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**AN EVALUATION OF OSMOTIC PRE-SOWING SEED TREATMENTS
AS A POTENTIAL METHOD FOR IMPROVING THE GERMINATION
PERFORMANCE OF *PINUS RADIATA* D. DON SEEDS**

**A thesis presented in partial fulfilment
of the requirements for the degree of
Master of Agricultural Science in Seed Technology
at Massey University, Palmerston North,
New Zealand**

KUSMINTARDJO

1992

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ABSTRACT

KUSMINTARDJO, 1992. **An Evaluation of Osmotic Pre-sowing Treatment as a Potential Method for Improving the Germination Performance of *Pinus radiata* D. Don Seeds**

Supervisor: Dr Peter Coolbear

This study was conducted to characterise optimum conditions for osmotic pre-sowing treatment as an effective means of improving the germination and/or emergence performance of *Pinus radiata* from different seed grades.

The results indicated that osmotic treatment could reduce the germination and/or emergence times of *Pinus radiata* seeds by 40% of controls, if treated seeds were not subsequently dried back to original moisture contents. Osmotic treatment did not alter both the uniformity and final percentage germination. Rapid germination at this rate was only obtained if seeds were treated in optimum treatment conditions, i.e. with a -1.0 MPa solution for 10 d at 20°C. The correct choice of water potential and treatment duration is crucial in determining the level of treatment benefits. At high water potential, seeds were lost due to pre-germination during treatment, while at low water potential treatment benefits were less. Treatment with salt solutions (KNO₃ + KH₂PO₄) was better than with polyethylene glycol, an effect which seemed to be a result of differing seed moisture content attained during treatment as no pre-germination occurred during PEG treatment while the moisture content of PEG-treated seeds attained following drying was less than that attained by salt-treated seeds.

Since seeds are kept in the imbibed state during treatment, the prevention of microbial proliferation is of prime importance. The use of Thiram at 1% seed weight and applied before osmotic treatment gave good protection against microbial attacks without losing treatment benefits in terms of rapid germination. Application of Thiram beyond its optimum rate should be avoided as it can delay seed germination.

Treated seed should not be dried back to low moisture contents rapidly, even at ambient temperatures (22-27°C, 50-6-% RH) as drying for 4 d in these conditions resulted in a complete loss of treatment benefit. However, slow drying of osmotically treated seeds at high relative humidity (20°C, 80-85% RH) prevented the adverse effects of desiccation on germination performance. Seed dried back in this way had 30% less in median germination times relative to untreated controls.

The response of osmotic treatment applied to different seed grades gave consistent results. Rapid germination due to osmotic treatment occurred in all seed grades at similar rates and was reflected in a significant increase in seedling dry weight. As the increases in seedling dry weight were more evident in larger or heavier seeds than in smaller or lighter seeds, it is suggested that osmotic treatment seems to influence relative growth at this stage of seedling development.

Osmotic treatment reduced the storability of seed, although applications of this treatment after storage restored the level of vigour of aged seeds which just begun to decline. Although total dehydrogenase activity in osmotically treated seeds was higher than in untreated controls, there was no difference in oxygen uptake between treated and untreated controls prior to radicle emergence. It was suggested that factors other than energy production are perhaps responsible for ensuring rapid germination of treated seeds.

The commercial implications of this study are potentially good. Osmotic treatment in tree seeds is no longer restricted to using only polyethylene glycol. Improved seedling growth as a result of early emergence can help low vigour/moderate vigour seedlings become more vigorous and meet standard specifications required for outplanting.

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I. INTRODUCTION

In the early days of forest establishment around the world, natural forests were viewed as an infinite resource to be utilised as markets demanded. Because they have not been managed sufficiently, the depletion of natural forest is inevitable. With important shifts of social and economic attitudes as well as increases in human populations, natural forests could no longer provide enough timber and other forest products.

New Zealand is no exception. Kauri, the indigenous tree, is no longer or hardly available in the market, the "kauri era" having finished a long time ago in the early 1900s. Since then the "radiata era" started to emerge as more and more timber was produced from the exotic radiata plantation (Simpson, 1973). New Zealand has perhaps the world's most intensively managed and productive forest resource. Radiata grows very well and performs better than if grown in its site of origin in central California (Roche, 1989).

Forestry in New Zealand is carried out on a huge scale by both the state-owned New Zealand Forest Service and private companies, notably New Zealand Forest Products Limited and Tasman Pulp and Paper Limited. Both these companies have extensive integrated forestry operations which include the care and development of forest stock, tree felling, timber milling, the manufacture of timber building boards, plywoods and veneers, pulp-processing and paper-making, as well as the production of other related forest products. Up until now New Zealand exports to 60 different countries. Therefore it is not surprising that timber and related products now generate about six percent of New Zealand's export earnings. These products, mainly from radiata plantations, are predicted to account for 15 percent by the end of this century when forestry production is projected to reach 300,000,000 cubic metres a year (Anonymous, 1986). This is an ambitious effort from the forestry sector.

