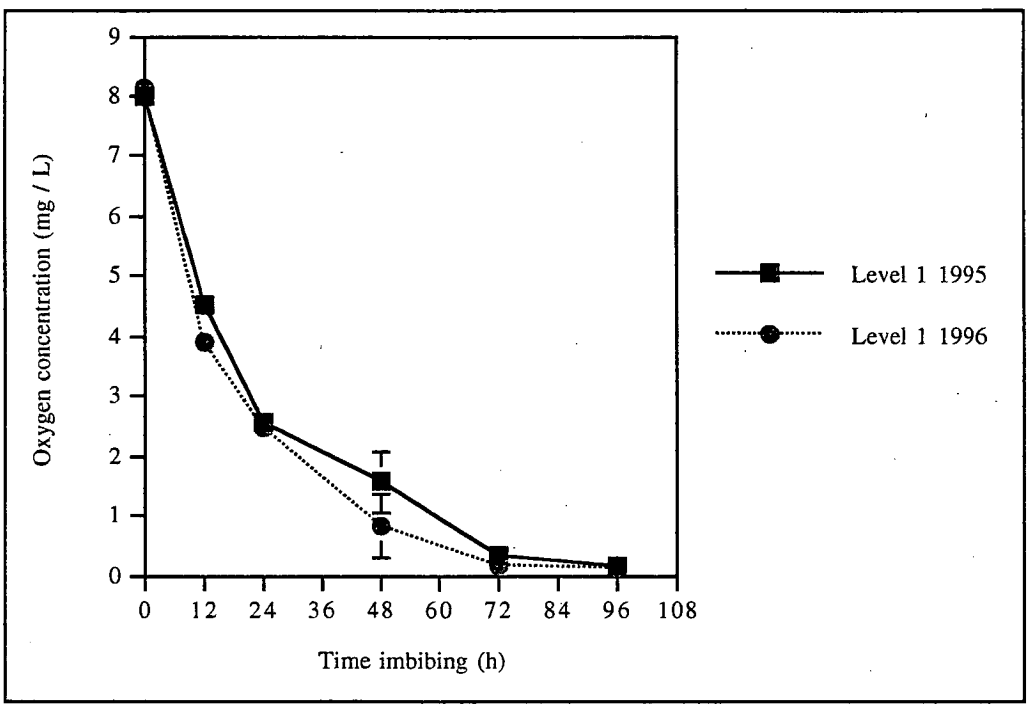

Figure 4.3 Dissolved oxygen concentration during soaking of 1000 seeds of two seedlots in 400 ml distilled water. Each point is the mean of three replicates. Standard errors are shown where larger than the symbol.

As available oxygen is depleted, the seeds must increasingly rely on anaerobic respiration to meet energy needs. Since the two seedlots showed similar rates of oxygen consumption (Figure 4.3), the ability of one seedlot (*STBA Level 1, 4 - 5mm, 1995*) to survive soaking better than the other suggests that the tolerant seedlot has mechanisms of coping with fermentation that are not available to the intolerant seedlot.

Drew (1997) reviewed root metabolism under anoxia and hypoxia and listed the possible causes of injury and death as: the accumulation of toxic end products of anaerobic metabolism including ethanol and lactate; reduced energy metabolism; and starvation of substrates for respiration. The reduced efficiency of anaerobic respiration compared with normal respiration may leave the seeds with insufficient energy to carry out processes critical to the seeds survival.

Cobb et al. (1995) found that maize seedlings exposed to hypoxia were able to survive subsequent anoxia. The period of hypoxia induced anaerobic metabolism that enabled the seedlings to survive. As the seeds in the present study was gradually starved of oxygen, the tolerant seeds may have acclimated to anoxia. Kennedy et al. (1992) and Drew (1997) emphasised the importance of avoiding accumulation of ethanol and acidification of the cytoplasm by lactate in preventing cell injury and death during anaerobiosis.

4.6 Discussion of presoaking experiments

Seedlots examined in this series of experiments varied in their response to soaking, but some were found to be susceptible to soak injury manifested as decreased germination capacity when soaked without adequate aeration. These results and evidence from the literature (Barnett 1971) suggest that an aerated soak will be less damaging than a non-aerated soak.

As imbibition proceeds and the tissues of the megagametophyte and embryo become rehydrated and reach positive turgor, respiration begins. Respiration increases linearly in imbibing seeds as the level of hydration increases (Bewley and Black 1994). As moisture content approaches the plateau phase, oxygen demand would be expected to reach its highest level. Where seeds respond negatively to soaking, the oxygen concentration of the soak water may be unable to meet the demand for oxygen. The longer the soak, the longer the seeds are dependent on the dissolved oxygen of the soak water.

The large seeds of seedlot *W & F old & S.O. 2, 3, 4* were most damaged by soaking. This may be a reflection of poor quality, whether due to genotype, handling, or environment. The limited quantity of oxygen available from the soak water is depleted in a relatively short time. A seedlot with a greater tissue volume would therefore be expected to deplete the available oxygen more rapidly. A given volume of water may therefore be able to sustain a seedlot of small seeds for a longer period of time than a seedlot of large seeds.

Where aeration was adequate or the seedlot was not susceptible to soak injury, soaking provided some benefits. In Chapter 4.2 seeds soaked for longer periods with maximum aeration had a shorter time until the first radicles emerged. Seeds of seedlot *STBA Level 1, 1995, 4-5mm* sown in the nursery (Chapter 4.3) had a significantly

shorter mean time to germination when soaked for more than 24 hours before sowing. It is likely that soaked seeds, when transferred to the incubator or sown in the soil of the nursery, were at a more advanced state of imbibition, thereby reducing the time to germination for individual seeds and the seedlot as a whole. If this is the case, prolonged soaking may lead to germination in the soak if adequate aeration is supplied, as all the requirements for germination have been met. However, as discussed in Chapter 4.3, the time in the soak was not equivalent to the reduction in FD seen in the field. This suggests that germination was unable to proceed due to the absence of factors critical to germination, including adequate oxygen and light.

5. Incubator and glasshouse germination of stratified seeds

Introduction

Stratification has long been used to overcome the dormancy of temperate conifer species. Many of these species shed their seeds in autumn or early winter (Krugman and Jenkinson 1974). Once shed, the seeds are able to take up water but cold winter temperatures and dormancy prevent germination. Over time, dormancy is overcome and the seeds are able to germinate with the onset of warmer weather. The process of stratification aims to replicate the conditions experienced by seeds during winter. It improves germination capacity and rate, and has also been reported to improve germination under less-than-ideal conditions including: dry soils (Outcalt 1991); the absence of light (Li et al. 1994); and low or high temperatures (Allen 1962b; Gosling 1988; Jones and Gosling 1994). The increased rate of germination can lead to greater seedling diameter at harvest as early emerging seedlings have a longer growing season before harvest date (Boyer et al. 1985; Barnett and McLemore 1984).

The requirements for successful stratification are sufficient moisture, low temperature, and good ventilation (Bonner et al. 1974). Stratification traditionally involved burying seeds in layers of moist sand and exposing to winter temperatures. In more recent times this treatment has been replaced by a method sometimes called naked stratification or prechilling. On a commercial scale naked stratification usually involves soaking seeds in water at 1 - 5 ° C for about 48 hours, draining the excess water, packaging the seeds in polythene bags or hessian sacks and storing at 1 - 5 ° C for 4 - 12 weeks. The seeds are usually soaked in three volumes of water to one volume of seed. In the laboratory, seeds are most commonly chilled on moist filter paper (Bonner et al. 1974; Aldhous and Mason 1994). These two methods were termed bulk and laboratory stratification respectively by Jones and Gosling (1990).

Increasing the duration of the stratification treatment has been found to increase the effect on germination of a range of species (Stanley 1958; Allen 1962b; Barnett and McGilvray 1971; Baron 1978; Hoff 1987; Jones and Gosling 1994; Mason et al. 1995). After a certain length of time, however, some seeds within the seedlot germinate at the stratification temperature (Grose 1958; Stanley 1958). Aldhous and Mason (1994) recommended stratifying shallowly dormant seeds for no longer than ten weeks, although they did not specify why the treatment should be restricted in this way.

Uncertainty exists over the dormancy status of radiata pine seeds, but Grose (1958) observed that radiata pine seeds did not germinate readily and concluded that a degree of dormancy was present. Krugman and Jenkinson (1974) recommended stratifying radiata pine seeds for 35 to 45 days, and Minko and Craig (1976) reported that stratification was the standard pre-sowing treatment for radiata pine seeds in north-eastern Victoria. Stratification was found by Grose (1958) and Minko and Craig (1976) to increase germination rate and capacity, decrease the time to achieve seedlings of a given size, and widen the range of temperatures over which the seeds germinated. More recent work in New Zealand (Anon. 1997) found that the germination rate of two radiata pine seedlots was increased by three weeks of stratification. In spite of evidence favouring stratification, Rimbawanto et al. (1988, 1989) reported that stratification did not significantly affect the germination of radiata pine seed, but it should be noted that these authors only stratified for one week.

The objectives of this study were to examine the effect of stratifying for up to 12 weeks using two stratification methods on germination of a range of seedlots in the incubator and glasshouse. Seedlings were grown in the glasshouse to determine the effect of stratification on seedling growth rates. An initial germination test was

conducted on six seedlots provided by the STBA to find seedlots to use in the stratification trial.

Preliminary Germination Test: Materials and Methods

Six seedlots, four open-pollinated and two control-pollinated were used in the initial experiment (Table 5.1). The seedlots were germinated under standard conditions in the incubator (see General Materials and Methods).

Table 5.1 Seedlots used in preliminary germination test

	Seedlot	Pollination
a	STBA Level 1, 4 - 5 mm, 1995	OP
b	STBA Level 1, 4 - 5 mm, 1996, heat damaged	OP
c	51246 x 51201	CP
d	51231 x 51235	CP
e	STBA Level 3.1, > 5 mm, 1994	OP
f	Unknown, 4 - 5 mm	OP

Results and Discussion

GC, MGT and FD

Treatment means are given in Table 5.2. GC, MGT and FD varied significantly between seedlots. Seedlot *b* had significantly lower germination capacity and longer mean time to germination than all other seedlots. Despite the significantly later time seedlot *d* began germination, it reached a higher germination capacity after 28 days than seedlots *a, b,* and *c*. Seedlot *f* had significantly higher germination capacity than seedlot *c*.

Table 5.2 Mean germination capacity (GC), mean germination time (MGT) and first germinant (FD) of six untreated seedlots used in the preliminary germination test. Means are of four replicates of 50 seeds.

Seedlot	GC (%)	MGT (days)	FD (days)
a	88.8	14.2	9.3
b	61.9	17.9	10.0
c	82.2	14.2	9.8
d	96.5	14.6	11.8
e	90.0	15.5	9.8
f	91.9	13.9	9.0
LSD (α = 0.05)	1.0	1.7	1.2

Percentage empty seeds

Counts of empty seeds (see General Materials and Methods) revealed that seedlots *a, b, e* and *f* had few, if any, empty seeds in the samples (Table 5.3). The control pollinated seedlots *c* and *d* had a substantial proportion of empty seeds. Germination values were calculated on the basis of the number of filled seeds.

Table 5.3 Mean percentage empty seeds in the preliminary germination test. Mean are of four replicates of samples of 50 seeds of six seedlots. LSD is 2.8 (α = 0.05).

Seedlot	% empty seeds
a	0
b	0
c	15.9
d	14.6
e	0
f	0.5

Stratification trial: Materials and Methods

Four of the seedlots from the germination trial were selected for use in the stratification trial (Table 5.4). The selected seedlots showed a range of germination behaviour and were selected so that the effect of stratification treatment on a range of germination characteristics could be examined.

Table 5.4 Seedlots selected from preliminary germination test for use in the stratification trial.

	Seedlot	Pollination
a	STBA Level 1, 4 - 5 mm, 1995	OP
b	STBA Level 1, 4 - 5 mm, 1996, heat damaged	OP
c	51246 x 51201	CP
d	51231 x 51235	CP

Stratification

The experiment was designed as a 2 x 4 x 5 factorial with 4 replicates, where the three factors were the method of stratification, seedlot and the duration of stratification respectively. The treatments are detailed in Table 5.5 below. Stratification was carried out on samples of 50 seeds. Bags and petri dishes were placed in a randomised arrangement in the cold room. Two control treatments were included, control A was untreated and control B was soaked for 48 hours, to indicate whether the seedlots were injured by soaking.

Table 5.5 Stratification treatments applied to seeds in the present study

Laboratory stratification method	*Bulk stratification method*
1. 50 seeds placed in each petri dish containing 3 filter papers and 5 ml distilled water **2.** Chilled at 5 ° C for 0 (control A), 2, 4, 8, or 12 weeks **3.** Petri dishes weighed periodically and water replaced where necessary	**1.** 50 seeds soaked in 20 ml distilled water in plastic bags for 48 hours at 5 ° C **2.** After 48 hours, soak water drained and seed sealed in fresh plastic bags **3.** Chilled at 5 ° C for 0 (control B), 2, 4, 8 or 12 weeks

Germination

Stratification treatments were staggered so that treatments finished at the same time. At the completion of chilling, seeds were transferred to fresh petri dishes and placed in the incubator to germinate under standard conditions (see General Materials and Methods).

Selected treatments were also sown in the glasshouse to observe post-germination stratification effects on growth. The selected treatments were seedlots *a* and *b* stratified using both methods for 0, 2 or 4 weeks. The seeds were sown in seed trays containing approximately 1.7 kg potting mix consisting of 70% pine bark, 20% peat and 10% sand. The trays had the dimensions 28 x 33 x 5 cm. Sixteen trays were divided amongst 4 blocks. Each tray was divided into 13 rows 2.5 cm apart. Twelve seeds were sown in each row, spaced 2.3 cm apart. Each tray contained 3 plots and treatments were assigned to plots according to a randomised block design. The blocks were placed on the greenhouse bench. The trays were watered before the seeds were sown, and seeds were sown just below the potting mix surface. The trays were irrigated by overhead sprinklers for 5 minutes 4 times per day. Care was taken not to place trays directly beneath sprinklers to prevent drip disturbance.

Seedlings were considered emerged once the seed coat had been lifted above the soil surface. Emergence counts were made daily for a three week period starting with the emergence of the first seedling for each plot. Plots were harvested individually, 3 weeks after the first seedling of that plot emerged. The harvested seedlings were dried in a drying oven at 70 ° C and weighed daily until constant weight was achieved. Comparisons between treatments were made using mean dry weight and coefficient of variability (CV) calculated by dividing the standard deviation by the mean, expressed as a percentage (Steele and Torrie 1980).

Seed water potential

Water potential (Ψ) of the seed at the completion of treatment was measured with a thermocouple psychrometer using the technique outlined in General Materials and Methods. The samples from the bulk treatments showed no sign of adherent water on the surface and a period of air-drying was considered unnecessary. As Ψ measurement is non-destructive, the seeds were returned to their respective samples after measurement.

Results

Laboratory germination: GC, MGT and FD

Germination capacity varied significantly with seedlot, stratification technique and duration. Seedlot interacted with stratification technique and duration of stratification, while stratification technique interacted with duration. Means, standard errors, and LSDs are given in Table 5.6(a) and (b). Treatment had little effect on GC of seedlots *a* or *d*, but stratification significantly increased the germination of seedlots *b* and *c*. Seeds bulk stratified for twelve weeks had a substantially higher GC than those stratified for shorter periods. Comparing the control soaked seeds of seedlots *b* and *c* (bulk stratified duration 0) with the control seeds (lab stratified duration 0) demonstrates that soaking the seeds without subsequent stratification significantly

reduced GC. Bulk stratified seedlot *c* had significantly higher GC than the control. Twelve weeks of stratification significantly increased GC over that of the seeds stratified for 2 or 8 weeks. Using the lab method however, only 12 weeks of stratification improved the germination capacity over that of the control. Comparing the seedlots given the same treatment, seedlots *a* and *d* regularly achieved higher germination capacity than seedlots *b* and *c*, and seedlot *b* always had significantly lower germination than the other seedlots.

Table 5.6 Mean germination capacity (GC), mean germination time (MGT) and first germinant (FD) for seeds of four seedlots stratified using two methods for 0, 2, 4, 8 or 12 weeks and germinated in the laboratory. Each value is the mean of four replicates of 50 seeds. For comparisons within and between tables LSDs (α = 0.05) are 14.2, 2.6 and 1.9 for GC, MGT and FD, respectively.

5.6(a) Bulk-stratified seed (continued overleaf)

Seedlot	Duration	GC (%)	MGT (days)	FD (days)
a	0	84.8	18.3	9.8
	2	86.1	12.1	5.8
	4	90.9	10.1	6.3
	8	79.7	9.8	4.8
	12	94.9	10.3	6.8

5.6(a) Bulk-stratified seed (continued)

Seedlot	Duration	GC (%)	MGT (days)	FD (days)
b	**0**	18.4	22.8	17.3
	2	51.5	18.0	6.0
	4	49.5	18.1	7.0
	8	58.7	15.6	6.0
	12	73.4	12.3	6.8
c	**0**	53.5	20.4	13.8
	2	68.6	15.6	6.8
	4	77.9	12.8	5.8
	8	72.8	9.9	5.3
	12	88.0	8.0	3.5
d	**0**	87.6	20.5	15.7
	2	94.4	12.2	6.3
	4	95.8	10.8	7.8
	8	85.7	12.6	8.0
	12	91.3	8.8	6.5

5.6(b) Lab-stratified seeds

Seedlot	Duration	GC (%)	MGT (days)	FD (days)
a	0	87.6	18.1	11.5
	2	94.9	14.4	6.3
	4	90.9	13.8	6.5
	8	95.0	10.1	4.0
	12	95.4	8.6	4.3
b	0	42.8	19.2	12.0
	2	65.4	17.2	6.3
	4	42.7	19.6	6.3
	8	61.2	15.0	5.0
	12	63.9	14.2	4.3
c	0	73.3	17.7	12.0
	2	83.1	13.4	6.0
	4	82.8	11.1	4.8
	8	79.2	9.3	4.0
	12	88.2	7.4	2.2
d	0	97.4	17.5	13.0
	2	97.4	13.0	6.8
	4	97.2	15.1	8.8
	8	95.4	10.0	4.5
	12	96.9	7.9	4.7

Seedlot and the duration of stratification significantly affected MGT, and significant interactions between seedlot and duration, duration and method, and method and seedlot were found. Generally, the stratified seeds of each seedlot had a shorter MGT than the unstratified seeds. Seeds stratified for 12 weeks in most cases germinated more rapidly than those stratified for a shorter period of time. The notable exception to this is the bulk stratified seedlot *a*. Seedlots *a*, *b*, and *d* lab stratified for 8 weeks, and seedlot *c* bulk stratified for 8 weeks also performed better than the seeds stratified for shorter periods of time. The rate of germination of seedlot *c* increased with bulk stratification such that increasing the duration of treatment progressively decreased MGT, although those stratified for 8 or 12 weeks took similar lengths of time to complete germination. The same trend was seen to a lesser extent in the lab stratified seeds, where 12 weeks of stratification significantly enhanced germination rate compared with 2 or 4 weeks of stratification, and 8 weeks led to faster germination than 2 weeks. Comparing the control treatments (bulk stratified duration 0 and lab stratified duration 0) demonstrates that soaking slowed the germination of unstratified seeds of seedlots *b*, *c* and *d*. Comparing the performance of the seedlots, seedlot *b* generally took the longest time to complete germination.

The time taken by the seedlot to begin germinating (FD) was also significantly affected by all three factors - seedlot, stratification technique and length. Once again there were significant interactions between seedlot and the method of stratification, method and duration, and seedlot and duration. In each seedlot the time taken for the first radicle to emerge was significantly decreased by stratification. The first emerged radicle of seedlots *a* and *b* was observed earlier in samples treated with lab stratification for 8 or 12 weeks, or 12 weeks respectively. The first radicle was observed earlier in samples of seedlot *c* bulk stratified for 12 weeks or lab stratified for 8 or 12 weeks. The latter result was also seen in seedlot *d*.

The seedlots all showed first germinants at the same time when unstratified and unsoaked (lab stratified duration 0), or stratified for 2 weeks, but as the stratification period was extended seeds of seedlot *c* began to germinate earlier than the other seedlots. The soaked unstratified control seeds of seedlot *b* had the first germinant recorded at a significantly later date than the other seedlots.

Laboratory germination: germination profiles

Germination profiles of seedlots *b* and *c* are reported as these seedlots showed the greatest response to stratification treatment. Figures 5.1 and 5.2 show the cumulative germination profiles of seedlot *b*.

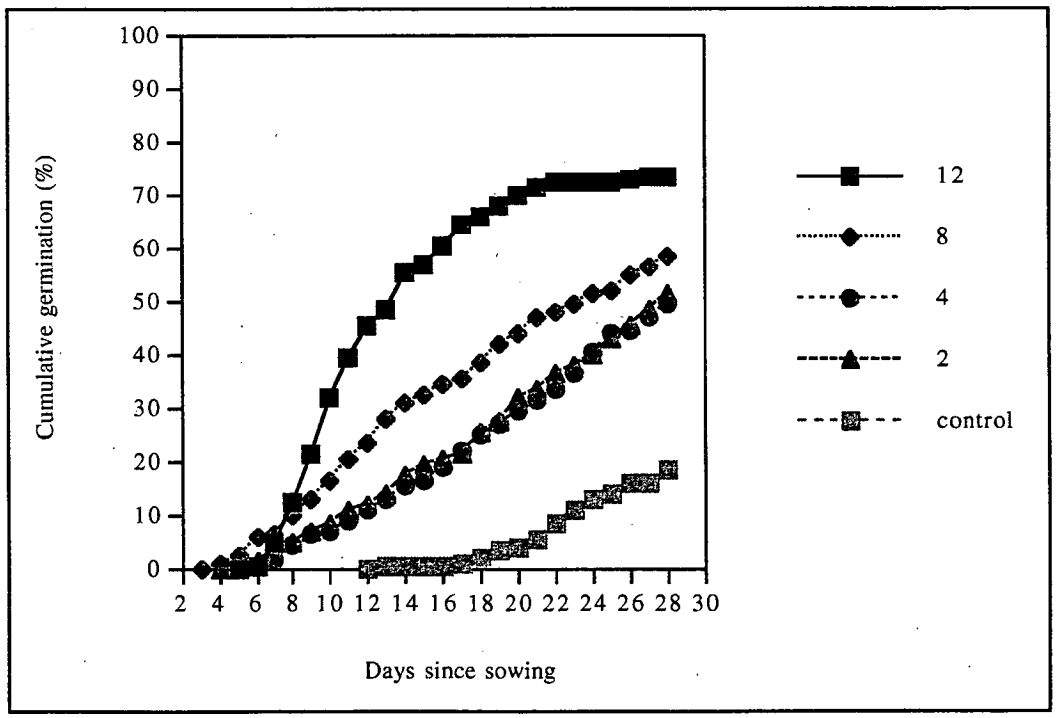

Figure 5.1 Cumulative germination profiles (as a percentage of the total number of seeds) of bulk stratified seedlot *b*, stratified for 0, 2, 4, 8 or 12 weeks. Each point is the mean of four replicates of 50 seeds.

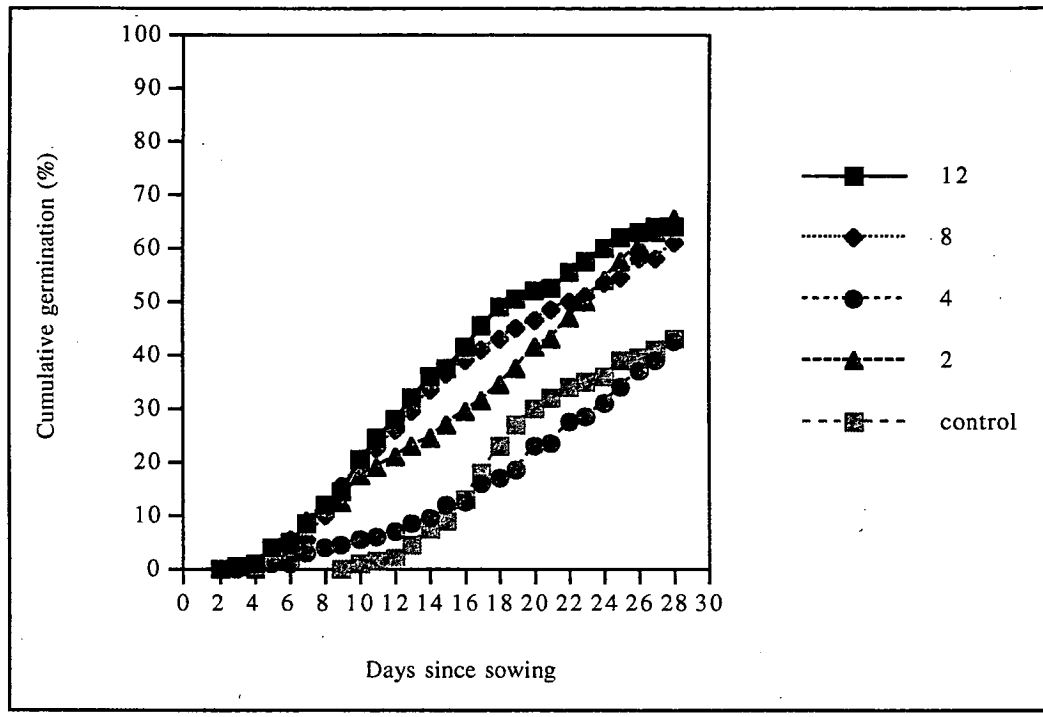

Figure 5.2 Cumulative germination profiles (as a percentage of the total number of seeds) of lab stratified seedlot *b*, stratified for 0, 2, 4, 8 or 12 weeks. Each point is the mean of four replicates of 50 seeds.

The germination of the soaked and unsoaked controls was still increasing at the end of the experiment, whereas after 12 weeks of stratification the germination profiles reach a plateau towards the end of the germination period, particularly in the case of the bulk-stratified seeds. This is less evident in Figures 5.3 and 5.4 for seedlot *c*, the germination of soaked-unstratified seeds is still increasing at the end of the germination test, but the untreated seeds attained a plateau by this time. Appendix II gives plots with standard errors.

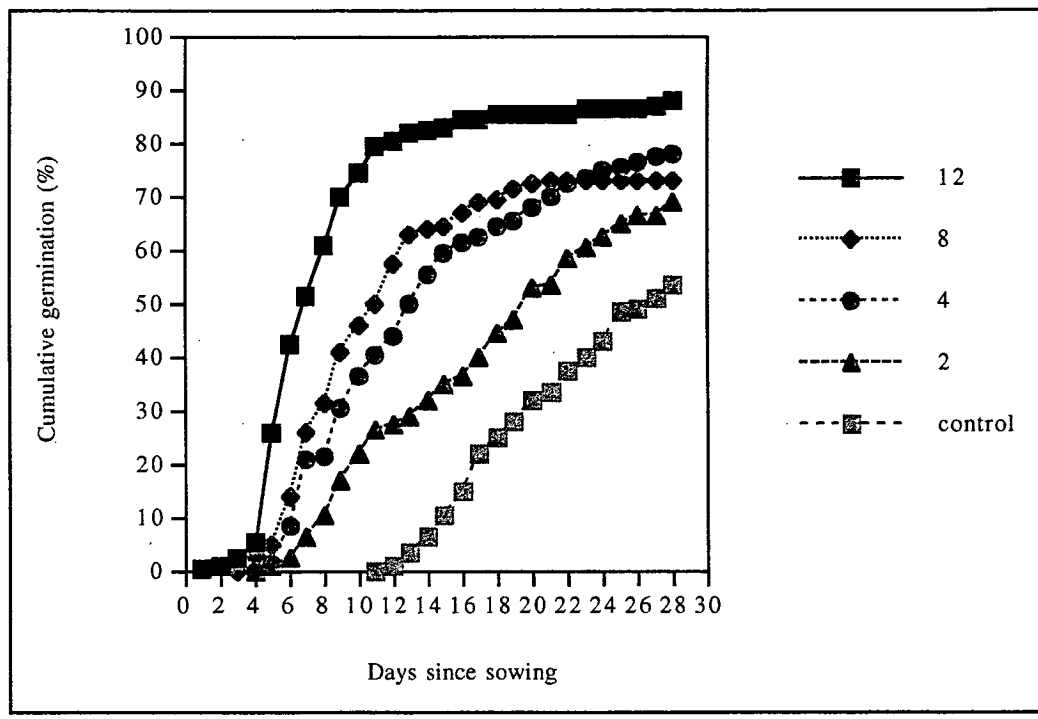

Figure 5.3 Cumulative germination profiles (as a percentage of the total number of seeds) of bulk stratified seedlot *c*, stratified for 0, 2, 4, 8 or 12 weeks. Each point is the mean of four replicates of 50 seeds.

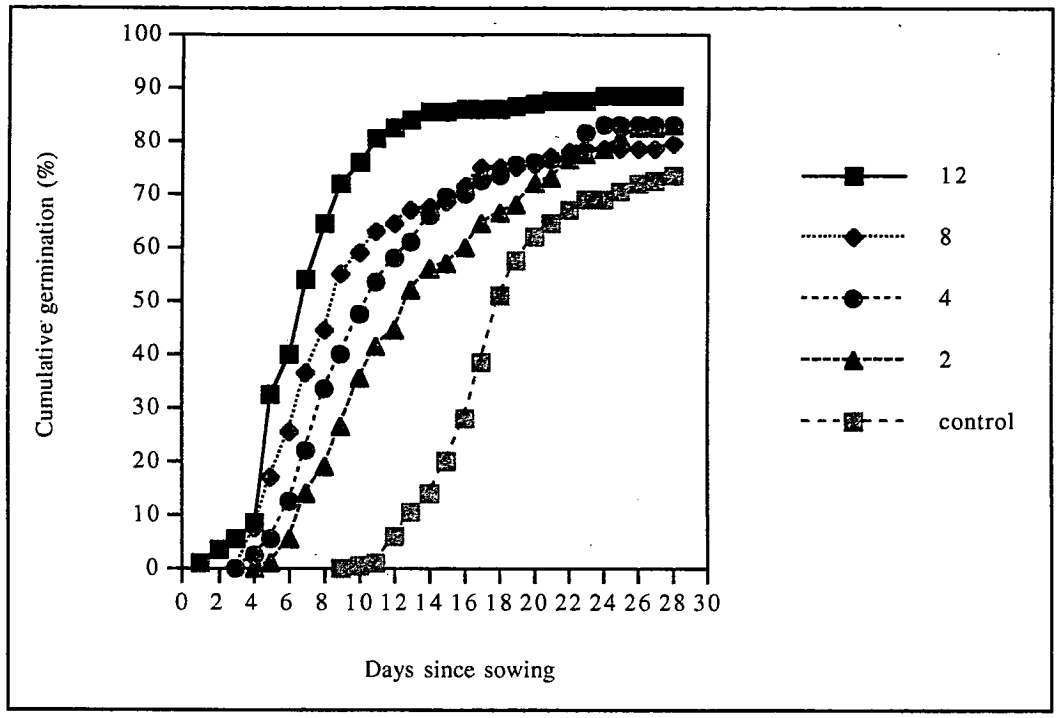

Figure 5.4 Cumulative germination profiles (as a percentage of the total number of seeds) of bulk stratified seedlot *c*, stratified for 0, 2, 4, 8 or 12 weeks. Each point is the mean of four replicates of 50 seeds.

Glasshouse germination: GC, MGT and FD

GC

In the glasshouse, germination capacity was significantly influenced by seedlot and the method of stratification. As the duration of the treatment did not significantly affect GC, results are averaged across the treatment duration. Treatment did not significantly affect the GC of seedlot *a*, but soaked seeds of seedlot *b* had significantly lower germination than unsoaked seeds, regardless of any subsequent stratification (Table 5.7). Seedlot *a* had significantly higher germination than seedlot *b* as has been the case with all other comparisons.

Table 5.7 Germination capacity (GC) of two seedlots germinated in the glasshouse. Means are taken across the duration of the treatment (0, 2 or 4 weeks) as the length of treatment did not significantly affect germination. Means are of 12 experimental units (three durations and four replicates) of 50 seeds. LSD (α = 0.05) is 9.41

Seedlot	Method	GC (%)
a	lab	77.5
	bulk	69.5
b	lab	46.7
	bulk	36.7

Table 5.8 Mean first germinant (FD) and mean germination time (MGT) of two seedlots stratified using two methods for 0, 2 or 4 weeks and germinated in the glasshouse. Each value is the mean of four replicates of 50 seeds.

Seedlot	Method	Duration	MGT (days)	FD (days)
a	lab	0	15.2	12.5
		2	14.1	10.3
		4	10.7	8.5
	bulk	0	15.9	11.8
		2	13.1	9.8
		4	13.4	11.3
b	lab	0	17.7	13.0
		2	15.2	10.8
		4	14.2	8.8
	bulk	0	18.1	13.8
		2	15.2	10.5
		4	17.5	11.5
LSD (α = 0.05)			1.1	1.7

MGT

Seedlot, method of stratification, and the duration of treatment had a significant effect on MGT in the glasshouse. There were significant interactions between the method of stratification and the duration, and between seedlot and duration. Generally, seedlot *a* germinated earlier than seedlot *b,* when comparing seeds given equivalent treatments (Table 5.8). Within each seedlot the unstratified control treatments (soaked and unsoaked) were not significantly different but the unstratified seeds germinated significantly later than the stratified seeds. An exception was seedlot *b*; seeds bulk stratified for 4 weeks was not significantly different to unstratified seeds. Lab stratified

seedlot *a* germinated more rapidly when stratified for 4 rather than 2 weeks, while bulk stratified seeds were not affected by the length of stratification. In contrast, lab stratified seedlot *b* was not affected by the length of stratification, while bulk stratification for 2 weeks led to more rapid germination than 4 weeks. Seeds of each seedlot stratified for 4 weeks had a shorter MGT when stratified using the lab rather than the bulk method but with only 2 weeks of stratification the treatments gave the same MGT.

FD

The time taken for the first seed of a treatment to germinate (FD) was significantly affected by seedlot, stratification method and duration (Table 5.8) and an interaction between stratification method and duration was found. Samples of each seedlot treated in the same way began germination at the same time, except in the case of the soaked unstratified seeds, where seedlot *a* was significantly earlier than seedlot *b*. Within each seedlot the trends in treatment effects were the same, there were no significant differences between the two control treatments but the FD of unstratified seeds was significantly later than stratified seeds. FD was reduced when the duration of lab stratification was increased.

Glasshouse germination: Seedling dry weight

Mean seedling dry weight after three weeks of growth was not significantly affected by stratification, means are given in Table 5.9. The dry weight of seedlings of seedlot *a* were significantly less variable (CV 24.0 +/- 0.9) than seedlings of seedlot *b* (31.3 +/- 1.0), but CV was not affected by stratification.

Table 5.9 Seedling dry weight three weeks after emergence. Two seedlots were stratified using two methods for 0, 2 or 4 weeks. Values are the mean of the number of germinated seeds for each of four replicates of 50 seeds. Treatment means are not significantly different (α = 0.05).

Seedlot	Duration	Lab stratified	Bulk stratified
a	0	48.8	52.9
	2	48.4	46.6
	4	46.6	52.6
b	0	46.0	48.3
	2	45.6	47.2
	4	42.6	48.8

Water potential

Seed water potential (Ψ_s) at the end of the stratification treatment was significantly affected by the seedlot, stratification method and duration. There was a significant interaction between seedlot, method of stratification and duration of stratification. The method of stratification seldom affected Ψ_s (Table 5.10). The Ψ_s of seedlots *a* and *d* stratified for 8 weeks was lower if stratified using the lab method, and seedlot *a* stratified for 4 weeks. In contrast, Ψ_s in seedlot *b* was lower after 4 weeks of treatment if the seeds were treated with the bulk stratification method.

Generally, Ψ_s was higher after longer periods of stratification. However, seedlots *a* and *b* bulk stratified for 4 weeks had significantly lower Ψ_s than those stratified for 2 weeks, after which Ψ_s began to rise. The Ψ_s of seedlot *c* did not change significantly during bulk stratification, and changed little during lab stratification. The Ψ_s of seedlots *a*, *b* and *d* did not change significantly from that reached after 8 weeks of bulk stratification when stratification was extended to 12 weeks.

When stratified using the lab method the seeds reached Ψ_s of -0.5 to -4.0 MPa after 12 weeks of treatment. However, the Ψ_s of seedlot *b* was unaffected by the duration of treatment. Once again, the Ψ_s of seedlot *a* was significantly lower at 4 weeks than at 2 weeks. The Ψ_s of seedlots with the same treatment varied considerably when stratified for less than 8 weeks when bulk stratified, or 12 weeks when lab stratified. With the longer durations of stratification Ψ_s did not vary significantly between seedlots.

Table 5.10 Ψ_s (MPa) of seeds of four seedlots after stratification using two methods for 0, 2, 4, 8 or 12 weeks. Each value is the mean of four replicates of 15 seeds. LSD is 4.5 (α = 0.05).

Seedlot	Duration	Lab stratified	Bulk stratified
a	2	- 13.4	- 9.3
	4	- 24.8	- 17.6
	8	- 14.9	- 2.5
	12	- 4.0	- 2.3
b	2	- 4.6	- 5.3
	4	- 6.2	- 20.7
	8	- 6.9	- 2.6
	12	- 2.0	- 2.3
c	2	- 3.9	- 4.1
	4	- 3.8	- 3.0
	8	- 5.7	- 1.4
	12	- 0.5	- 2.1
d	2	- 9.0	- 9.4
	4	- 13.8	- 13.2
	8	- 8.5	- 2.7
	12	- 1.2	- 2.7

Split seeds were observed in the samples stratified for 12 weeks, particularly those stratified using the laboratory method (Table 5.11). Seedlot *c* had the greatest percentage of split seeds at the end of treatment, and this was reflected in FD.

Table 5.11 Mean percent split seeds after 12 weeks of bulk or lab stratification. Means are of four replicates of 50 seeds.

Seedlot	Bulk stratified	Lab stratified
a	0	1.5 ± 1.0
b	0	1.5 ± 1.0
c	2.9 ± 2.3	6.4 ± 1.4
d	0	0.5 ± 0.5

Discussion

The stratification treatments applied in this study were found to benefit the germination of the radiata pine seedlots tested. Germination was enhanced by an increased rate of germination in the incubator and glasshouse, higher germination capacity of some seedlots germinated in the incubator, and the overcoming of soak injury in some seedlots. The longest duration of treatment (12 weeks) led to the most rapid germination.

The germination capacity of seedlots *b* and *c* was increased by stratification and decreased by soaking. The effect of soaking on germination capacity was overcome if soaking was followed by stratification in all cases but one (seedlot *c* stratified for 2 weeks). In the glasshouse, lab stratified seeds of seedlot *b* achieved a higher germination capacity than bulk stratified seeds when stratified for 4 weeks. This suggests that the seeds may require a relatively long period of stratification to overcome soak injury when grown under glasshouse conditions.

The dry weight of glasshouse grown seedlings was measured 3 weeks after the emergence of the first seedling in that plot. The analysis of dry weight indicated that stratification decreased the time to germination but did not improve seedling growth rates, as there were no differences between treatments. This result is consistent with that reported by Barnett and McLemore (1984) who found that pine seedlings grown from stratified seeds emerged earlier and hence had a longer growing season before harvest date than later-emerging unstratified seedlings, and the seedlings from stratified seeds were therefore larger at harvest.

The Ψ_s of the stratified seeds increased from the fourth week of stratification to reach Ψ_s above -5 MPa after 12 weeks of stratification (Table 5.10) apart from seedlot *c* where Ψ_s did not vary greatly between treatments. Rising Ψ_s suggests that the hydration of the seed increased during stratification. Stanley (1958) and Schneider and Gifford (1994) found that the water content of sugar and loblolly pine seeds, respectively, increased during stratification and the increase in water content correlated well with increased germinability. Germination is thought to occur in the range of approximately -2 to 0 MPa (Bradford 1995). It is clear that some of the seeds in the samples stratified for 12 weeks were within this range as split seed coats were observed (Table 5.11). Split seed coats are most likely to be caused by the expansion of tissues within the seeds due to water uptake, as the seed progresses from phase II to phase III of imbibition. The reduced MGTs of stratified seeds may have been the result of the increased level of hydration, as less time was required for imbibition once the seeds were placed in conditions suitable for germination.

The germination rate of lab stratified seedlot *c* was significantly faster when stratified for 12, 8, or 4 weeks rather than 2. When bulk stratified for 12 or 8 weeks the seeds germinated earlier than did those stratified for 4 weeks, and 4 weeks of treatment led to

earlier germination than 2 weeks. As the water potential of seedlot *c* did not change significantly during treatment it cannot be said conclusively that increased hydration was wholly responsible for decreasing the time to germination of this seedlot. For germination to be improved by treatment without any evidence of increased imbibition, some other mechanism must be involved.

Since it cannot be said with any confidence whether or not radiata pine seeds are dormant, any discussion of the mechanisms by which stratification acts must be speculative. Carpita et al. (1983) proposed that stratification increased the "growth potential" of loblolly pine embryos, enabling the radicle to overcome the resistance of the seed coat. They suggested that stratification induced a change in sucrose absorption and metabolism in the embryo, allowing an increased rate of embryo elongation. Downie et al. (1997b) found that the resistance of the megagametophyte and nucellus of white spruce seed was decreased by stratification, probably as a result of enzyme activity. Radiata pine seed germination may be controlled to some extent by the tissues surrounding the embryo, as seen in other seeds of the Pinaceae (Kozlowski and Gentile 1959; Barnett 1972, 1976; Baron 1978; Carpita et al. 1983; Hoff 1987; Downie and Bewley 1996; Downie et al. 1997b). These tissues may have been weakened during stratification which would allow the seeds to take up sufficient water to initiate radicle expansion. Weakening during the shorter treatments may not have proceeded as far, leading to longer MGTs in seeds stratified for less than 12 weeks.

Stratification increased the GC of the seedlots affected by soaking and untreated seeds of seedlots *b* and *c*. Germination capacity can be an indicator of seedlot quality. Poor seed quality has been attributed to a range of factors including genetics and handling (Delouche 1980; Coolbear 1995; Finch-Savage 1995). The poor quality of seedlot *b* had been induced by overheating. At least a proportion of the ungerminated seeds were

viable, as an increased proportion of the seedlot was able to germinate following stratification.

Stratification may improve GC in a number of ways. Stratification conditions may allow repair processes to proceed within the seed. Alternatively, deterioration may make the seed more susceptible to stress conditions during germination. Rising water potential indicated seeds imbibed slowly during stratification, so they were able to germinate rapidly once exposed to conditions suitable for germination, thereby reducing the time of exposure to stressful conditions. Another explanation may be that a fraction of the seedlot was dormant, dormancy alleviation by stratification would thus allow more seeds to germinate.

A fourth explanation may lie in the improvement of germination rate by stratification. Increased germination capacity may be due to more of the seedlot germinating within the test period, such that the germination curve is shifted forward in time. There is some evidence that shorter MGTs allowed more of the seeds to complete germination within the test period (Figures 5.1 to 5.4).

The method of stratification had little effect on Ψ_s, GC or MGT. The seeds stratified on moist filter paper had a moisture source available throughout the stratification period, while the bulk stratified seeds were only provided with moisture for the first 48 hours of stratification. This suggests that the seeds were able to absorb sufficient water during the soak to enable the embryo to reach positive turgor during stratification, given enough time. In sugar pine seeds stratified at 5 ° C the seedcoat became fully hydrated within 30 days of chilling, after which time the moisture content decreased (Stanley 1958). The embryo and endosperm continued taking up moisture until chilling ceased. It is possible that the seedcoat became hydrated very quickly, and then acted as

a reservoir for the tissues contained within, as Schneider and Renault (1997) reported in soybean seeds.