Since seedling propagation seems to be the most efficient and economic method for plant establishment, a large number of seeds are needed for annual planting, particularly genetically improved seed from selected trees. The "268" multinodal breeds, for instance, are those trees which are considered to have fast growth, straight stems, an even multinodal branch habit, freedom from forking and resistance to *Cyclaneusma* needle cast. Similarly, the "875" breeds, despite having the same growth characteristics as the "268" breed, has higher wood density (Shelbourne, 1986).

At the moment, production from seed orchards is around 4000 kg a year which is numerically enough to supply current requirements for reforestation and afforestation throughout the country (Shelbourne, 1986), where planting activities are targeted at 40,000 hectares a year (Roche, 1989). Although seeds produced from orchards have quite high percentage germination as indicated from laboratory tests (Section 4.2.2), field performance seems to be quite low. Poor survival of planted seedlings has been reported, sometimes being less than 50% (Dale, 1981). Causes of poor survival in pine could include:

- i. poor quality of planting stock;
- ii. improper lifting, handling, culling, storage before planting and transportation;
- iii. poor planting techniques, and
- iv. unfavourable site conditions during and after planting (e.g. Rasanen, 1980; Venator, 1981; Xydias, 1980).

Poor seedling quality could be due to variation in seed size and weight, since large seeds or heavy seeds of *Pinus radiata* produces larger and more vigorous seedlings needing

a shorter period in the nursery compared to those from small or light seeds. This has resulted in *Pinus radiata* seeds being sold commercially in four different seed size grades, A, B, C and D; A being the largest seed and D the smallest one. Since seedlings produced from low grade seeds very often do not survive well after planting (perhaps due to small size) and the production of large grade seeds may be limited, the long term impact of any poor survival in large scale plantations for 40,000 hectares a year would be very significant.

It has been realised that seedling growth is governed by seed size, emergence rate, available space for growth and genetic components. Nursery practice should therefore minimise the adverse effects independently associated with those factors. In loblolly pine, late emergence adversely affected seedling morphology and increased mortality from < 1% in seed emerged early to around 23% for those emerged later, while in *Pinus ponderosa* comparable figures were < 23% for early emergents to over 50% for late (Mexal and Fisher, 1987). The same effect of late emergence on seedling morphology has also been observed in larch and *Pinus caribaea* (Venator, 1973; Logan and Pollard, 1979). It is possible that the problems of low vigour and poor seedling establishment owing to late emergence may be overcome by applying pre-sowing treatments to improve seed performance as have been developed in agriculture and horticulture. Such pre-sowing treatments include hydration-dehydration methods, also called seed hardening (May *et al.*, 1962), low temperature pre-sowing treatments (Coolbear *et al.*, 1984, 1987) and osmotic pre-sowing treatment or seed priming (Heydecker and Coolbear, 1977; Bradford, 1986; Haigh and Barlow, 1987a). Significant improvements caused by pre-sowing treatments in horticultural seeds in particular include: (i) advanced germination times, (ii) reduction in the spread of germination within a seed population (Heydecker and Coolbear, 1977; Heydecker and Gibbins, 1978); (iii) increased tolerance of seeds or seedlings to adverse conditions during seedling establishment (Coolbear and McGill, 1990) and probably (iv) increased yield or plant weight (Khan *et al.*, 1980; Brocklehurst and Dearman, 1983b).

Despite the successes of using osmotic treatment for enhancing germination performance of agricultural and horticultural seeds, large scale applications have not been adopted widely. This is, perhaps, partly due to the fact that every species of seed and even individual seed lots require specific conditions for optimum results. For large seeds sown in huge amounts, the cereals, legumes and many tree seeds for example, setting up a good priming system has not been explored far enough, and other problems may also occur, such as: toxicity of osmotica, low availability and mobility of oxygen in PEG solutions (Mexal *et al.*, 1975), microbial contamination during treatments, and also problems in drying back the seed after treatment. The mechanism of improved germination performance of treated seeds is very often suggested to be the initiation of repair mechanisms during treatment, however, firm evidence underlying this hypothesis is sparse, although some biochemical changes have been reported (see Literature Review, Section 2.2.3.2).

So far no one has published on the effects of osmotic treatment in *Pinus radiata*. This study was thus designed to identify and overcome some of the problems associated with osmotic treatment which might be encountered in developing a system for *Pinus radiata* in New Zealand as well as some physiological aspects relating to the treatment. The specific objectives of the study are:

- i. To characterise the best conditions of osmotic treatment for *Pinus radiata* seeds.
- ii. To investigate proper drying techniques which can retain maximum benefits of treatment.
- iii. To determine the effects of seed treatment on different seed grades and their effects on seedling growth.

- iv. To evaluate the interactions of osmotic treatment with seed storage.

- v. To conduct an investigation on some physiological aspects of osmotic treatment with particular emphasis on respiration and the activity of dehydrogenase enzymes.