Although stratification proved to be a beneficial treatment by decreasing MGT and increasing GC of seedlots with relatively poor germination, this experiment highlighted the problems long associated with stratification, in particular premature germination. The splitting of the seedcoat seen in the longer stratification treatments may present difficulties if stratified seeds were to be sown in a nursery. While radicles did not emerge, the Ψ_s readings and observation of split seed coats suggests that this would have occurred had stratification been prolonged. This presents a problem if, for example, sowing must be delayed due to bad weather. Seed coat splitting also increases the susceptibility of the embryo to drying out while sowing.

6. Stratification in atmospheres of controlled water potential

Introduction

Moist radiata pine seeds stratified for sufficiently long periods of time will eventually germinate at stratification temperature, as the seeds move from phase II to phase III of imbibition (see previous chapter). During phase II, at low temperature, germination processes are able to proceed, leading to hastened radicle emergence once seeds are transferred to conditions more suitable for germination (Downie et al. 1997b). Levitt and Hamm (1943) speculated that it may be possible to supply seeds with sufficient moisture to enable them to undergo the processes associated with germination occurring in phase II but insufficient to permit germination. Wilson (1971, 1973) applied this principle by imbibing crested wheatgrass (*Agropyron desertorm* Fisch. ex Link) seeds in vapour over solutions and observed that the seeds germinated more rapidly than untreated seeds. Downie and Bergsten (1991) and Jones and Gosling (1994) took a different approach and supplied eastern white pine; and Douglas-fir, lodgepole pine and sitka spruce seeds, respectively, with a known quantity of water to bring them to the required moisture content. The treated seeds germinated more rapidly than did the controls.

It is important that the moisture content at which the seeds are held is at the right level. If the moisture content is too low, the processes associated with germination may not proceed. Alternatively, if seed moisture content is too high the seeds may germinate. This requires an understanding of the process of imbibition. The desired seed moisture content for chilling is at, or near, the plateau moisture content of phase II. The required moisture content is sometimes called the target moisture content (TMC) (Jones and Gosling 1994). The same principle would apply to seeds held in vapour at a water potential above the threshold at which cells elongate. Although the threshold water potential and content for germination varies within a population of seeds (Hegarty

1978), the TMC approach has been applied successfully to prevent germination during treatment (Jones and Gosling 1994). Falleri (1994) reported that the germination capacity of maritime pine (*Pinus pinaster* Ait.) seeds was reduced when the external water potential was -0.4 MPa or below, but 50% of the seeds were still able to germinate at water potentials of -0.8 MPa. Similarly, Barnett (1969, cited in Falleri 1994) found that an external water potential of less than -0.8 MPa was required for any great effect on germination of longleaf and slash pine seeds.

Jones and Gosling (1994) and Downie and Bergsten (1991) held relative humidity at 80% and 90% respectively. As the seeds were held in containers open to the atmosphere it can be assumed that relative humidity would have played a role in determining equilibrium moisture content, as atmospheric Ψ is determined by relative humidity and temperature (Nobel 1974), and slow movement of moisture between the seeds and their environment would be expected in response to a Ψ gradient.

Pretreatments that limit moisture availability in this way share the principle with priming, which is often applied to vegetable seeds to improve germination. Simak (1985) stated that pretreating Scots and lodgepole pine seeds by controlling moisture content during incubation was at least as efficient as priming in PEG, and was easier to apply and less risky to the seeds as extra aeration need not be supplied. The optimal moisture content range for incubating Scots and lodgepole pine was found to be slightly less than the moisture content obtained by priming seed in PEG.

The aim of this study was to develop a treatment that would allow radiata pine seed germination to benefit from pretreatment by limiting water uptake through control of Ψ of the atmosphere in which the seeds were held, while avoiding the problems associated with other treatments, in particular soak injury and premature germination associated with prolonged stratification.

Materials and Methods

The experiment was a 4 by 5 factorial with 4 replicates. Seeds were chilled for 4, 8, 12 or 16 weeks at 5 ° C over NaCl solutions of 0, 0.7, 1.4, 2.0 and 2.7 *m*. Seeds were sampled from seedlot *STBA Level 1, 4 - 5 mm* for use in the trial. The sample was divided into 80 subsamples of 100 seeds for each plot.

Controlled Ψ environments. NaCl solutions were prepared according to Lang (1967). Solution water potential was measured using the psychrometer at room temperature (23 ° C). Measured water potential was corrected to 5 ° C using the temperature correction equation given in the psychrometer handbook (see General Materials and Methods). Molalities and water potentials are given in Table 6.1. Due to inconsistencies between the theoretical water potential values, and the measured values, Ψ_{sol} are referred to as molalities in the text.

Table 6.1 Solution molalities and water potentials (MPa)

Molality	Ψ (Lang 1967)	Measured Ψ at 23 ° C	Measured Ψ corrected to 5 ° C
0	0	-0.12 ± 0.03	-0.05 ± 0.02
0.7	-3	-2.81 ± 0.02	-1.54 ± 0.01
1.4	-6	-5.66 ± 0.06	-3.10 ± 0.03
2.0	-9	-8.71 ± 0.06	-4.77 ± 0.03
2.7	-12	-11.93 ± 0.07	-6.53 ± 0.04

Each subsample of seeds was chilled in a 150 ml sealed plastic container. A shelf with a wire mesh floor was placed 2.5 cm below the top of the container. The shelves were made by cutting 2 cm off the tapered end of a container, using a scalpel blade heated in a bunsen burner flame. A window was cut into the base of the shelf leaving a frame of 1 cm around the edge. The wire mesh floor was placed over the window. The seeds

were placed in a wire mesh cage of approximately 4.5 x 4.5 x 1 cm which was placed on the shelf so that the seeds were held above the solution. Each container (plot) held 50 ml of the appropriate solution.

Chilling. The containers were placed in a 5 ° C coldroom in a completely randomised design. The seeds were chilled for 4, 8, 12 or 16 weeks. Treatments were commenced at different times so that all treatments finished together. Three experimental units, each consisting of one of the controlled atmosphere containers holding 150 seeds, were placed in the cold room with the 16 week treatment. These experimental units were included so that Ψ_s and moisture content could be measured during chilling. Seeds were sampled from these replicates during chilling, and Ψ_s and moisture content of the sampled seeds was measured.

Measuring Ψ and moisture content. Ten seeds were sampled from each of the 3 replicates after 1, 3, and 7 days of chilling and thereafter at weekly or fortnightly intervals. Water potential was measured using the psychrometer, as described in General Materials and Methods. Surface drying was unnecessary as the seeds were not in contact with the solution. Moisture content was determined following Ψ_s measurement using the method described in General Materials and Methods and expressed as a percentage of the dry weight of the ten seeds. Moisture content was not expressed as a percentage of fresh weight because the seeds were randomly sampled after imbibition and the initial (fresh) weight was therefore not known. Ψ_{sol} was measured at intervals during chilling but was found to remain constant.

Germination. Following chilling, the seeds were removed from containers. The number of mouldy, split and germinated seeds was recorded. Mouldy seeds were discarded. The seeds were rinsed in distilled water, 50 seeds sampled and added to

petri dishes to germinate under standard conditions in the incubator (see General Materials and Methods).

Results

GC, MGT and FD

Germination capacity was significantly affected by the length of stratification. A significant interaction between factors was not found. Means are therefore taken across all Ψ_{sol}, and are given in Table 6.2. Seeds stratified for 12 weeks had significantly higher germination than those stratified for 4, 8 or 16 weeks.

Table 6.2 Mean germination capacity (GC) of seeds stratified for 4, 8, 12 or 16 weeks. Means are of 20 experimental units (five solution concentrations and 4 replicates) each with 50 seeds. LSD = 3.9 (α = 0.05).

Weeks chilling	GC (%)
4	88.6
8	91.6
12	96.4
16	89.5

Mean germination time was significantly influenced by the two factors - length of chilling and Ψ_{sol}. There was also a significant interaction between the two factors, shown in the plot of MGT versus solution concentration (Figure 6.1). Seeds chilled over solutions with concentrations of 0 - 1.4 *m* germinated much more rapidly than seen in previous experiments. Ψ_{sol} did not have a significant effect on MGT when seeds were stratified for 4 weeks, but when stratified for 8, 12 or 16 weeks those stratified with solutions of 0 or 0.7 *m* had a significantly shorter MGT than those stratified over more concentrated solutions and hence lower Ψ_{sol}.

Generally, the longer the seeds were held at low temperature, the earlier they completed germination. Germination was not significantly hastened when chilling was extended from 4 weeks to 8 except when held at a Ψ_{sol} of 0, but those seeds stratified for 12 weeks germinated earlier than those stratified for 4 or 8 weeks at all Ψ_{sol}. It appears that 12 weeks was the ideal duration of chilling as seeds chilled for 16 weeks germinated more slowly than those chilled for 12 weeks except at Ψ_{sol} of 0. The seeds chilled for 16 weeks over solutions of 0.7, 1.4, 2.0 and 2.7 *m* germinated significantly later than those chilled for 12 weeks.

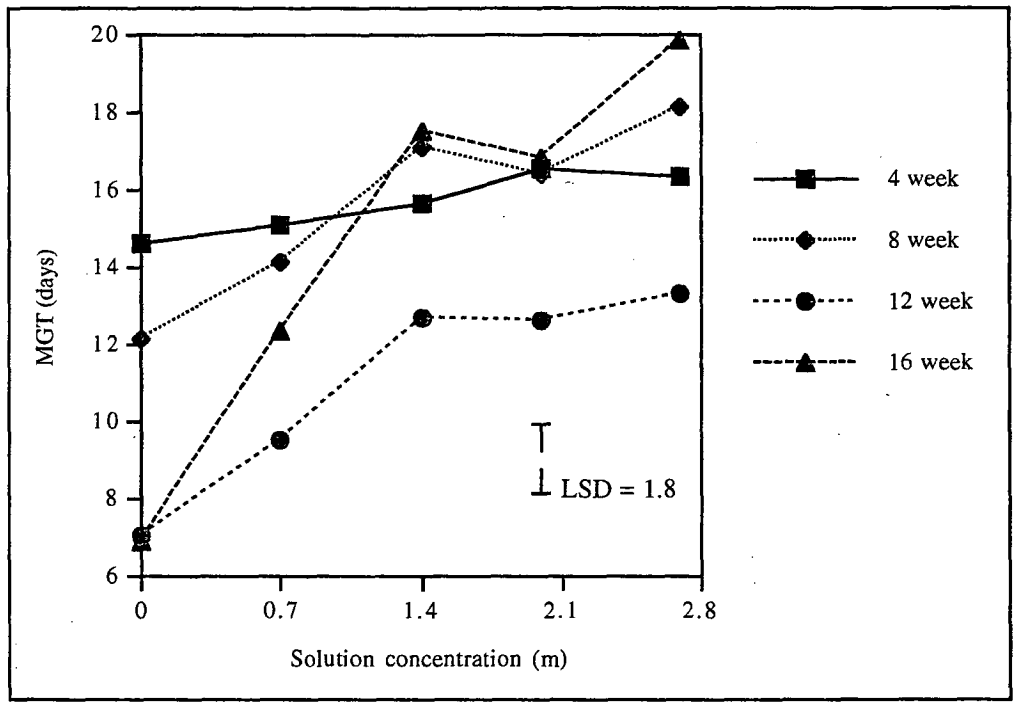

Figure 6.1 Mean germination time (MGT) of seeds chilled for 4, 8, 12 or 16 weeks over 0, 0.7, 1.4, 2.0 and 2.7 *m* NaCl solutions. Each point is the mean of four replicates of 50 seeds. The LSD is given at the lower right corner of the graph.

The time to the first germinant (FD) was significantly affected by the factors chilling time and Ψ_{sol}. There was a significant interaction. Treatment means are shown in Figure 6.2. Once again, FD of seeds chilled over solutions of 0 to 1.4 *m* was substantially earlier than seen in previous experiments. Ψ_{sol} did not significantly affect

FD when seeds were stratified for 4 weeks, but when stratified for 8, 12 or 16 weeks those stratified with solutions of 0 or 0.7 *m* NaCl the first seed germinated earlier than those stratified with lower Ψ_{sol}.

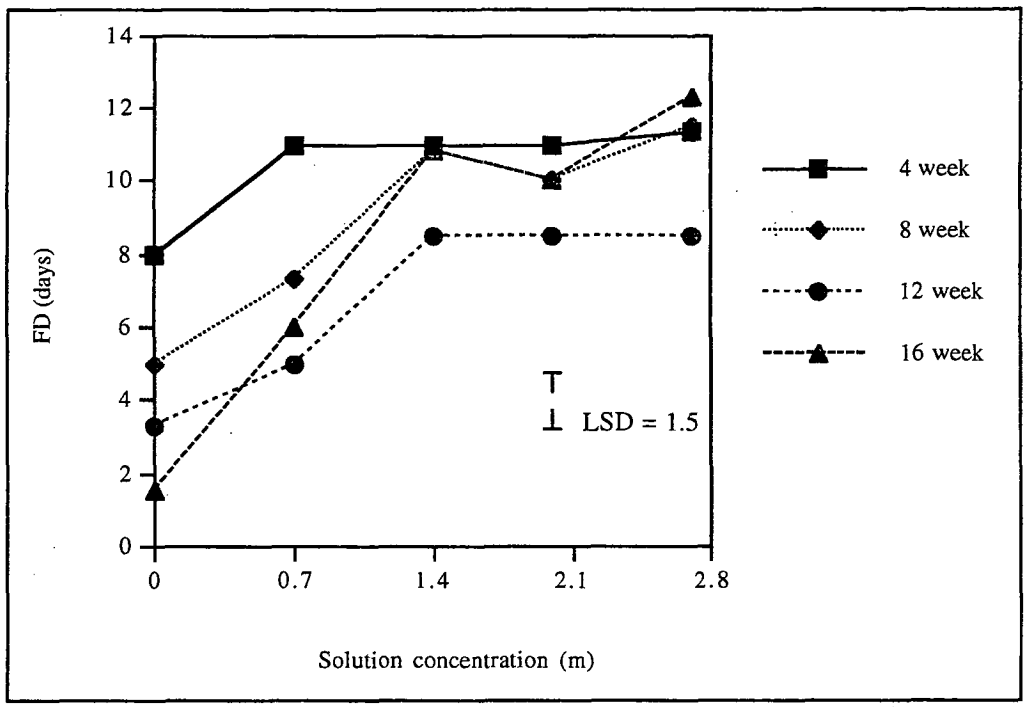

Figure 6.2 Mean day of first germinant (FD) of seeds chilled for 4, 8, 12 or 16 weeks over 0, 0.7, 1.4, 2.0 and 2.7 *m* NaCl solutions. Each point is the mean of four replicates of 50 seeds. The LSD is given at the lower right corner of the graph.

When Ψ_{sol} was 0, FD was significantly earlier when chilling was extended. This was also the case when chilled for 12 weeks over solutions of 0.7 *m* concentration and above, compared with 4 or 8 weeks. However, there were no significant differences between those chilled for 4 or 8 weeks. Seeds chilled for 16 weeks when solution concentration was 1.4 or 2.7 *m* had a later FD than those chilled for 12 weeks at the same Ψ_{sol}.

It appears that 12 weeks was the ideal duration of chilling as seeds chilled for 16 weeks only germinated earlier than those chilled for 12 weeks if the Ψ_{sol} was 0. The

seeds chilled for 16 weeks over NaCl solutions germinated significantly later than those chilled for 12 weeks.

Germination profiles

Germination profiles of seeds chilled for 4 and 12 weeks are included to illustrate the results discussed above. The germination profiles of seeds chilled for 4 weeks varied little with Ψ_{sol} (Figure 6.3).

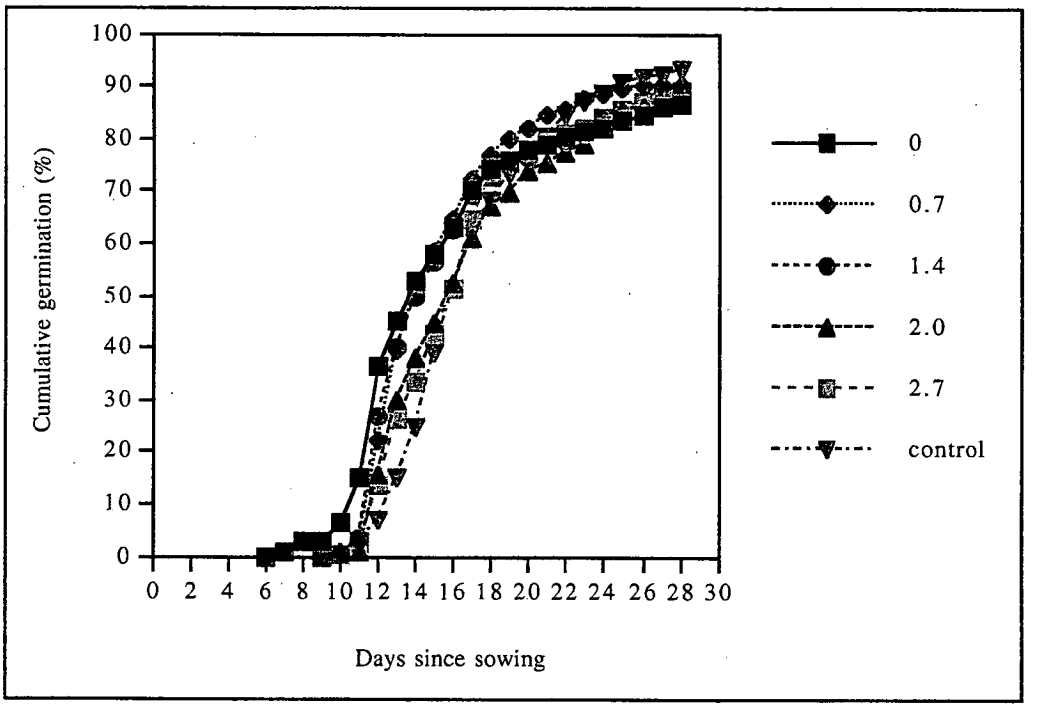

Figure 6.3 Cumulative germination (as a percentage of the total number of seeds) of seeds chilled for 4 weeks over 0, 0.7, 1.4, 2.0 and 2.7 *m* NaCl solutions. Each point is the mean of four replicates of 50 seeds.

As the chill duration was extended, however, Ψ_{sol} affected the timing of germination. The germination of seeds chilled over 0 or 0.7 *m* solutions for 12 weeks was more rapid than the control (Figure 6.4), but the curves of seeds chilled over more concentrated solutions cannot be distinguished from one another. Plots with standard errors are given in Appendix III.

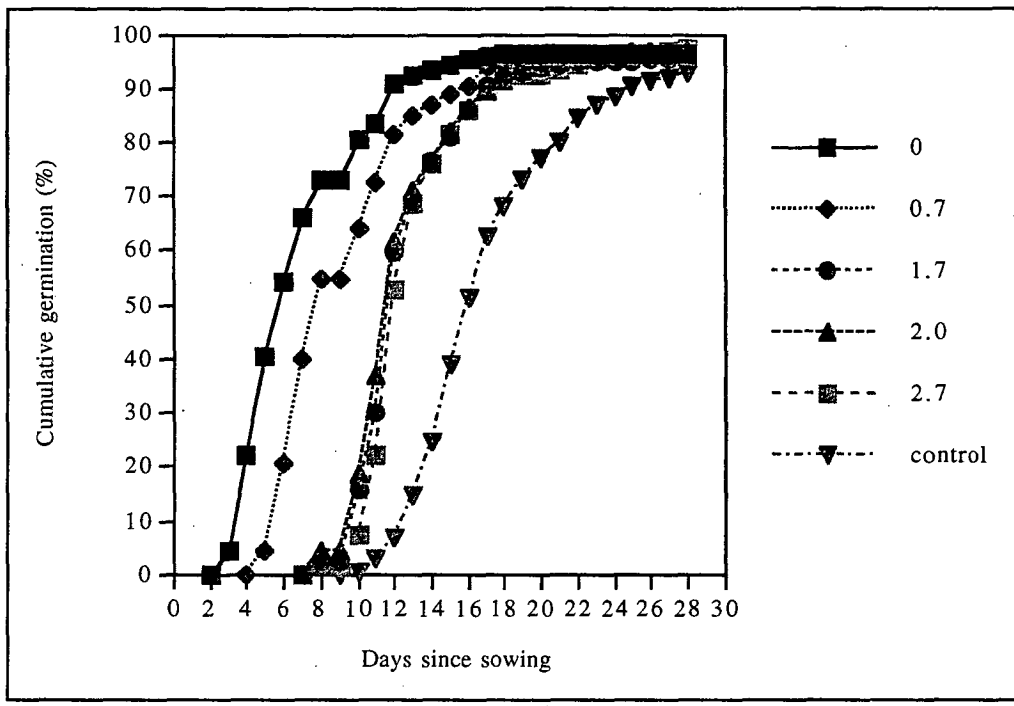

Figure 6.4 Cumulative germination (as a percentage of the total number of seeds) of seeds chilled for 12 weeks over 0, 0.7, 1.4, 2.0 and 2.7 *m* NaCl solutions. Each point is the mean of four replicates of 50 seeds.

Seed moisture content and Ψ during chilling

Figure 6.5 gives Ψ_s during chilling. Figure 6.6 shows the same data without the results from the first two weeks of treatment, allowing data from Fig. 6.5 to be expressed on an expanded axis

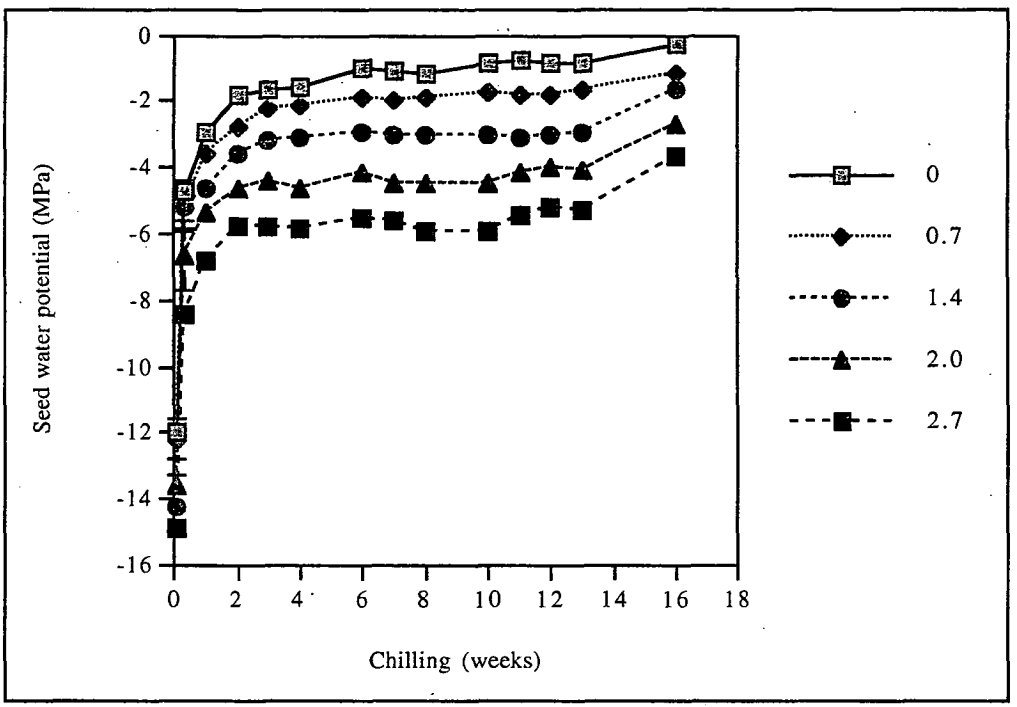

Figure 6.5 Ψ_s of seeds chilled over 0, 0.7, 1.4, 2.0 and 2.7 *m* NaCl solutions for 16 weeks. Each point is the mean of three replicates of 10 seeds. Standard error bars are shown where larger than the symbol.

All seeds showed a rapid rise in water potential when placed in chilling containers. Solution water potential had little effect on the rate of increase until 7 days of chilling had passed. By the second week of chilling the rate of change had slowed substantially and any further rise in water potential occurred at a more gradual rate. Most treatments showed a small decline in Ψ_s between weeks 6 and 8. Seeds chilled over solutions of 1.4, 2.0, 2.7 *m* showed a second rapid increase in Ψ_s between weeks 13 and 16.

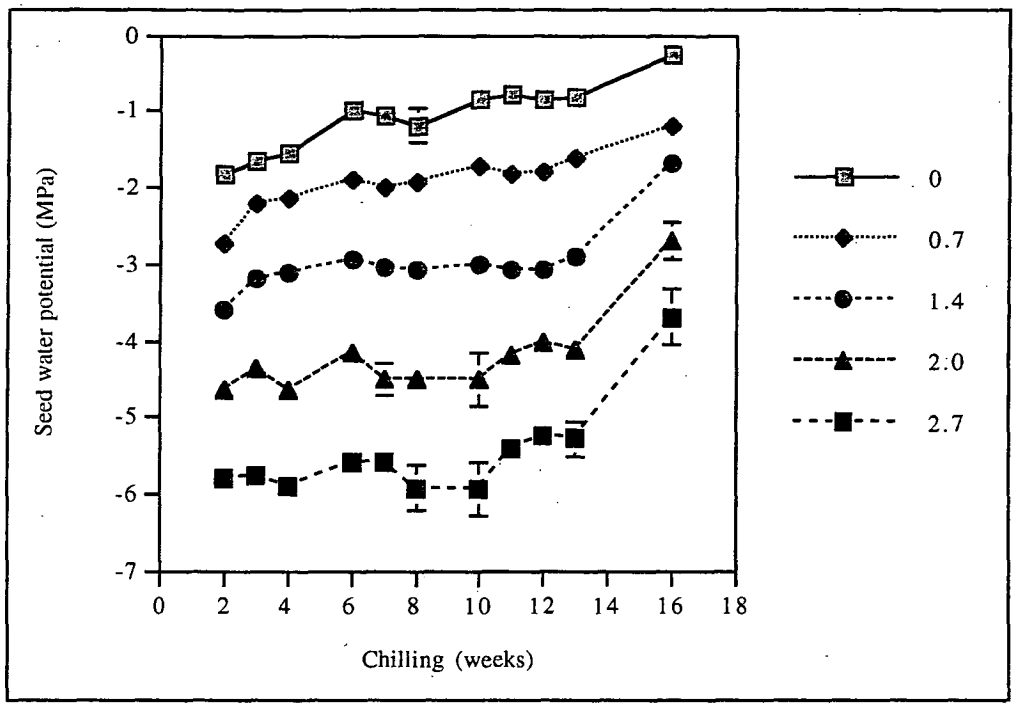

Figure 6.6 Ψ_s of seeds chilled over 0, 0.7, 1.4, 2.0 and 2.7 *m* NaCl solutions for 2 to 16 weeks. Each point is the mean of three replicates of 10 seeds. Standard error bars are shown where larger than the symbol.

Moisture content, like Ψ_s, initially rose at a rapid rate until about 2 weeks of treatment at which time the rate of uptake slowed (Figure 6.7).

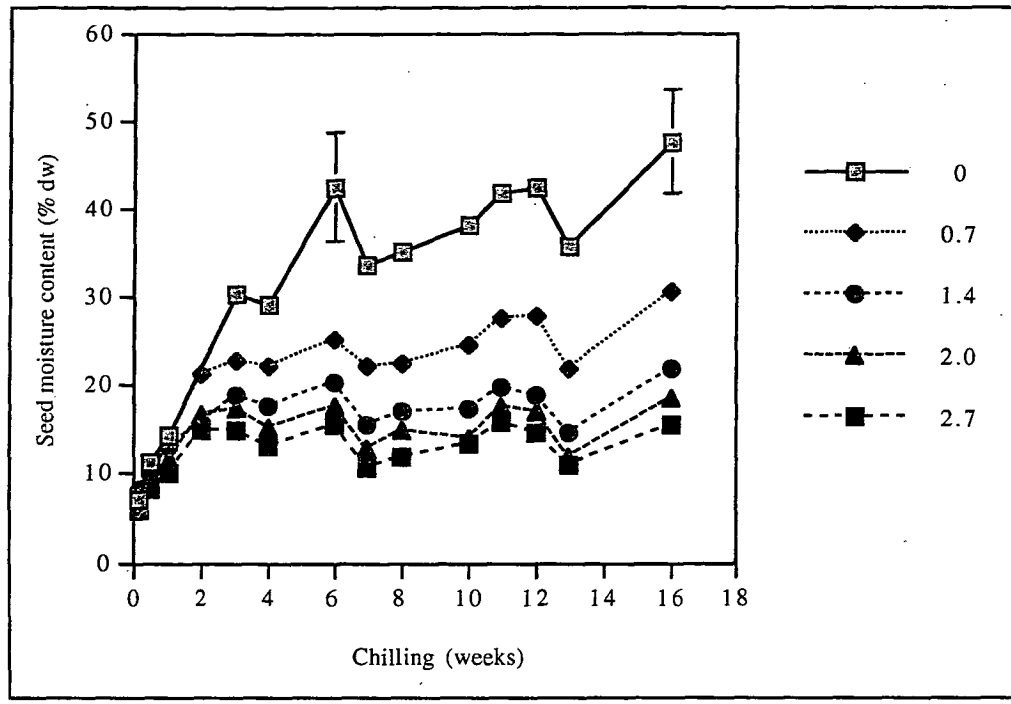

Figure 6.7 Moisture content of seeds chilled over 0, 0.7, 1.4, 2.0 and 2.7 *m* NaCl solutions for 16 weeks. Each point is the mean of three replicates of 10 seeds. Standard error bars are shown where larger than the symbol.

The maximum moisture content during chilling was strongly influenced by the Ψ_{sol}. There was little difference in the moisture content of seeds chilled over solutions of 1.4 *m* concentration or greater, but those chilled over 0 or 0.7 *m* solutions showed a substantially higher moisture content which continued to rise during treatment. However, there is some indication of a decrease in moisture content in most treatments concurrent with the drop in Ψ_s between weeks 6 and 8. Dry weight did not decrease during treatment (Figure 6.8), suggesting that respiration rates were low enough to minimise utilisation of seed reserves.

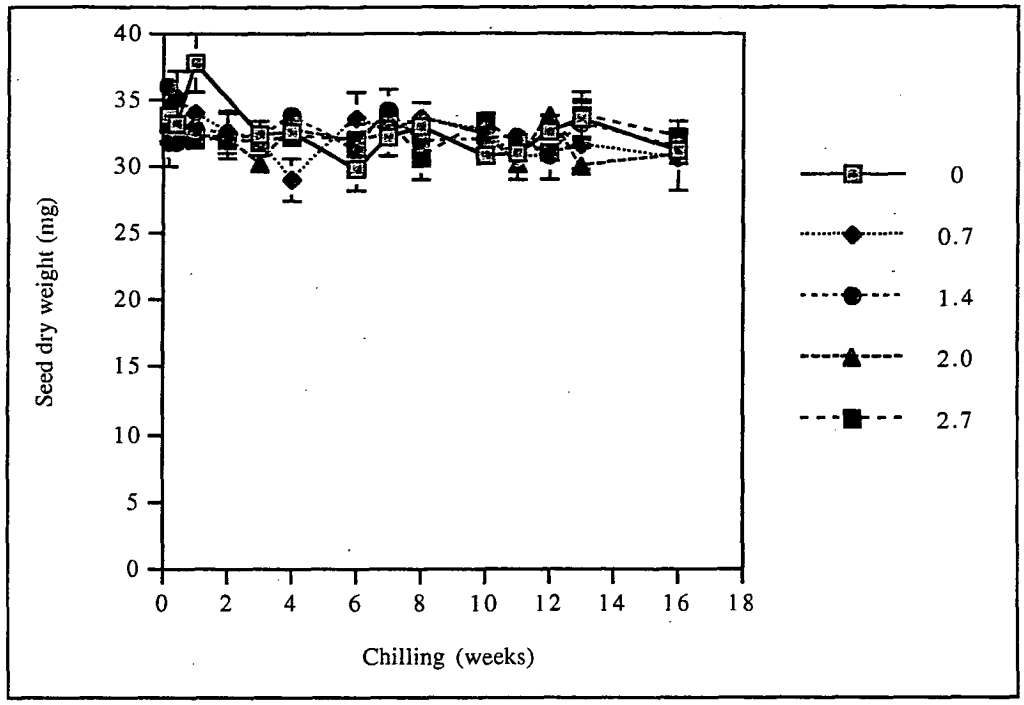

Figure 6.8 Dry weight of seeds chilled over 0, 0.7, 1.4, 2.0 and 2.7 *m* NaCl solutions for 16 weeks. Each point is the mean of three replicates of 10 seeds. Standard error bars are shown where larger than the symbol.

Percentage of split, germinated and contaminated seeds after treatment

The percentage of seeds discarded due to fungal growth was influenced by Ψ_{sol} and the duration of treatment, results are given in Table 6.3.

Table 6.3 Mean contaminated seeds (%) after 4, 8, 12 or 15 weeks of stratification over 0, 0.7, 1.4, 2.0 and 2.7 molal NaCl solutions. Means are of four replicates of 100 seeds.

Ψ_{sol} (MPa)	4 wk (% seed)	8 wk (% seed)	12 wk (% seed)	16 wk (% seed)
0	1.25 ± 0.95	0.75 ± 0.75	0.75 ± 0.75	2 ± 1.35
0.7	0.25 ± 0.25	2.25 ± 1.03	2.75 ± 1.38	2.75 ± 0.95
1.4	0	0	2 ± 1.41	1 ± 0.58
2.0	0	0	0.25 ± 0.25	0.25 ± 0.25
2.7	0	0	0	2.25 ± 1.31

Fungal growth was most severe when solution concentration was 0.7 *m*. Very little growth occurred at lower Ψ_{sol}. Little fungal growth occurred when seeds were chilled for 4 weeks, but growth was more severe when the chill duration was extended.

The number of split seeds was influenced by Ψ_{sol} and duration of chilling. Means are given in Table 6.4. The seeds chilled for 16 weeks over distilled water had a high proportion of split seeds present at the end of treatment. Table 6.5 shows that 2% of the seeds chilled over distilled water had germinated after 16 weeks of treatment.

Table 6.4 Mean percentage of split seeds after 4, 8, 12 or 15 weeks of stratification over 0, 0.7, 1.4, 2.0 and 2.7 molal NaCl solutions. Means are of four replicates of 100 seeds.

Ψ_{sol} (MPa)	4 wk (% seed)	8 wk (% seed)	12 wk (% seed)	16 wk (% seed)
0	0	0.75 ± 0.48	1 ± 0.71	12.5 ± 2.10
0.7	0	0	0	0.25 ± 0.25
1.4	0	0	0	0
2.0	0	0	0	0
2.7	0	0	0	0

Table 6.5 Mean percentage germinated seeds after 4, 8, 12 or 15 weeks of stratification over 0, 0.7, 1.4, 2.0 and 2.7 molal NaCl solutions. Means are of four replicates of 100 seeds.

Ψ_{sol} (MPa)	4 wk (% seeds)	8 wk (% seeds)	12 wk (% seeds)	16 wk (% seeds)
0	0	0	0	2 ± 1.08
0.7	0	0	0	0
1.4	0	0	0	0
2.0	0	0	0	0
2.7	0	0	0	0

Discussion

Stratification at controlled high relative humidity was tested on radiata pine seeds to determine if it was possible to give the seeds the benefits of stratification while preventing them from proceeding with germination to the point of embryo expansion. Only the seeds suspended over distilled water showed any signs of germination. It is concluded that seeds held over NaCl solutions with concentrations of 0.7 *m* and above equilibrated at moisture contents below that required for embryo expansion.

Although water uptake had reached a plateau by 4 weeks, chilling for this length of time had little effect on subsequent germination. In contrast, the germination of seeds chilled for 12 weeks was substantially improved, even though the water potential of the seeds changed little during 8 weeks of further treatment. This suggests that improvements to germination are not the consequence simply of an advanced state of hydration. It suggests that when held at high moisture content and low temperature for a prolonged period of time, the seeds are able to undergo processes that decrease the time to radicle emergence when the temperature is raised.

Figure 6.7 showed that the seeds continued to take up water after 4 weeks of treatment, particularly those held over distilled water or a 0.7 *m* solution, even though there was relatively little change to seed Ψ after this time. Weakening of the tissues surrounding the embryo by enzyme action could be one of the mechanisms involved in improving germination. Downie et al. (1997b) found that the force required to penetrate the megagametophyte and nucellus of white spruce seeds declined during stratification, and the activity of the enzyme endo-β-mannanase in the seed coat and nucellar layers increased during treatment. Similar processes could be taking place in *Pinus radiata* seeds during the current stratification.

The seeds chilled over distilled water or a 0.7 *m* NaCl solution plateaued at a Ψ_s below Ψ_{sol} (Table 6.1, Figure 6.6). Continued water uptake can thus be explained by the presence of a Ψ gradient between the seed and the atmosphere. Seed chilled over 1.4 and 2.0 *m* solutions plateaued at Ψ_s close to Ψ_{sol}. There was little further movement of moisture between the seed and the atmosphere once the plateau had been reached due to the absence of a Ψ gradient. The seed chilled over 2.7 *m* NaCl plateaued at a Ψ_s above Ψ_{sol}. This suggests the presence of a Ψ_s gradient between the seed and its environment that would lead to the loss of moisture from the seed. There is little evidence of sustained moisture loss which suggests there are barriers to the movement of moisture out of the seed. Such barriers would be a selective advantage if periods of transient moisture stress were experienced during seed germination in the natural environment.

Most treatments showed a decline in Ψ_s between weeks 6 to 8, and this was accompanied by a decline in moisture content. Hallgren and Wu (1995) discussed preliminary results that showed declining loblolly pine Ψ_s during priming. In the case of Hallgren and Wu (1995), the decline was from -1.0 to -2.0 MPa, and was explained as osmotic adjustment to maintain water flow into the embryo. Seed moisture content increased from 70 to 90% over the same period. However, in the present study moisture content declined during the period of decreasing Ψ_s .

The results suggest that seed quality began to deteriorate between 12 and 16 weeks of chilling. This is reflected in the relatively high proportion of seeds of this treatment that had to be discarded because of fungal contamination (Table 6.3). Fungi were absent from seeds held at the lowest Ψ_{sol} (2.7 *m* solution) but chilled for less than 16 weeks, as would be expected in an environment in which relatively little moisture is available to support growth. Hegarty (1978) discussed deterioration of seeds at low Ψ, and speculated that below Ψ of about -5 to -8 MPa seed activation and repair mechanisms

cannot operate effectively and processes of deterioration dominate. Deterioration may increase the likelihood that the seed will be attacked by storage fungi that are able to grow at lower Ψ than field fungi (Christensen 1973).

It is interesting to compare the germination of this seedlot following conventional stratification (see the previous chapter) with the results obtained when treated for similar durations in controlled water vapour environments. The three parameters, GC, MGT and FD, were similar for a given length of treatment (Table 6.6) indicating that the treatments were equally effective in improving germination.

Table 6.6(a) Germination capacity (GC), mean germination time (MGT) and first germinants (FD) of conventionally stratified seeds given 4, 8 or 12 weeks treatment (see previous chapter). Values are calculated from eight experimental units (two stratification methods and four replicates) of 50 seeds.

	GC (%)	MGT (days)	FD (days)
4	90.9 ± 1.8	11.9 ± 1.0	6.4 ± 0.3
8	87.4 ± 6.3	9.9 ± 0.5	4.4 ± 0.2
12	95.2 ± 0.8	9.5 ± 0.5	5.5 ± 0.5

Table 6.6(b) Germination capacity (GC), mean germination time (MGT) and first germinants (FD) of seeds stratified for 4, 8 or 12 weeks over distilled water in the present study. Values are calculated from four replicates of 50 seeds.

Weeks chilled	GC (%)	MGT (days)	FD (days)
4	86.6 ± 2.1	14.6 ± 0.1	8.0 ± 0.7
8	94.5 ± 2.1	12.1 ± 0.4	5.0 ± 0.4
12	96.5 ± 1.0	7.0 ± 0.7	3.3 ± 0.3

The solutions used in this experiment differed greatly in measured Ψ_{sol} from their expected values (Lang 1967). Water potential was measured at room temperature (23 ^{0}

C). At 5 ° C water potential readings for a given salt solution would be expected to be higher than readings taken at a higher temperature. The expected water potentials at room temperature are given in Table 6.7. This suggests the temperature correction equation used here is not suitable for converting Ψ measured at room temperature to their values at lower temperatures.

Table 6.7 Expected solution water potential (MPa) (Lang 1967) compared with water potential of 0.7, 1.4, 2.0 and 2.7 molal NaCl solutions measured with a psychrometer at 23 ° C.

Molality	Ψ at 23 ° C (Lang 1967)	Measured Ψ at 23 ° C
0.7	-3.19	-2.81 ± 0.02
1.4	-6.58	-5.66 ± 0.06
2.0	-9.70	-8.71 ± 0.06
2.7	-12.92	-11.93 ± 0.07

7. Water relations and respiration during germination

7.1 Introduction

Seed hydration follows a triphasic curve. During the first phase, seeds imbibe rapidly as matric potential drives uptake, then as the cells become fully hydrated uptake slows (Bewley and Black 1994). A third phase of rapid uptake follows, once cellular expansion begins and the embryo pushes through the tissues surrounding it. Embryo expansion is thought to be turgor driven, similar to cellular expansion in other plant tissue. Seeds will not germinate until the P of the embryo cells exceeds a threshold. The threshold is determined by the resistance of the embryo cell walls to expansion, and the resistance of the tissues surrounding the embryo to penetration by the extending radicle (Bradford 1990).

The period of time over which phase I and phase II of imbibition take place varies greatly between species, and may be as short as an hour or as long as two weeks (Salisbury and Ross 1992). Bradford (1990) related lettuce germination rates directly to the rate of weakening of the tissues surrounding the embryo, which determined the length of phase II. During phase II, lettuce (Bradford 1986) tomato (Haigh and Barlow 1987) and muskmelon (Welbaum et al. 1995) seeds were reported to be in water potential equilibrium with the imbibing solution and no net movement of water occurred. In contrast to this, Bewley and Black (1983) suggested that a water potential gradient of 1.0 MPa existed between the seed and the solution during phase II, with the seed at a lower water potential.

Earlier experiments have shown that radiata pine seeds germinate slowly, even in the ideal conditions of an incubator. According to the criteria of Gosling (1988) the seeds can thus be characterised as exhibiting relative dormancy. Apart from the obvious exception of seeds with hard coats, dormant seeds often undergo phases I and II of

imbibition, but the transition to phase III is delayed. Hegarty (1978) suggested that dormancy could be seen as a block to the initiation of cellular expansion, as phase III begins with the emergence of the radicle from the seed coat. The mechanisms involved in pine seed dormancy are not yet clearly understood, but there is increasing evidence that one or more of the tissue layers surrounding the embryo (the megagametophyte, nucellus and seedcoat) resist embryo expansion and this resistance declines during pretreatments that overcome dormancy (Downie et al. 1997b).

The water potential, moisture content and respiration of entire seeds and isolated embryos in the period prior to radicle emergence were examined in this study. The aim of the work was to describe these processes to gain a better understanding of seed physiology during germination. Four separate experiments examined: water relations, moisture uptake and respiration of entire seeds during imbibition; water potential of embryos excised from imbibing seed; and water relations parameters of excised embryos.

7.2 Water potential, moisture uptake and respiration of imbibing seed

Introduction

The progression of imbibition has the ability to affect seedling success by determining germination rate and the quantity of reserve tissue available to the growing seedling. The length of the second phase of imbibition is critical in determining the rate of germination of a seedlot, as germination occurs once the seed progresses from phase II to phase III. It also influences the amount of reserve material available to the seedling as respiration during phase II relies on the seed reserves to provide substrates. The growing seedling depends on these reserves for nutrition until photosynthesis begins.

Respiration increases with increasing hydration of the seed (Bewley and Black 1994). The course of respiration during imbibition, like moisture content, is triphasic. The first phase is one of rapid increase in oxygen consumption. During the second phase the rate of respiration remains more or less constant. The third phase begins as the diffusion barrier of the seedcoat is compromised (Bewley and Black 1994). A rapid increase in respiration continues as the seedling begins to grow. The plateau of respiration in the second phase is thought to occur in part because of temporary hypoxia inside the seed due to partial or complete impermeability of seed tissues to oxygen (Bewley and Black 1994). Botha et al. (1992) concluded that during this plateau phase, accumulation of pyruvate exceeded utilisation, resulting in the production of ethanol.

This preliminary study examined respiration, water potential and moisture uptake of entire seeds during the time taken to reach the third phase of imbibition which was earlier found to take up to ten days in this seedlot.

Materials and Methods

Seedlot *STBA Level 1, 1995, 4 - 5 mm* was used in this trial. Seeds were imbibed in petri dishes and vials at 20 ° C in darkness. Petri dishes contained 5 ml distilled water, 3 filter papers and 50 seeds. Seeds were imbibed in vials using the standard soaking method (see General Materials and Methods). Seeds were sampled every 12 hours until emerged radicles were observed. At each sample time 4 petri dishes and 4 vials were randomly selected, one from each replicate. Respiration and volume measurements were taken (see below), then seeds were surface dried for imbibed weight measurement (see General Materials and Methods). Fifteen seeds were sampled from each lot of 50 seeds and placed in the psychrometer for water potential measurement. The remainder were transferred to the drying oven. Following water potential measurement the seeds were added to the appropriate sample in the drying oven and dried to constant weight. The fresh (initial) weight of all samples was recorded before imbibition.

Respiration rate

Oxygen consumption was measured by manometry using a Warburg respirometer as described by Sestak et al. (1971). The procedure was given in detail in Umbreit et al. (1972). In summary, 50 seeds were added to each manometer flask. The flask contained 1 ml of 10 % KOH and a folded piece of filter paper in the centre well. The flasks were attached to manometers filled with Brodie's solution, and stoppers placed in the sidearm. The respirometers were placed in a water bath at 20 ° C and agitated. After 5 minutes the solution in the manometer was adjusted to the 15 mm mark and the stopcock closed. The position of the solution in the manometer was recorded. Following 15 minutes of agitation the solution in the closed arm was re-adjusted to the 15 mm mark and the change in the height in the open arm was recorded. Another reading was taken 15 minutes later.

Pressure change was converted to volume change using the following relationship

$$x = hK$$

Where x is the volume in microlitres, h is the change in height of the liquid in the open arm, and K is the apparatus constant which is determined from the equation

$$K = \{(V_g * 273/T) + (V_f * \alpha_T)\} / 10\,000$$

where V_g is the volume in microlitres of the gas phase in the respirometer, V_f is the volume in microlitres of the liquid contents of the flask, T is the temperature in degrees Kelvin, and α_T is the solubility of the gas being exchanged in the reaction. The term $(V_f * \alpha_T)$ was ignored in these calculations as the seeds were not in solution. V_g was obtained by subtracting the volume of the flask contents from the total volume of the respirometer, which had previously been determined. The volume of the seeds was measured by displacement. The seeds were placed in a mesh cage and immersed in a beaker of water to a given depth. The volume of the cage was subtracted from the change in weight of the beaker of water when the seeds were added, to give the volume of the seeds.

A thermobarometer consisting of an empty flask attached to a manometer was set up to account for variation in barometric pressure and temperature. Any change in the height of the solution in the thermobar was subtracted from the readings.

As seeds of the Pinaceae commonly carry internal and surface microbes and the presence of respiring microbes will affect respiration measurements, a preliminary examination of the effect of surface disinfestation on respiration measurement was conducted. Calcium and sodium hypochlorite have been shown to remove surface

infestations on slash, longleaf, and shortleaf pine seeds but calcium hypochlorite is preferred over sodium hypochlorite as sodium ions have been found to adversely affect pine embryos as reported by Sweet and Bolton (1979).

Radiata pine seeds were soaked under standard conditions for 20 hours before surface sterilisation. The seeds were sterilised in solutions of calcium hypochlorite with 0.05 % available chlorine for 0.5, 1, 1.5, 2, 3, 10, 20, and 40 minutes, with frequent agitation. The seeds were rinsed in three changes of distilled water, placed in the respirometer, and oxygen consumption measured. There was no appreciable effect on respiration measurement and surface disinfestation was considered unnecessary for future work.

Water potential and Moisture Content Determination

The low temperature oven method, as described in General Materials and Methods, was used to determine moisture content as a percentage of fresh weight. Water potential was measured using the psychrometer (see General Materials and Methods).

The results were plotted as three-point rolling averages to reduce some of the variation caused by the distribution of germination times within a population of seeds.

Results

Seed moisture content rose rapidly with imbibition to reach a plateau by about 24 hours of exposure to moisture (Figure 7.1). There is some evidence that moisture content declined slightly between days 1 and 3. The plateau value was maintained for the remainder of the trial. Although radicles began to emerge from seeds on day 9 of the experiment, there is little evidence of an increase in moisture content at this time. The plateau moisture content of the seeds imbibed in petri dishes was higher than those imbibed in vials.

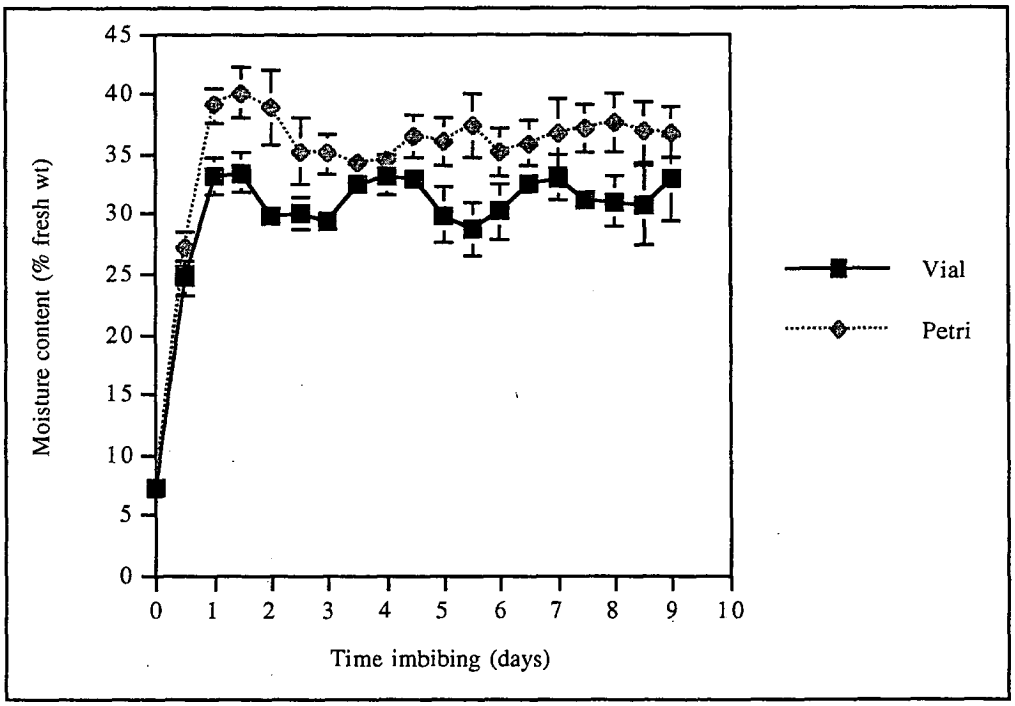

Figure 7.1 Moisture content of imbibing seeds. Seeds were imbibed in bulk water (vial) or on moist filter paper (petri). Points are three-point rolling averages of 12 replicates of 50 seeds. Standard error bars are shown where larger than the symbol.

Respiration rate increased rapidly with imbibition, but plateau respiration was attained later than plateau moisture content (Figure 7.2). The seeds imbibed in petri dishes achieved a plateau respiration rate substantially higher than those imbibed in vials. A second increase in respiration rate was observed from about day seven onwards, which coincided with the observation of split seed coats. The respiration rate of "dry" seeds was negative, possibly due to displacement of adsorbed gas from the surface. It is generally accepted that seed moisture content is too low to allow respiration in unimbibed seed (Priestly 1986).

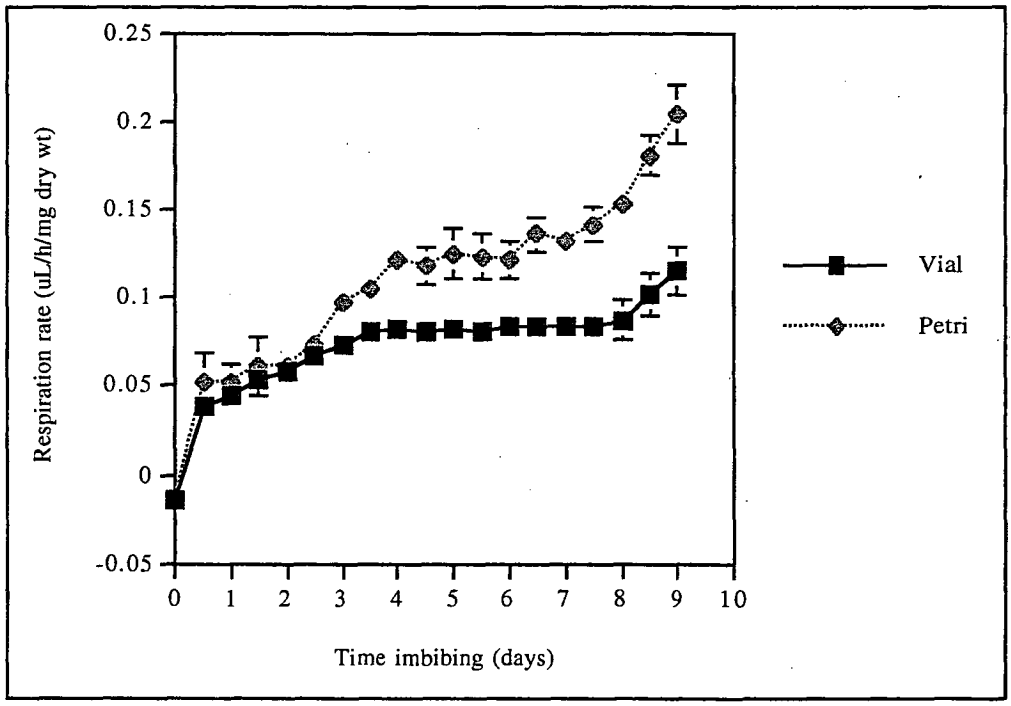

Figure 7.2 Respiration rate of imbibing seeds. Seeds were imbibed in bulk water (vial) or on moist filter paper (petri). Points are three-point rolling averages of 12 replicates of 50 seeds. Standard error bars are shown where larger than the symbol.

When dry seed is included, the plot of water potential during imbibition (Figure 7.3) reveals little detail about the plateau phase of imbibition, as dry seed has a very low Ψ. Figure 7.4 gives seed water potential during imbibition excluding dry seed. The graph shows that seed Ψ dropped to below -10 MPa early in the plateau phase of imbibition, then began to rise again, after about 4 days of imbibition.

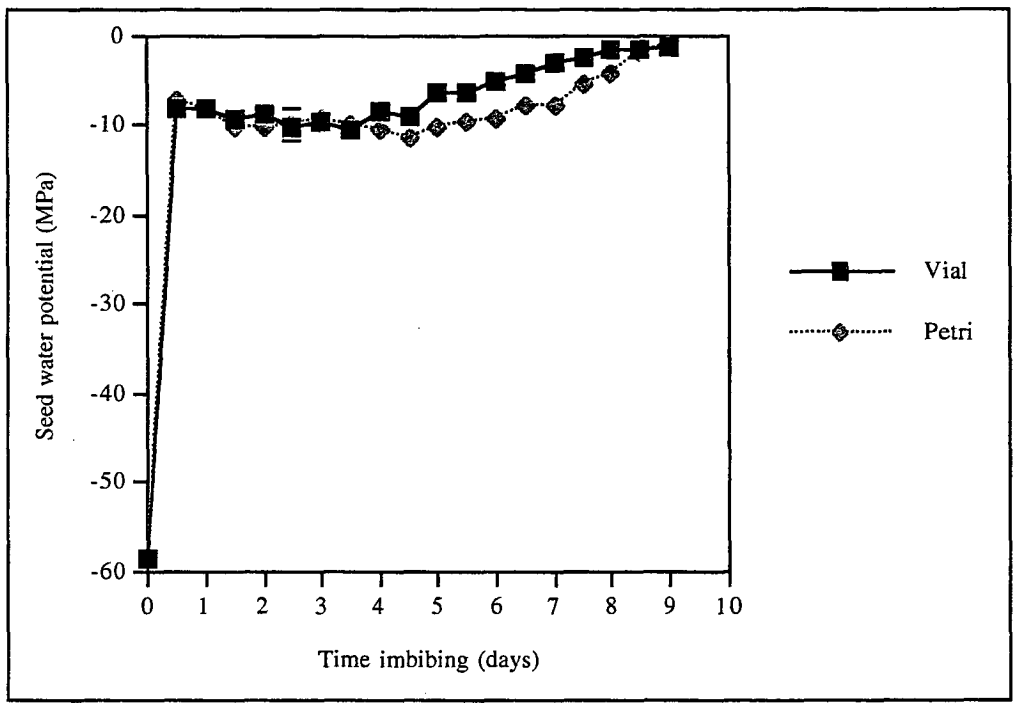

Figure 7.3 Water potential of imbibing seeds. Seeds were imbibed in bulk water (vial) or on moist filter paper (petri). Points are three-point rolling averages of 12 replicates of 15 seeds. Standard error bars are shown where larger than the symbol.

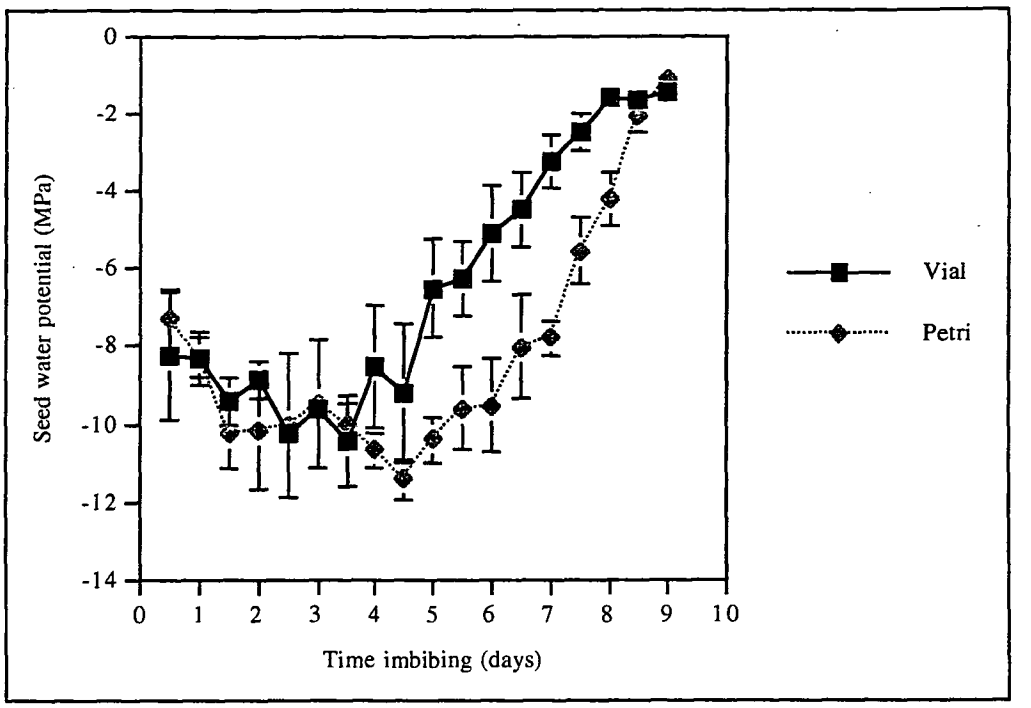

Figure 7.4 Water potential of imbibing seeds, dry seeds excluded. Seeds were imbibed in bulk water (vial) or on moist filter paper (petri). Points are three-point rolling averages of 12 replicates of 15 seeds. Standard error bars are shown where larger than the symbol.

Discussion

The initial rate of water uptake was rapid, driven by the gradient in water potential between the dry seeds, which was close to -60 MPa, and that of distilled water, which is theoretically zero. Water uptake during phase I was linear and respiration increased with increasing hydration. An initial rise until 24 hours was the same in seeds imbibed in either petri dishes or vials. There followed a similar fall in water potential in both treatments. On day 3, the rapid rise in respiration in the vial-soaked seeds plateaued, corresponding with the fall in available oxygen to near zero in non-aerated soak water found earlier (Figure 4.3). There was a similar plateau in respiration in seeds imbibed in petri dishes but onset was delayed 24 hours. The plateau in respiration rate began on day 4. The reversal in the downward trend in seed water potential occurred from day 3.5 in the seeds in vials, and day 4.5 in the petri dish seeds. In both treatments, water

potential then rose quickly to just above -2 MPa on day 8 for the vial soaked seeds and day 9 for the petri dish seeds.

Earlier work demonstrated exhaustion of oxygen supplies from soak water at about day 3. Seeds transferred from vials to the manometer flasks were introduced to normoxic conditions, but recovery was not immediate as the rate of respiration was lower than the seeds in petri dishes imbibed in normoxic conditions.

The systems for aerobic respiration may not be as well developed in seeds in hypoxic or anoxic conditions, for example, there may be fewer mitochondria. Crawford (1977) found that species that were tolerant of anoxia had a lower rate of respiration under anoxia than those that were not. This seedlot is tolerant of anoxia, that is, it does not develop soak injury. It may therefore have a reduced respiration rate under anoxic conditions. As there is a gradual transition from normoxia to anoxia there may be the opportunity for seeds to acclimate to the absence of oxygen, which would allow the seeds to survive periods of anoxia, as reported by Drew (1997) in maize and wheat seedlings.

Haigh and Barlow (1987) and Welbaum and Bradford (1990) reported that the onset of radicle emergence in tomato and muskmelon seeds, respectively, was not associated with a measurable increase in moisture content. In the present study, seeds began to split on day 7 of treatment. This corresponded with an increase in respiration rates. However, there was no evidence of an increase in moisture content. It is possible that the increase in moisture content required for a radicle to elongate sufficiently to force the seed coat to split was small enough that it was masked by the sample mean moisture content. As seeds within a population have a range of times to gemination, the mean moisture content may not increase appreciably until a substantial number of seeds of a sample are germinating.

There was no significant change in moisture content during the plateau phase, but water potential began to rise after 4.5 days of imbibition, continuing until radicles emerged on day 9. Change in seed water potential without a change in water content per unit dry weight implies a change in either osmotic potential or elasticity. A reduction in the elasticity of the tissue would affect water potential. Reduced elasticity increases turgor pressure, thereby increasing water potential without a concomitant increase in moisture content. Such a change in elasticity could occur if the embryo and megagametophyte expanded to fill the cavities in which they are contained. As the internal structure of the seed expands the seed coat may begin to influence elasticity, and if the seed coat does not expand, may become an overriding influence on water potential. The driving force for uptake will decrease unless elasticity changes once more, perhaps as the result of enzyme action on tissues surrounding the embryo.

An alternative explanation for the increased total potential is increased osmotic potential as soluble carbohydrates are utilised by respiration, or inorganic ions are leached to the surrounding water. Although respiration continued during the plateau phase of imbibition, it did not increase (Figure 7.2). The rapid rise in respiration at about the time seeds began to split was not accompanied by a similar discontinuity in water potential. This suggests that a change in elasticity, caused by the seed coat restriction of megagametophyte and embryo expansion from day 4.5, is the more likely explanation. Further support for this argument comes from the observation that the water potential of seeds imbibed in anoxic conditions (Figure 7.4) was higher, although it would be expected that carbohydrate reserves would be less depleted in these seeds due to the lower respiration rate.

The lag of one day or more observed between seed coat splitting and emergence of the radicle may represent the time required to take up sufficient water to allow cell elongation.

Welbaum and Bradford (1990) found that the measured water potential of muskmelon seeds was sensitive to the pressure applied to the seed when moisture was blotted from the surface of the seed. Applying pressure removed some of the water held in the seed coat. The seed then re-equilibrated during the measurement period in the psychrometer which led to lower and variable readings. This may have occurred in the present study when measuring radiata pine seed water potential. The blotting and air-drying protocol applied to seed may have removed water from the seedcoat thus lowering measured water potential.

The seeds began respiring as soon as imbibition began. Respiration rates increased to reach a maximum rate at about day 4. Respiration rates would remain constant if cell division and growth was absent or if oxygen diffusion was limiting. Higher respiration rates have been recorded in decoated radiata (Rimbawanto et al. 1989), loblolly (Barnett 1972), and eastern white (Kozlowski and Gentile 1959) pine seeds compared with intact seeds. In the present study, the rate of respiration rose once the seedcoats had split, which suggests that the seedcoat presents a barrier to oxygen diffusion. It is difficult, however, to separate cause and effect as splitting of the seedcoat might allow rapid growth of the embryos and increased oxygen consumption.

7.3 Seed water potential from 0 - 18 hours of imbibition

Introduction

Seed water potential in the previous experiment appeared to rise to a peak at the end of phase I of imbibition, and then fall immediately after this. The present study was conducted to examine seed water potential and moisture content, during the transition from phase I to phase II, in more detail.

Materials and Methods

Seedlot *STBA Level 1, 4 - 5 mm, 1995* was soaked in samples of 15 seeds to 6 ml distilled water using standard soaking conditions. Fresh weight of each sample was recorded. Water potential and moisture content were measured after 0, 2, 4, 6, 8, 10, 12, 14, 16 and 18 hours of soaking. Each soak duration was replicated 9 times.

Samples were drained, surface dried, weighed and placed in the psychrometer. The method of surface drying varied slightly to the method described in General Materials and Methods as seeds were air-dried for 40 min rather than 30 min. Sample water potential was measured using the method described in General Materials and Methods. The trial was designed so that the readings were taken at a different time of day for each replicate. The seeds were placed in the drying oven and dried to constant weight (see General Materials and Methods).

Results and Discussion

Seed water potential and moisture content during the first 18 hours of imbibition were plotted firstly with the complete data set (Figure 7.5), and secondly with the data from two hours of imbibition onwards (Figure 7.6) to reveal more detail of the transition period.

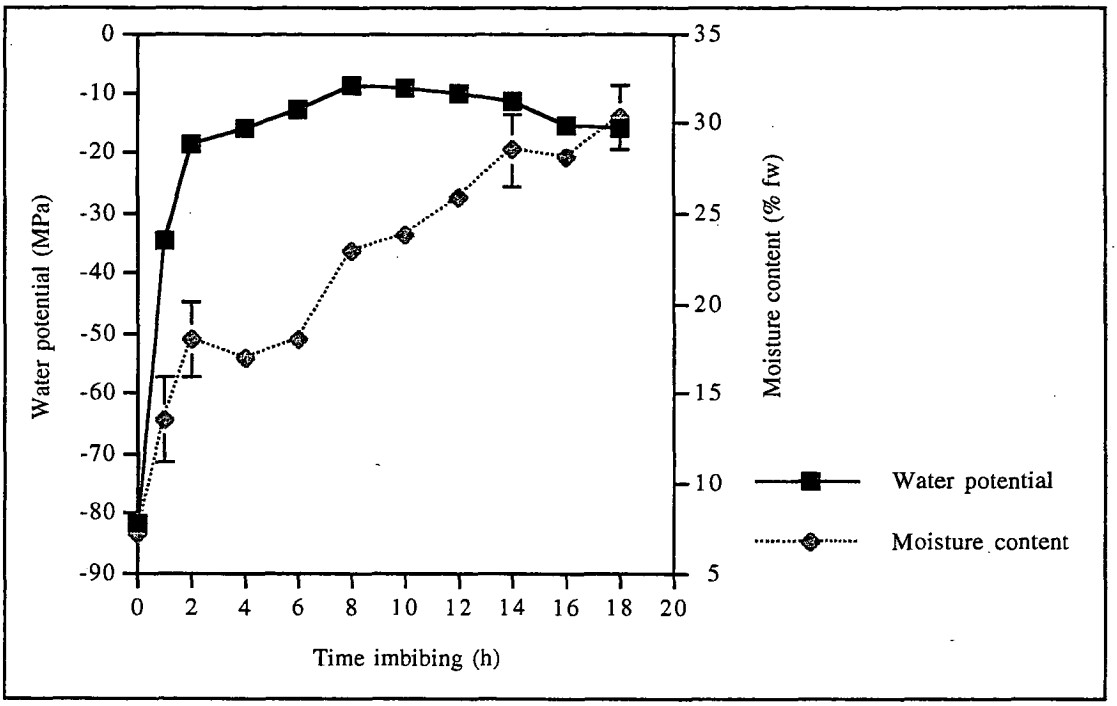

Figure 7.5 Water potential and moisture content of 15 seeds imbibed in 6 ml distilled water. Points are the mean of nine replictes. Standard error bars are shown where larger than the symbol.

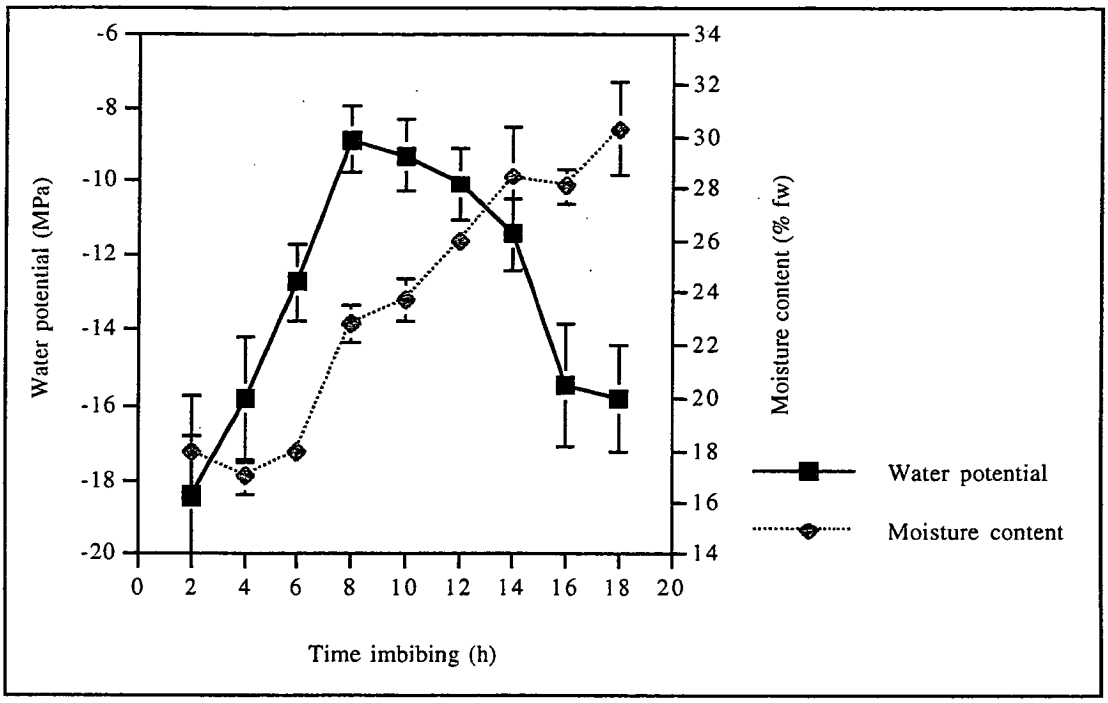

Figure 7.6 Water potential and moisture content of 15 seeds imbibed in 6 ml distilled water, 2 hours of imbibition onwards. Points are the mean of nine replicates. Standard error bars are shown where larger than the symbol.

The seeds were rapidly hydrated during the first eight hours of imbibition. Within this period of rapid imbibition the rate of water potential increase changed after two hours of imbibition. This is reflected in seed moisture content which increased rapidly in the first two hours and then changed little from two to six hours. The decline in water potential between eight and 16 hours was not accompanied by a decrease in moisture content as would be expected.

7.4 Embryo water potential during imbibition

Introduction

Water relations measurements on entire seeds give an average value for seed tissues including the seedcoat and megagametophyte, the characteristics of which may differ considerably from the embryo tissue. For instance, the seedcoat consists of non-living tissue and matric potential is the major component contributing to the water potential.

As noted earlier, Welbaum and Bradford (1990) found that water potential measurement on entire seeds was highly sensitive to pressure applied during blotting of surface moisture from the seeds, due to the large amount of water held in the seedcoat. Measuring water potential of excised embryos should therefore give a more accurate indication of imbibition by the embryo than measurements taken on entire seeds.

Haigh and Barlow (1987) found that the water potential of entire tomato seeds during phase II was in equilibrium with the imbibition solution, but the water potential of embryos excised from imbibing seeds plateaued at a much lower value (-1.5 MPa). However, when embryos were excised from dry seeds and then imbibed, there was little difference between the entire seed water potential, and embryo water potential. The authors suggested that the apparent existence of a water potential gradient within the seed could be explained if the embryo was restrained from expansion by the tissues surrounding it. Restraint on the embryo would increase the turgor pressure, bringing the embryo into water potential equilibrium with the rest of the seed. When the restraining tissues were removed, the embryo was able to reach the water potential of embryo expansion within one hour.

If radiata pine embryos are under restraint from the surrounding tissues, the plateau water potential of embryos excised from imbibing seeds should be lower than the entire-seed measurements taken in the previous experiment. The objective of this study was to examine the water potential of embryos excised from imbibing seed.

Materials and Methods

Preliminary work indicated that six embryos were required for each 1.25 ml pyschrometer cup to obtain accurate measurements. It was established that one hour was a sufficient length of time to allow the embryos to equilibrate with the psychrometer chamber atmosphere. Embryos were excised from imbibing seeds in a humidified box to minimise water loss from exposed tissues before sealing them in the psychrometer (Boyer 1995). Bradford (1986) described the use of a humidified box, which allowed water potential values higher than -0.2 MPa to be measured consistently.

Seeds were imbibed under standard germination conditions in the incubator. Each replicate consisted of 128 seeds divided between four petri dishes. Replicates were placed in the incubator at 24 hour intervals. At each sample time eight seeds were randomly selected from each replicate. The embryos were excised using a scalpel, placed in psychrometer cups, and sealed in the psychrometer. Readings were taken after equilibration. Two readings were taken on each sample. A NaCl calibration solution was included in each run of the psychrometer.

To ensure that excision did not harm the embryos or prevent radicle elongation, ten excised embryos were placed on moist filter paper in a petri dish in the incubator. Radicle elongation was examined under standard germination conditions. The embryos were able to elongate satisfactorily.

Results and Discussion

Embryo water potential rose rapidly in the first six hours of imbibition (Figure 7.7). After 24 hours imbibing the embryos had reached phase II of imbibition. Figure 7.8 shows the detail of embryo water potential during the plateau phase. Water potential continued to rise throughout this phase, from -3 MPa after 24 hours of imbibition, to near -0.6 MPa after 10 days of water uptake. The split seeds observed on day 6 indicated some embryos had commenced expansion by this time. The first germinated seed was observed on day 8 (Table 7.1).

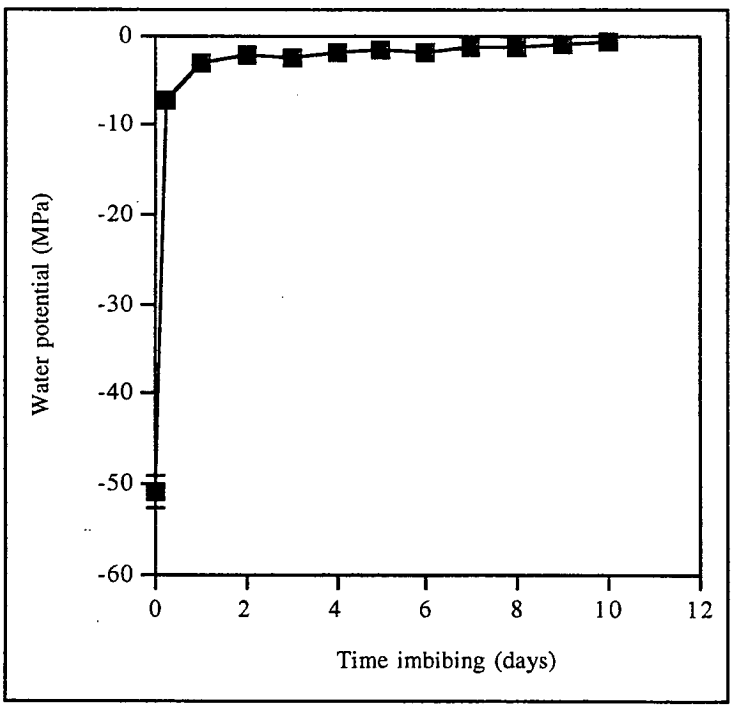

Figure 7.7 Water potential of embryos excised from seeds imbibing in petri dishes. Points are the mean of four replicates of eight embryos. Standard error bars are shown where larger than the symbol.

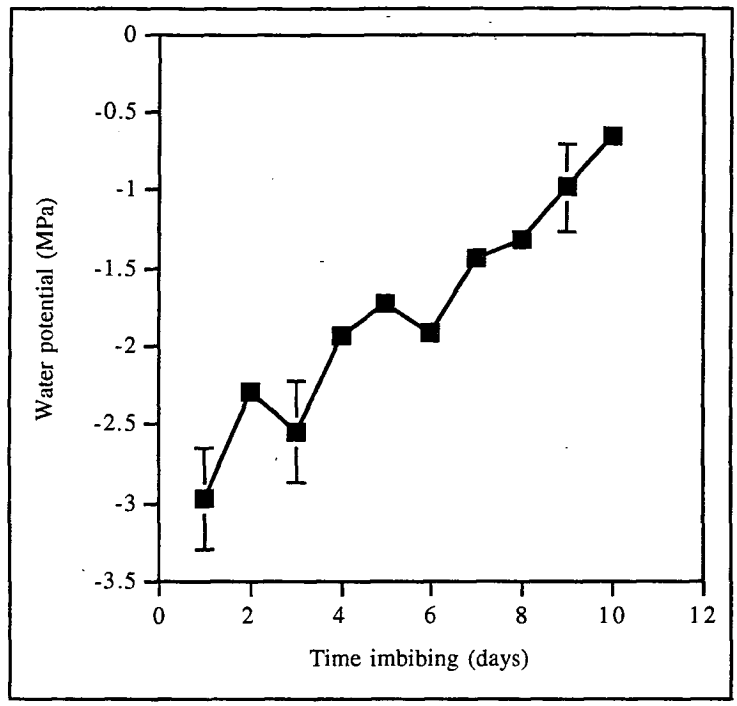

Figure 7.8 Water potential during phase II of imbibition. Embryos were excised from seeds imbibing in petri dishes. Points are the mean of four replicates of eight embryos. Standard error bars are shown where larger than the symbol.

Bradford (1986) suggested that because water uptake by the seed ceases with the transition to the plateau phase of imbibition, the seeds are in equilibrium with the water potential of the imbibition solution. This was supported by the results of Haigh and Barlow (1987) with tomato seed and Welbaum et al. (1995) with muskmelon seed. Figure 7.8 suggests that this is not the case with radiata pine seed embryos. Although uptake slowed there is no evidence of a plateau. The graph suggests that embryo water potential continues to rise throughout this phase.

Table 7.1 Proportion of embryos used for water potential measurement taken from split or germinated seed

Days imbibing	% embryos from split seed	% embryos from germinated seed
5	0	0
6	4.2	0
7	21.2	0
8	42.8	3.1
9	36	28
10	65	30

The presence of split and germinated seeds in a sample (Table 7.1) could reasonably be expected to raise the water potential of that sample above that of ungerminated seeds. Haigh and Barlow (1987) found that a sample of tomato seeds imbibed for a given period of time contained both germinated and ungerminated seeds. The water potential of the embryos from germinated seeds was higher than that of embryos from ungerminated seeds. The ungerminated seeds had the plateau water potential. However, in the present study linear water potential increase was observed between days 1 and 5 (Figure 7.8), before any split seeds were observed. It would thus be difficult to estimate the water potential of ungerminated embryos and the contribution of germinated and ungerminated seeds to the sample water potential.

Bradford (1990) suggested the length of phase II of imbibing lettuce seeds, and thus the rate of germination, is primarily determined by the time required for resistance to expansion to be reduced below the turgor pressure of the embryo. The resistance is determined primarily by the tissues surrounding the embryo. The rate of weakening of these tissues therefore affects the germination rate. Weakening of the tissues surrounding the embryo prior to germination has been observed in species of the Pinaceae (Downie et al. 1997b).

Embryo water potential in the present study was higher than entire seed water potential (see Figures 7.3 and 7.4). This contrasts with the results of Haigh and Barlow (1987) who found that tomato embryos excised from imbibing seeds had a lower water potential than the entire seeds. Haigh and Barlow (1987) attributed the difference to the effect of endosperm restraint on embryo elasticity. It is evident from the present study that either radiata pine embryos are not under a similar restraint, or that the method of water potential measurement influenced the results.

7.5 Water relations of embryos

Introduction

There have been few studies on embryo water relations during germination, and none specifically dealing with radiata pine seeds. Water relations measurements taken on entire seeds give averages across all seed tissues. Given the large proportion of the seed represented by the seedcoat and megagametophyte tissue, processes taking place within the embryo may well be masked in entire-seed water relations measurements. Alternatively, the control of embryo water potential may be imposed by the effect of seed tissue on the elasticity of the embryo, as discussed earlier.

The pressure-chamber technique originally developed by Scholander et al. (1964) is commonly used for estimating the water relations parameters of intact living tissue such as leaves. In this technique, tissue is placed under pressure and exuded water collected and weighed. Water release curves are generated, from which components of total water potential may be estimated. The pressure chamber technique cannot be used on embryos, but it is possible to generate water release curves by suspending tissue over unsaturated salt solutions of known molality and measuring the change in tissue moisture content. This technique has been used by Livingstone et al. (1992) on European larch embryos, and Dumont-BeBoux et al. (1996) on western larch, loblolly pine and white spruce embryos.

Once the tissue has reached vapour equilibrium with the air above the solution the relative water content can be measured and plotted against the reciprocal of the solution water potential to give a composite "pressure-volume" curve from which it is possible to estimate embryo water relations parameters (Livingstone et al. 1992). The technique was used in this study to examine the water relations parameters of mature radiata pine embryos excised from dry seed.

Materials and Methods

The method of Livingstone et al. (1992) was modified for use in this study. Standard 1.5 ml microcentrifuge tubes had their caps removed, and their tapered basal end cut off just below the beginning of the taper. Fine stainless steel mesh was attached to the base of the tubes using a soldering iron to melt the mesh into the plastic. An embryo was placed on the mesh inside each microcentrifuge tube, and the tubes weighed. The microcentrifuge tubes were placed inside 6.5 ml plastic tubes of 12 x 55 mm lined with filter paper. The tubes were held in place by the upper rim of the microcentrifuge tube which pushed against the inside wall of the larger tube.

Table 7.2 Molality and water potential of NaCl solutions (Lang 1967)

Molality	Ψ	Molality	Ψ
0.1	- 0.431	1.1	- 4.713
0.2	- 0.852	1.2	- 5.160
0.3	- 1.272	1.3	- 5.611
0.4	- 1.693	1.4	- 6.068
0.5	- 2.115	1.5	- 6.529
0.6	- 2.539	1.6	- 6.996
0.7	- 2.967	1.7	- 7.460
0.8	- 3.398	1.8	- 7.940
0.9	- 3.832	1.9	- 8.430
1.0	- 4.270	2.0	- 8.920

Three ml of unsaturated NaCl solution of known osmotic potential was placed in each of the larger tubes. NaCl solutions with Ψ of 0 to -8.9 MPa were prepared according to Lang (1967) (Table 7.2). When the smaller tubes were in place, the embryos were suspended about 5 mm above the solution. Each solution was replicated three times.

The tubes were tightly capped and placed in a completely randomised design in a cold room at 5 ° C for 72 hours to allow equilibration of the embryos with the atmosphere. The microcentrifuge tubes were reweighed to obtain the equilibrium weight and then dried at 105 ° C to determine dry weight.

Tissue relative water content (RWC) was calculated as:

$$RWC = (M - M_d) / (M_t - M_d)$$

where M is the mass at a given Ψ, M_d is the dry mass, and M_t is the mass at full turgor. M_t was determined by suspending embryos over distilled water at 5 ° C for 72 hours. This treatment was replicated 12 times.

Composite pressure-volume curves were obtained by plotting 1 - RWC of each embryo against $1/\Psi$ of the solution. Below the RWC at which the embryos lose turgor the relationship between 1 - RWC and $1/\Psi$ is linear, as embryo water potential is equal to osmotic potential. Extrapolation of this line to 1 - RWC = 0 gives the value of $1/\pi$ at full turgor. Extrapolated to $1/\Psi = 0$ gives the apoplastic water content (A). Three separate composite pressure volume curves were plotted. Parameter estimates were taken as means from the three curves and standard errors calculated for each mean.

Results and Discussion

Pressure-volume curves generated for each replicate showed linear regions at low tissue water content. Extrapolation of the line beyond the data to the x and y axes gave π at full turgor and A values of - 0.94 ± 0.07 and 0.024 ± 0.008, respectively. Osmotic potentials were in agreement with those presented in the literature (see Table 7.3), but the apoplastic relative water content in the present study was substantially lower than values found elsewhere (Livingstone et al. 1992; Dumont-BeBoux et al.

1996). Thus, the maximum turgor pressure in fully hydrated embryos imbibed *ex vitro* is 0.94 MPa.

The pressure-volume curves departed from linearity when solution water potential was -1.7 MPa and seed was above 0.48 ± 0.04 RWC, indicating the embryos attained positive turgor at RWC greater than this value. This is within the range found for other species (Table 7.3).

Table 7.3 Water relations parameters of mature embryos excised from dry seed or embryonic tissue of a range of species.

Species	π	*A*	RWC at *P* loss	Reference
western larch	- 0.59 ± 0.06	0.15 ± 0.01	0.47 ± 0.06	Dumont-BeBoux et al. (1996)
loblolly pine	- 0.54 ± 0.10	0.18 ± 0.02	0.61 ± 0.08	Dumont-BeBoux et al. (1996)
European larch				
hypocotyl	- 2.08 ± 0.23	0.155 ± 0.021	0.78 ± 0.04	Livingstone et al. (1992)
root	- 1.26 ± 0.27	0.072 ± 0.013	0.62 ± 0.07	Livingstone et al. (1992)
lettuce	- 0.89	-	-	Bradford (1986)
tomato	- 0.65 ± 0.03	-	55 %	Haigh and Barlow (1987)

These results indicate that when imbibed in the absence of external tissues, embryos will begin to expand once the water potential reaches -1.7 MPa. However, the presence of surrounding tissues will affect embryo water relations parameters. The absence of the megagametophyte may affect osmotic potential due to the absence of this source of soluble carbohydrates. As discussed earlier, tissues surrounding the embryo may affect embryo water relations by changing the tissue elasticity.

7.6 Discussion of water relations studies

Seed hydration is generally considered to be a triphasic process. Rapid imbibition characterises the first phase as matric potential drives uptake. The moisture content plateaus during the second phase as the cells become fully hydrated and uptake slows. A third phase of rapid uptake follows, once cellular expansion begins and the embryo pushes through the tissues surrounding it (Bewley and Black 1994).

Seed water potential in seeds allowed to imbibe at room temperature increased when wetted, rising to near -8 MPa within 24 hours. The final water potential just prior to splitting and germination 6 days later was also -8 MPa. Water content as percent fresh weight also reached near its maximum level within the first 24 hours of imbibition. Thus, measured as either water potential or water content as percent dry weight, seeds reached full hydration within the first 24 hours.

After initial water uptake there appears to have been a re-adjustment of water potential, with a decline to near -12 MPa before increasing to -8 MPa before splitting. Seeds allowed to imbibe at low temperature (Chapter 5) also showed a marked decline in water potential after the initial rise. There was then a gradual increase as stratification continued. Samples were taken at 2 - 4 week intervals in this experiment but the downward trend in Ψ_s was clearly evident from week 2 to week 4 in all seedlots in both stratification methods. When stratification was carried out under controlled water vapour pressure, there appeared to be no comparable adjustment, except perhaps at the two lowest vapour pressure treatments. There are few reports in the literature of such a re-adjustment of water potential after initial imbibition. McDonough (1975) reported changes in water potential during imbibition in a range of grasses and legumes. Water potential rose rapidly followed by a gradual decline until just before germination. The decline in the range of -1.5 to -2.0 MPa was attributed to solubilisation of carbohydrate reserves during hydration. Close examination of data presented by Haigh and Barlow

(1987) suggests a slight decline in water content in imbibing tomato seeds from about 24 hours of imbibition until phase III began. This was not commented upon by the authors.

Possible explanations for the reduced water potential include outward movement of water against an osmotic potential gradient, osmotic adjustment due to solubilisation of crystalline or otherwise dried cell contents suggested by McDonough (1975), and increased elasticity of seed content cell walls or of the seed coat itself. Water uptake by the seed in phase II of imbibition is usually regarded as initially driven by osmotic potential (Hallgren and Wu 1995). Cells within the seed also expand as moisture content increases. An increase in resistance of the cell walls and surrounding tissues leads to increased turgor pressure and water potential, which reduces the driving force for water uptake (Hallgren and Wu 1995). Thus Ψ_s during phase II appears to be controlled by both osmotic potential and elasticity of megagametophyte cells. Welbaum et al. (1995) suggested that further increase in the driving force for uptake occurs in muskmelon seeds when the tissues surrounding the embryo are gradually weakened allowing a decrease in water potential and further embryo expansion. Downie et al. (1997b) found that the micropylar megagametophyte and nucellar tissues of white spruce seeds are weakened prior to germination.

Throughout this period of adjustment of seed water potential, there remained a potential difference of 7-8 MPa between the extracted embryos and the remainder of the seed, suggesting that the embryo remains isolated from the seed throughout the imbibition process. Tillman-Sutela and Kauppi (1995a, 1995b) reported that the nucellar tissues of Scots pine and Norway spruce seeds regulate moisture movement into the embryo. Such tissue layers may prevent moisture loss from the embryo and megagametophyte.

Reports on changes in osmotic concentration and tissue elasticity can explain a short term decline in measured Ψ_s. In the present study it is difficult to explain how a decline in Ψ_s could be sustained for several days in a seed which can apparently fully hydrate in 24h. Further, although there was clearly some metabolic activity during this readjustment period (Chapter 7.2) the low measured Ψ_s, well below Ψ for metabolic activity in leaf tissue (Goode and Higgs 1973), and the magnitude of the change (about 4 MPa) suggest that regulation of Ψ_s was not related to metabolic activity. It is also notable that seeds allowed to imbibe in water vapour rather than liquid did not exhibit the same marked changes in Ψ_s during phase II. There was also a suggestion in Experiment 7.2 that there was a small decrease in water content (% FW) during the decline in Ψ_s.

Combined the results probably confirm the inadequacy of Ψ_s as a measure of seed water status, particularly during imbibition in liquid water. One possible explanation for the observed changes is that an initial influx of liquid fills the spaces in the seed, raising Ψ_s to the levels measured after 24 h. Hydration of megagametophyte tissue then proceeds slowly and tissue expands possibly expelling free water leading to a decline in Ψ_s. During the subsequent period Ψ_s, as measured, probably reflects the water potential of the megagametophyte as it hydrates, but again a sustained decline in Ψ_s is difficult to explain. Nevertheless, the delay in embryo hydration and the dye studies by Tillman-Sutela and Kauppi (1995a, 1995b) which demonstrated a long delay in water movement into the embryo may indicate that while hydration is apparently complete in 24h a much longer period of water redistribution is required before the seed and embryo are fully hydrated.

Unfortunately further exploration of seed water relations was beyond the scope of this study, but these observations underline the areas of uncertainty about seed water

relations in general. They may also indicate that assumptions, mainly based on studies on Angiosperms may not be generally applicable in the more primitive Gymnosperms.

8. General Discussion

Seed pretreatments have long been applied to temperate zone tree seeds, particularly Northern Hemisphere conifers, in an effort to overcome dormancy and improve germination rate, uniformity and capacity. The North American species *Pinus radiata* is currently grown in plantations across the globe, including extensive areas in Australia, New Zealand, Chile and South Africa. Germination of seedlots from Australian seed orchards has proven at times to be low and variable in field nurseries. Pretreatment application has been suggested as a means of improving germination and overcoming seedlot limitations. This project examined a range of pretreatment options to improve radiata pine germination and aimed to further our understanding of germination and the mechanisms of action of pretreatment in radiata pine seed, primarily by examining seed water relations during germination and pretreatment. Germination testing determined that some, if not all, seedlots exhibited relative dormancy and would thus benefit from pretreatment.

Initial work examining the effect of a short presoak on subsequent germination demonstrated that seedlots varied in their response to soaking. Thus, the treatment has the potential to be used as a seed quality or vigour test. Decreased germination capacity resulted when susceptible seedlots were soaked without adequate aeration. Respiration increased rapidly in imbibing seeds and oxygen in soak water had reached very low levels after 48 hours at 20 ° C. Seeds reached full hydration after 24 hours at this temperature and oxygen demand would be expected to rise rapidly once seed tissues were fully hydrated. When oxygen levels in soak water become limiting the seeds must rely on anaerobiosis to provide the energy required to complete the processes required for radicle protrusion. Seed injury in non-aerated soaks may be associated with anaerobiosis.

Presoaking is currently widely used in radiata pine nurseries. Care is usually taken to provide the seeds with oxygen, either by using running water or changing the water several times. In the present study soaking improved germination to some extent when aeration was adequate. Long soak durations with aeration of the soak water led to a reduction in the time to germination (Chapter 4.2). Seeds of seedlot *STBA Level 1, 1995, 4-5mm* sown in the nursery (Chapter 4.3) had a significantly shorter mean time to germination when soaked for more than 24 hours before sowing. Laboratory experiments confirmed that this seedlot was not susceptible to soak injury when soaked at 20 ° C without aeration. Soaked seeds were apparently fully imbibed when sown in the field or placed in the incubator, which effectively reduced the time to germination for individual seeds and the seedlot as a whole. Presoaking with aeration proved to be a rapid and simple method of improving germination rate in both incubator and field studies. This confirms the benefit of the general industry approach, provided adequate attention is applied to aeration.

Treating conifer seeds by stratification has a lengthy history, but the mechanisms involved in the improvement of germination are still poorly understood. Recent studies of conifer seeds have examined metabolism in the megagametophyte (Murphy and Hammer 1993; Schneider and Gifford 1994) and weakening of tissues surrounding the embryo (Downie and Bewley 1996; Downie et al. 1997b) during stratification treatment. In the present study stratification was found to benefit germination. Germination rate was increased in the incubator and glasshouse, and germination capacity of some seedlots increased in the incubator. It was found that given a sufficient length of treatment (at least 4 weeks), stratification had the capacity to improve the number of seeds capable of germination after a non-aerated soak treatment that negatively affected germination.

Seedling dry weight studies indicated that stratification does not improve seedling growth rates. However, it is likely that for a given harvest date stratified seedlings will have greater biomass than untreated seedlings, due to the more rapid rate of emergence from the soil, which effectively lengthens the growing season. This corresponds with the findings of Barnett and McLemore (1984), who reported that loblolly pine seedlings grown from stratified seeds emerged earlier and were larger at harvest than seedlings from unstratified seeds.

Rising Ψ_s demonstrated that hydration of the seed increased during stratification. Stanley (1958) and Schneider and Gifford (1994) found that the water content of both sugar and loblolly pine seeds increased during stratification. Germination is thought to occur in the range of approximately -2 to 0 MPa (Bradford 1995). The presence of seeds with split seedcoats in samples stratified for 12 weeks suggests that these seeds had reached the water potential threshold for germination, as splitting of the seedcoat usually precedes radicle emergence by about one day. Consequently, the increased rate of germination of stratified seeds may simply be the result of the increased hydration. As the moisture content of the seed is raised, it is closer to the threshold level required for embryo expansion and it could reasonably be expected that the seed will germinate relatively quickly once the temperature is raised. However, the water potential of stratified seedlot *c* did not differ significantly from the unstratified seed, yet the mean time to germination was decreased by stratification and the greater the duration of treatment, the more rapidly germination proceeded. This suggests that other processes as well as increased hydration are involved in improving germination.

A range of mechanisms of action of stratification have been suggested in the literature. Carpita et al. (1983) proposed that stratification induced a change in sucrose absorption and metabolism in the embryo, enabling the embryo to increase the rate of elongation. Downie and Bewley (1996) found that the seedcoat is largely responsible for

dormancy in white spruce seeds, but the megagametophyte and nucellus also contribute. Subsequent work by Downie et al. (1997b) with the same species demonstrated that the megagametophyte and nucellus is weakened by enzyme action prior to radicle emergence from non-dormant seed, and after stratification treatment to overcome dormancy given to both dormant and non-dormant seed. Resistance to penetration of the tissues surrounding the embryo therefore plays a role in both germination and the imposition of dormancy. Radiata pine seed germination may be controlled to some extent by the tissues surrounding the embryo, as seen in other seeds of the Pinaceae. These tissues may have been weakened during stratification. Increased germination rate results as turgor pressure does not need to build to as great an extent to overcome the resistance of the tissues surrounding the embryo. Thus the effect of duration of chilling on germination rate may be due to a greater degree of weakening in the restrictive tissues of seeds treated for a greater length of time.

The germination capacity of two seedlots was improved by stratification. One of these seedlots had been deteriorated by overheating. Improvement of germination capacity indicates either that a greater number of seeds were able to germinate within the test period due to increased germination rate, or that the viability of the seedlot increased. Increased viability may result if stratification conditions allow repair processes to proceed within the seed as dormancy is alleviated.

Two methods of stratification were used. One involved soaking seeds for 48 hours, draining and chilling in plastic bags. The second method involved placing the seeds on moist filter paper in petri dishes and chilling. The method of stratification had little effect on Ψ_s measured at intervals during stratification, germination capacity or rate. This suggests that the seeds were able to absorb sufficient water during the soak to enable the embryo to reach positive turgor during stratification, given enough time stratifying. Stanley (1958) reported that the seedcoat of sugar pine seeds stratified at 5

° C became fully hydrated within 30 days, but the embryo and endosperm continued taking up moisture until chilling ceased.

While stratification proved to be a beneficial treatment by increasing germination rate and germination capacity of seedlots with relatively poor germination, the splitting of the seedcoat seen in the longer stratification treatments may present difficulties if stratified seeds were to be sown in a nursery. While radicles did not emerge, the Ψ_s readings and observation of split seedcoats suggests that this would have occurred had prechilling been further extended.

Jones and Gosling (1994) overcame this problem by controlling seed moisture content during stratification by limiting the quantity of water available. The principle behind this treatment differs little from priming. The quantity of moisture imbibed by the seed is sufficient to permit processes that speed germination to occur, but moisture is insufficient to allow embryo expansion to proceed to the point of radicle emergence. The difference between the two methods is that priming is usually carried out at higher temperatures and seed moisture content is determined by the water potential of the imbibition solution. In the present study seed moisture content during stratification was controlled by chilling seeds in atmospheres of relatively high humidity, above salt solutions of known osmotic potential.

At chilling temperature it took at least 4 weeks for seed water potential to equilibrate with the atmosphere. The seeds chilled in 100 % relative humidity (above distilled water) germinated during treatment, but lowering the solution water potential to -3 MPa was sufficient to prevent germination. However, the seeds were still able to benefit from the treatment by increased germination rate when transferred to germination conditions. Despite little change in water potential from 4 weeks onwards, there was a rapid rise in germination rate with increasing treatment duration. This is

further evidence supporting the hypothesis that improvements to germination are not simply the consequence of an advanced state of hydration at the end of treatment. When held at high moisture content and low temperature for a prolonged period of time, the seeds were able to undergo processes that decreased the time to radicle emergence when the temperature was raised. Despite relatively little change to water potential from 4 weeks onwards, seed moisture content continued to rise slowly. Weakening of tissues surrounding the embryo by enzyme action during chilling would be expected to allow embryo expansion, reduced embryo water potential and further moisture uptake.

Extending treatment from 12 to 16 weeks gave little or no benefit to germination. At reduced water potential, 16 weeks of treatment reduced germination capacity and increased the time to germination compared with 12 weeks. As germination was improved by 12 weeks of treatment, processes occurring within the seeds at low water potential between 12 and 16 weeks of treatment must counteract the improvement. Hegarty (1978) suggested that below Ψ of about -5 to -8 MPa, seed activation and repair mechanisms cannot operate effectively and processes of deterioration dominate.

Water relations of seed samples suggested that the physical changes occurring within the seed during phases I and II of imbibition before germination are more complex than would have been expected from previously published literature.

During germination Ψ_s increased rapidly when the seeds were wetted, rising from near -60 MPa to -8 MPa within 24 hours. The final water potential just prior to splitting and germination 6 days later remained low at -8 MPa. Thus, seeds reached full hydration within the first 24 hours of imbibition. Moisture content as percent of dry weight also reached near its maximum level within the first 24 hours of imbibition. After the initial water uptake there was a re-adjustment of water potential downwards and moisture

content measurements revealed some evidence of moisture loss. Other explanations for the apparent change in water potential include osmotic adjustment and changes in elasticity of all or part of the tissue. Presumably cells within the seed expand as moisture content increases until resistance of the cell walls and surrounding tissues such as the megagametophyte, nucellus and seedcoat prevent further uptake. Relaxation of these tissues would decrease water potential until the seedcoat prevented further expansion and decreased the driving force for water uptake (Hallgren and Wu 1995). Welbaum et al. (1995) related changes in tissue elasticity in muskmelon seeds where the tissues surrounding the embryo are gradually weakened allowing a decrease in water potential and further embryo expansion. Downie et al. (1997b) found that the micropylar megagametophyte and nucellus tissues of white spruce seeds are weakened prior to germination. Weakening of the tissues surrounding the embryo would thus lead to a decrease in water potential until further water uptake and a rise in water potential. Turgor pressure would once more build to the point at which the tissues enveloping the embryo restrain further expansion.

Changes in osmotic concentration sufficient to allow such a large change in Ψ_s at a potential well below that normally expected for metabolic activity seem unlikely. That the large change in Ψ_s occurred with little measurable change in water content also remains unexplained. A further unexpected result from the water relations study was the apparently high resistance to movement of water between embryo and the remainder of the seed. Embryo water potential remained relatively constant changing from -3 to -1 MPa during a period when measured Ψ_s changed from below -8 to -2 MPa.

Imbibition in liquid water may result in the filling of air spaces between the seedcoat and megagametophyte. Expansion of the seed tissues within the seedcoat would force water held in this space out through the seedcoat, effectively reducing seed moisture

content and Ψ_s. This theory is supported by evidence from the stratification experiments. Three of the 4 stratified seedlots (Chapter 5) showed a re-adjustment of seed water potential downwards between 2 and 4 weeks of treatment. In contrast, seeds stratified over salt solutions (Chapter 6) showed no re-adjustment of Ψ_s. Importantly, these seeds imbibed water as vapour rather than liquid, thus preventing the pooling of water within the seedcoat.

It is also worth noting that the seeds stratified over solutions with a water potential of -3 MPa were not able to germinate during treatment. This corresponds fairly closely with the finding that embryos entered phase II of imbibition above -3 MPa (Figures 7.7 and 7.8). Germination occurred once embryo water potential had risen above about -2 MPa, which corresponds with the value cited by Bradford (1995).

In conclusion, this study has indicated that seed pretreaments have the potential to improve radiata pine nursery emergence percentage and rate, and hence improve nursery seedling yield and uniformity, and seed use efficiency. The results have indicated that the response to pretreatment depends on seedlot quality and therefore the need for pretreatment depends to an extent on seedlot history. Studies of water relations during imbibition and pretreatment have given some preliminary insights into mechanisms that may be involved in the improvement of germination.

9. Conclusions and Recommendations

9.1 Conclusions

1. Germination testing under optimal conditions demonstrated that seedlots exhibited relative dormancy (slow germination rate) which may be at least partly responsible for variable emergence observed in field nurseries.

2. The germination of some seedlots was negatively affected by presoaking without aeration while other seedlots were unaffected. This suggests that presoaking could be used to indicate seedlot quality. When adequate aeration was supplied, presoaking increased germination rate, probably as a result of increased moisture content. Increased germination rate may thus be due to a decrease in the difference between the seed moisture content and the threshold for germination.

3. Stratification increased the germination rate of all seedlots, and germination capacity of some seedlots. The treatment also increased the germination of seedlots damaged by unaerated presoaking. Twelve weeks of stratification was optimal, but seeds had begun to germinate at 5 ° C towards the end of this time. Controlling seed moisture content during stratification, by reducing atmospheric water potential to -3 MPa, prevented germination during treatment and increased germination rate after treatment. Embryos were found to begin phase II of imbibition above -3 MPa, holding the seeds at this water potential therefore prevented progression onto the second phase of imbibition. Germination occurred once embryo water potential had risen above about -2 MPa.

4. Rising Ψ_s demonstrated that hydration of the seed increased during stratification. However, some seedlots showed a substantial improvement in germination with

increased length of treatment without an increase in water potential or moisture content. This suggests that improvements to germination are not simply the consequence of an advanced state of hydration at the end of treatment, and raises questions about water relations during hydration. Mechanisms involved in improvement of germination by stratification are still poorly understood.

5. Stratification improved the germination capacity of a deteriorated seedlot, indicating that either: a greater number of seeds germinated within the test period due to increased germination rate; or seedlot viability increased as repair processes were able to proceed during treatment or dormancy was overcome. This indicates stratification has the capacity to improve poor quality caused by both relative dormancy and deterioration.

6. Measurements of water potential of seed samples during imbibition confirmed that seed water relations are still poorly understood. The seeds apparently reached full hydration within 24 hours of wetting, but did not germinate until at least 6 days later. After the initial water uptake there was a re-adjustment of water potential downwards. The reduced water potential could be explained by moisture loss, and there was some evidence of a small moisture loss in moisture content measurements. Other explanations for the apparent change in water potential include osmotic adjustment and changes in elasticity of all or part of the seed tissues. Changes in osmotic concentration sufficient to allow such a large change in Ψ_s at a potential well below that normally expected for metabolic activity seem unlikely. That the large change in Ψ_s occurred with relatively little change in water content also remains unexplained. A further unexpected result from the water relations study was the apparently high resistance to movement of water between embryo and the remainder of the seed. Embryo water potential changed from -3 to -1 MPa during a period when Ψ_s changed from below -8 to -2 MPa.

Recommendations

Recommendations for industry

1. Current industry practice of presoaking can be maintained, but attention must be paid to aeration during treatment.

2. Stratification, particularly with controlled moisture availability, could be adopted by industry to improve rate, uniformity, and potentially capacity of germination.

3. Care should be taken with thiram application. Further studies on the effect of thiram application on germination and emergence of seedlings grown in nursery conditions would be useful to determine best rates of application in the nursery, and the effect of leaching and weathering on thiram toxicity.

Recommedations for further study

1. The project determined that stratification at controlled moisture content for extended periods of time improved germination. It would be useful to examine the effect of controlling moisture content at room temperature for a short period of time prior to sowing. Such a treatment would be similar to priming. This would determine if the effects of stratification include a chilling effect. The treatment would be more practical for industry use as it would require shorter treatment times.

2. While it was found that germination benefited from stratification treatment, we still have little understanding of the mechanisms involved. An examination of water relations, megagametophyte and seedcoat weakening and carbohydrate metabolism during treatment might further our understanding of the processes involved.

3. Nursery trials and laboratory germination tests, at temperatures closer to those experienced in the field, of the most beneficial seed pretreatments could provide valuable information about the effectiveness of the treatments on nursery emergence and growth. Tests were not possible in the present study due to the seasonal climate. Such tests would be more valuable if a range of seedlots were used as the number of seedlots used in the present study was limited by practicalities.

4. Further water relations studies need to be carried out to improve our understanding of processes taking place during imbibition. Some of the issues raised by the present work include: is there equilibration between all seed parts and the environment during phase II of imbibition; is the readjustment seen during imbibition "real", and if so, what are the mechanisms involved; what is the effect of measuring the water potential of a population (sample) of seeds compared with measuring the water potential of individual seeds during imbibition; and is the water potential differential between the embryo and the whole seed "real", and if so, how does the embryo resist water loss to other seed tissues?

5. In addition, the effect of blotting and air-drying on water potential measurement, as used in the present study, needs to be clarified. Seeds and embryos that had not been in contact with liquid water did not need to be blotted and air-dried before water potential measurement as they had no adherent water. These measurements were generally closer to those expected in tissues in water potential equilibrium. This suggests that the blotting and air-drying may have affected measurements. The effect could be examined by measuring the water potential of whole seeds blotted and air-dried for a range of times.

6. A further soaking experiment needs to be carried out to detemine that seeds were responsible for the loss of oxygen from soak water.

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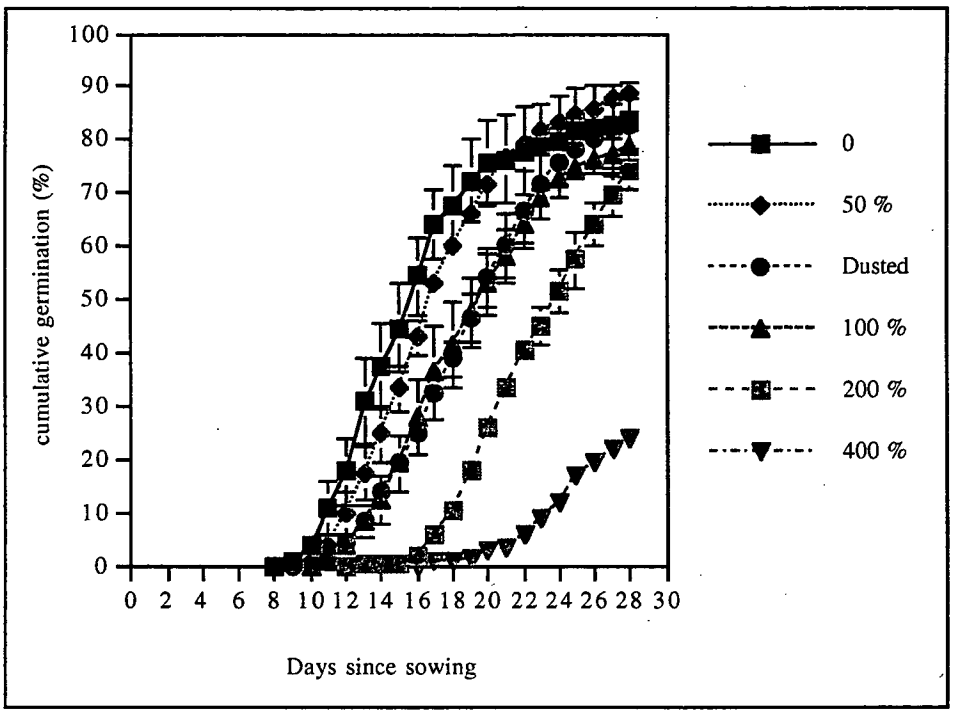
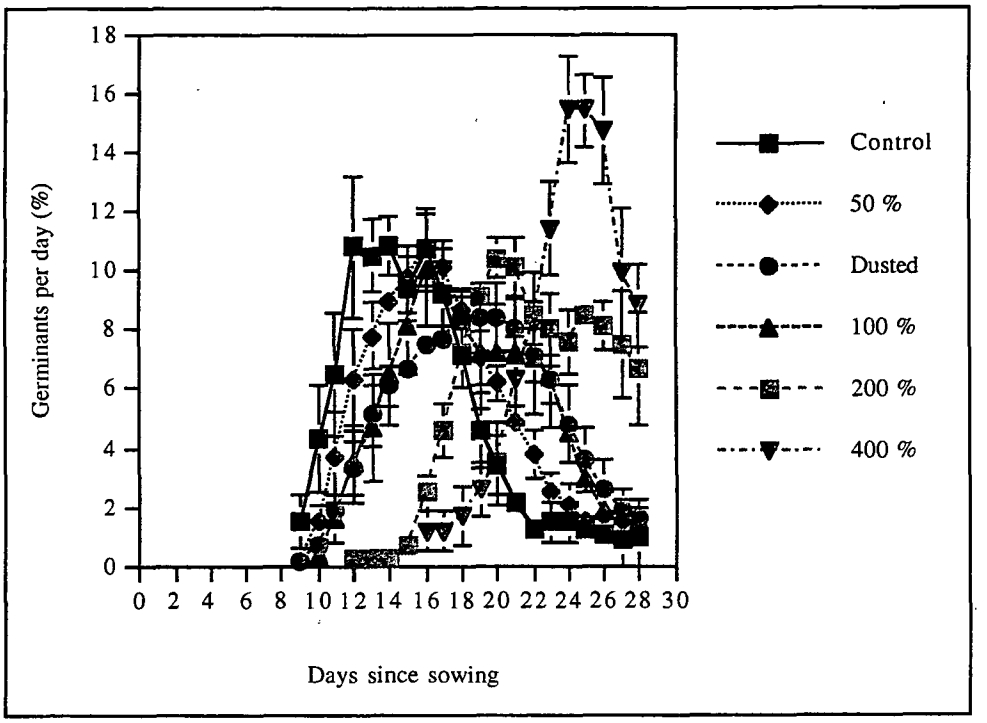
11. Appendices

Appendix I. Germination of thiram treated seed (Chapter 4.4)

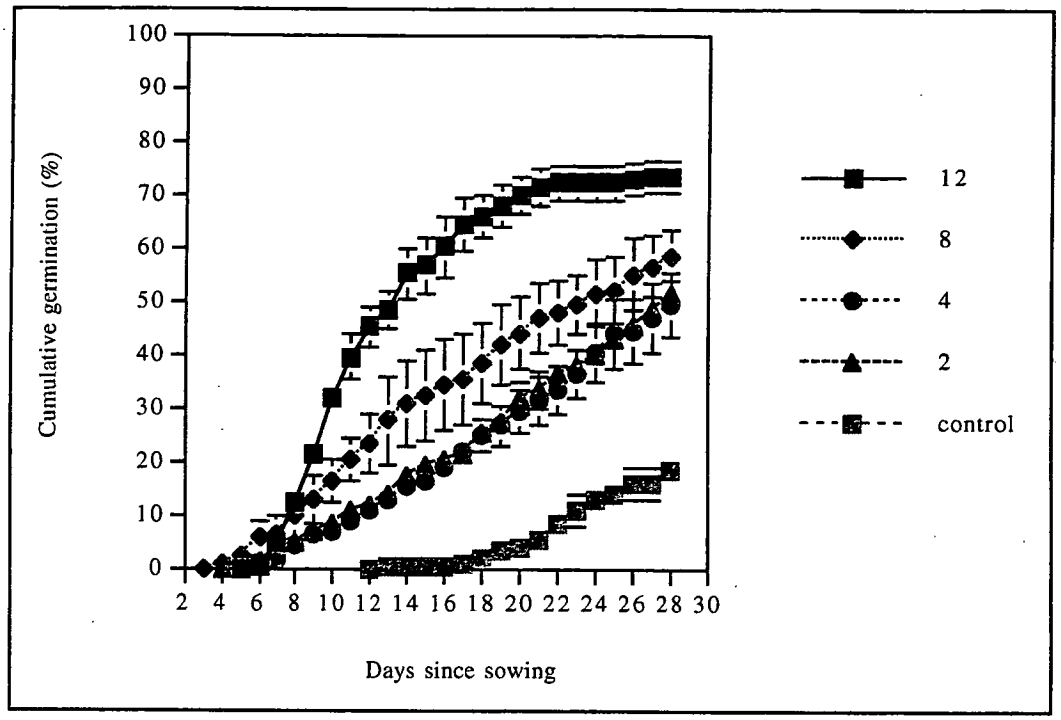


Cumulative germination profiles (as a percentage of the total number of seeds) of seeds treated with 0 (treatment a), 50% (treatment b), 100% (treatment c), 200% (treatment d), 400% (treatment e) the recommended level of thiram or dusted with thiram (treatment f). Each point is the mean of four replicates of 50 seeds.

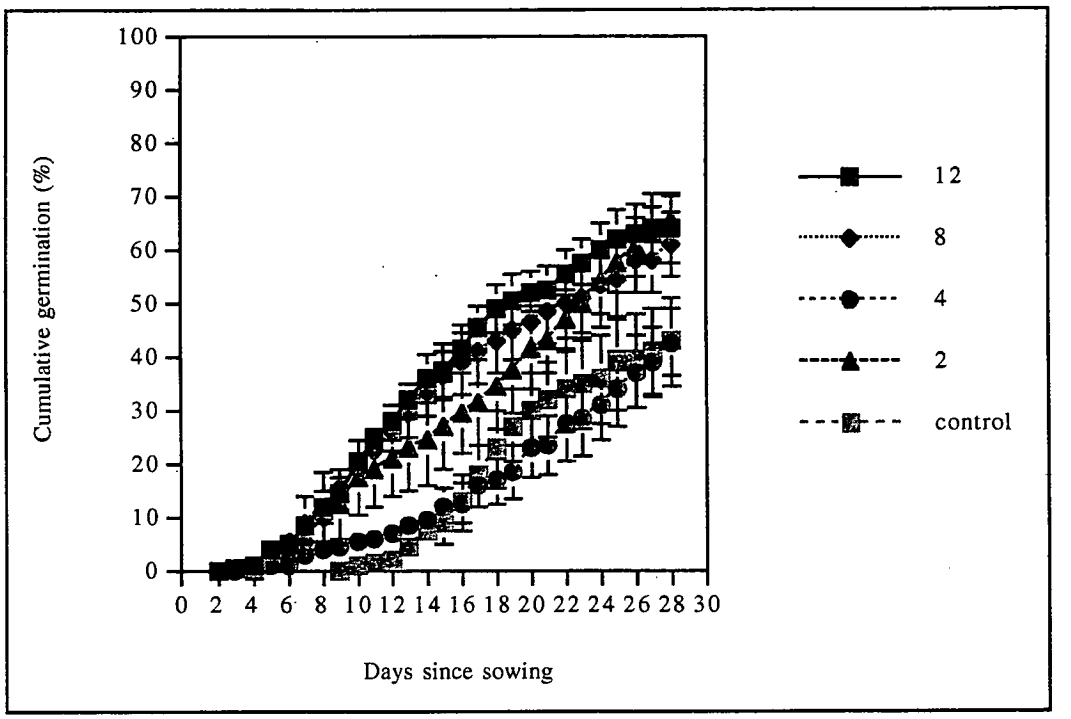


Daily germination profiles (as a percentage of the number of seeds that completed germination), of seeds treated with 0 (treatment a), 50% (treatment b), 100% (treatment c), 200% (treatment d), 400% (treatment e) the recommended level of thiram or dusted with thiram (treatment f). Germination is expressed as a three-day moving average. Each point is the mean of twelve replicates of 50 seeds.

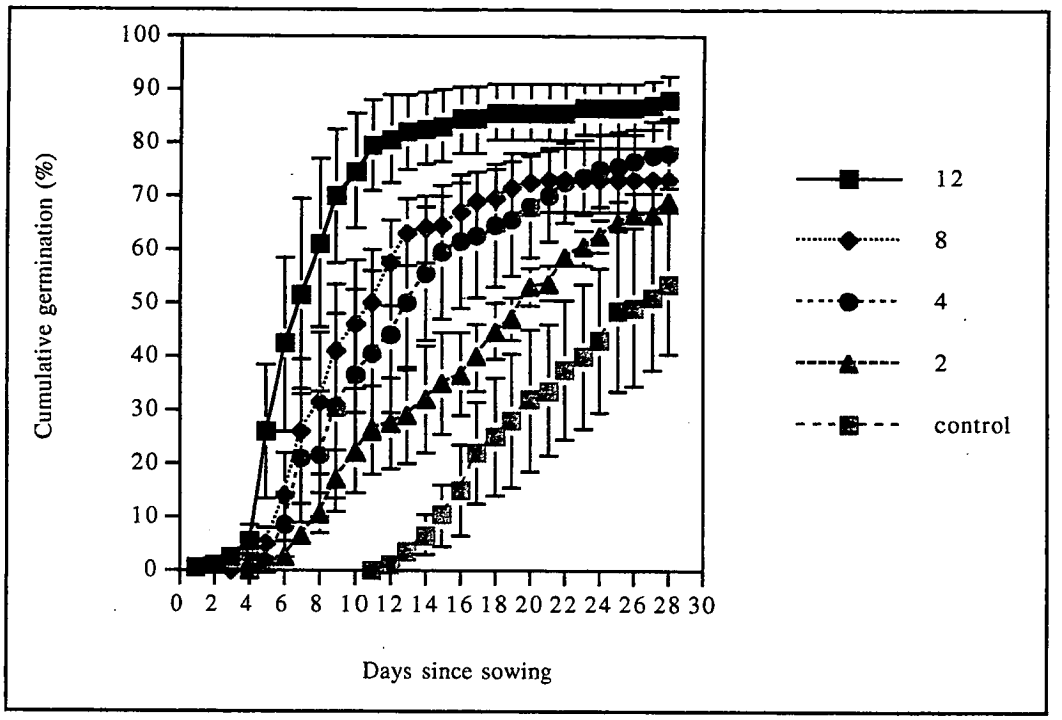
Appendix II. Germination of stratified seedlots b and c (Chapter 5).



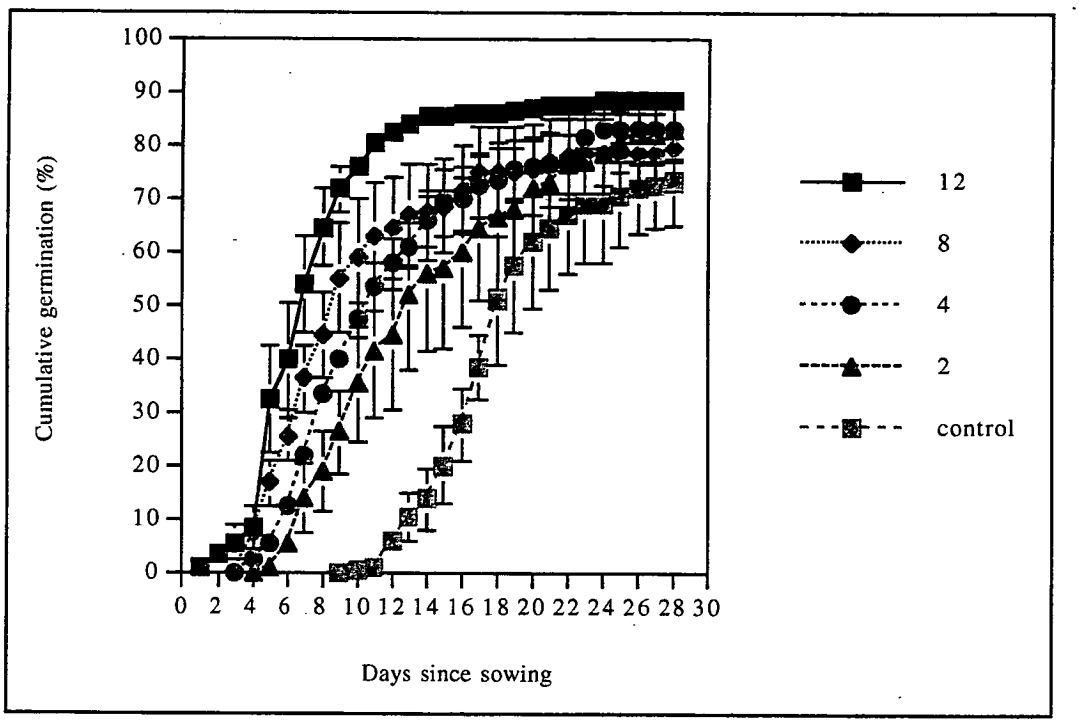
Cumulative germination profiles (as a percentage of the total number of seeds) of bulk stratified seedlot *b*, stratified for 0, 2, 4, 8 or 12 weeks. Each point is the mean of four replicates of 50 seeds.



Cumulative germination profiles (as a percentage of the total number of seeds) of lab stratified seedlot *b*, stratified for 0, 2, 4, 8 or 12 weeks. Each point is the mean of four replicates of 50 seeds.

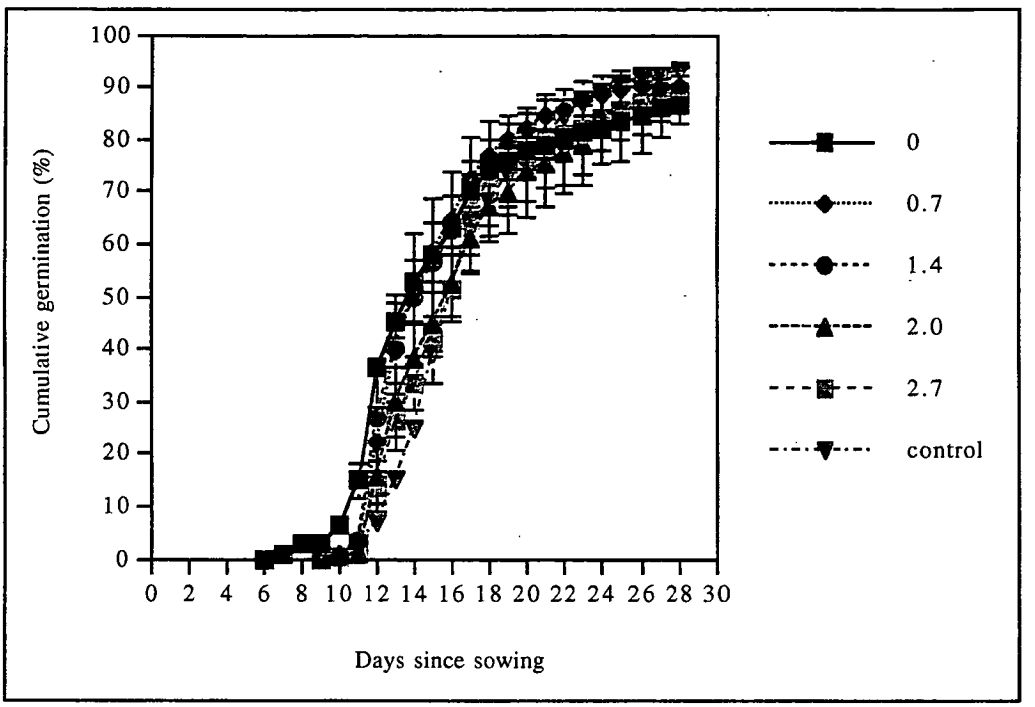


Cumulative germination profiles (as a percentage of the total number of seeds) of bulk stratified seedlot *c*, stratified for 0, 2, 4, 8 or 12 weeks. Each point is the mean of four replicates of 50 seeds.

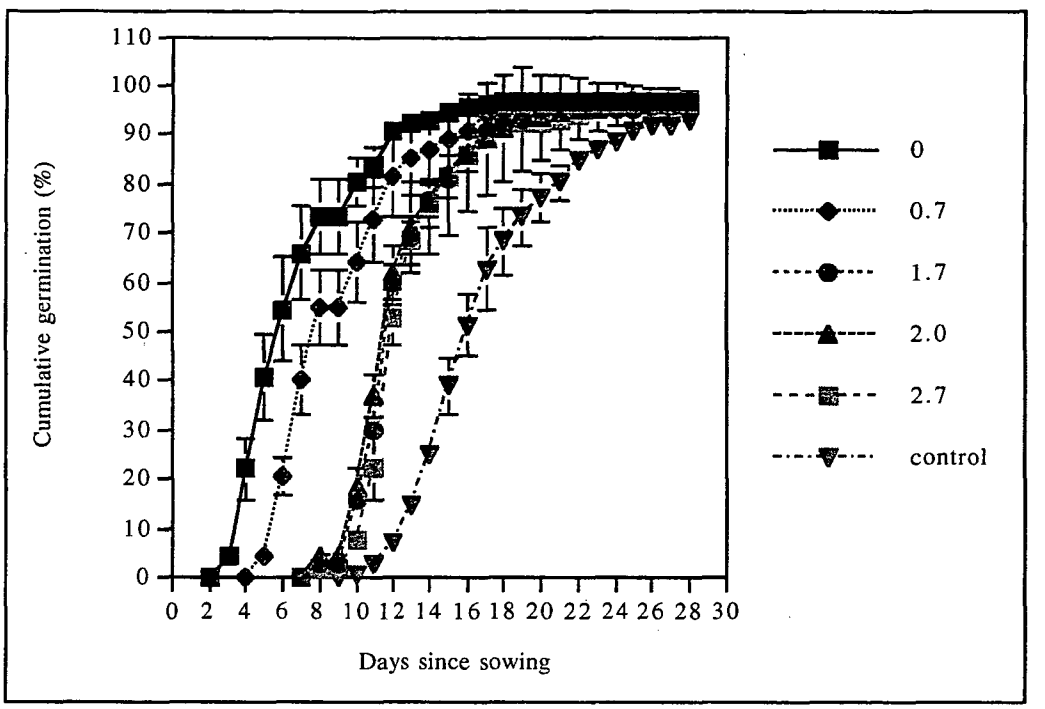


Cumulative germination profiles (as a percentage of the total number of seeds) of bulk stratified seedlot *c*, stratified for 0, 2, 4, 8 or 12 weeks. Each point is the mean of four replicates of 50 seeds.

Appendix III. Germination of seed chilled for 4 or 12 weeks over salt solutions (Chapter 6)



Cumulative germination (as a percentage of the total number of seeds) of seeds chilled for 4 weeks over 0, 0.7, 1.4, 2.0 and 2.7 *m* NaCl solutions. Each point is the mean of four replicates of 50 seeds.



Cumulative germination (as a percentage of the total number of seeds) of seeds chilled for 12 weeks over 0, 0.7, 1.4, 2.0 and 2.7 *m* NaCl solutions. Each point is the mean of four replicates of 50 seeds.