II. LITERATURE REVIEW

It is generally realised that the time from sowing to seedling establishment is a crucial phase during which seed are exposed to environmental factors which are often very hostile. Since those factors can all affect germination performance and seedling establishment, seed quality and vigour will be very important for planting purposes. Seeds which produce substantial amounts of weak and abnormally growing seedlings in the standard germination test should be considered low quality seeds not to be used for planting. Similarly, low vigour seeds may germinate slowly and thus remain longer in the soil and become more prone to pest and disease attacks (Bewley and Black, 1985). Consequently, there have been many attempts devised to improve germination performance in the field, the aim being to accelerate germination, so decreasing the time during which seedlings are exposed to environmental and biological stresses (Heydecker and Coolbear, 1977; Thomas, 1981).

This review is divided into two sections. The first section highlights general concepts on seed quality and vigour, the physiological basis of poor seed vigour and the current problems of low vigour in forestry tree seeds. The second section deals with pre-sowing treatments for improved seed performance with special emphasis on pre-sowing hydration treatment and/or osmotic pre-sowing treatments.

2.1 Seed Quality and Vigour

2.1.1 General concepts

Although Thomson (1979) defined 10 components of seed quality, i.e.: analytical purity, cultivar purity, freedom from weeds, high germinability, high vigour, seed size and

uniformity of size, seed health and moisture content; not all of these components have equal value, while the relative order of importance varies according to different situations (Salunkhe *et al.*, 1987). Seed health, as an example, is an important component anywhere, while seed vigour would be much more important in bad weather than any other seed quality components and uniformity of seed size probably becomes more a matter of consideration in mechanical planting than in manual planting. A similar situation applies to the relative importance of other seed quality components.

As a seed's viability or germinability dictates its ability to establish seedling under favourable conditions, seed vigour reflects its ability to do so even under poor or unfavourable conditions (Salunkhe *et al.*, 1987; McDonald, 1980). Therefore ideally seed vigour will have greater effects on seedling performance under stressful environments than any other seed quality component. It is not the aim to discuss all of the many varying seed quality components but emphasis will be given to seed vigour in this review.

2.1.1.1 Vigour concepts: ISTA vs AOSA

There are two similar definitions of seed vigour which are internationally accepted. In 1977 (Perry, 1978) the Vigour Test Committee of the International Seed Testing Association (ISTA) defined that "Seed vigour is the sum total of those properties of seeds which determine the level of activity and performance of the seed or seed lot during germination and seedling emergence. Seeds which perform well are termed high vigour seeds and those which perform poorly are called low vigour seed." Various aspects of seed performance in this definition include: (1) biochemical processes and reactions such as enzyme activity and respiration, (2) rate and uniformity of seed germination and seedling growth, (3) rate and uniformity of seedling emergence and growth in the field, and (4) emergence ability of seedlings under unfavourable environmental conditions.

In 1979 (McDonald, 1980) the Association of Official Seed Analysts (AOSA), Vigour Testing Sub-Committee proposed the definitions of seed vigour as "Seed vigour comprises of those seed properties which determine the potential for rapid, uniform emergence and development of normal seedling under a wide range of field conditions."

Although those two concepts have a similarity in emphasis on rapid and uniform seedling emergence under a wide range of environmental conditions which are of special importance for planting purposes, there are dissimilarities between them. The ISTA concept seems to be more "academic" because of comprehensively discussing, identifying and describing what seed vigour is. While the AOSA concept, on the other hand, is more direct, shorter and more operational. However, as described in the AOSA Vigour Test Handbook (1983) there is no need to select one definition over the other, all that can be said is that the definition described by ISTA is perhaps more ambiguous than the definition described by AOSA.

2.1.1.2 Vigour variations: The effect of seed size

There are many factors known to influence vigour potential and they may be classified as: genetic constitution, environment and nutrition of mother plant, stage of maturity at harvest, seed size or weight, mechanical integrity, deterioration and ageing (Perry, 1978).

Seed size or weight and uniformity are important quality parameters of seeds. Both genetic and environmental factors cause variations in seed size, particularly in cross-pollinated species (Thomson, 1979). Other factors influencing seed size are nutrition and moisture to the developing seed, and so depend on factors which vary from plant to plant throughout the crop (shading, soil moisture and fertilisers) and which are modified by local competition (Delouche, 1980; Thomson, 1979).

Reports describing an association between seed size and germinability and/or vigour are abundant. Large seeds of hybrid maize have been shown to germinate more rapidly than from small seeds, and although there was no difference in final germination, increased seed size was associated with improved field emergence (Hong *et al.*, 1982). Similarly, in *Chenopodium* sp., tomato and barley increases in the rate of germination were observed from large seed (Hall and McNeill, 1982), although opposite findings for tomatoes showed a slower germination for large seeds but with faster seedling growth (Withington *et al.*, 1965; Whittington and Fierlinger, 1973). In cabbage seeds cv. "Wisconsin All Season" and turnip cv. "Purple Top", large seeds of both vegetables were significantly higher in germination and seedling vigour than in small seeds (Hanumaiah and Andrew, 1973). Although many studies in pine indicated similar advantages of large sized seeds over small seeds (Table 2.1), in *P. taeda* (Struve *et al.*, 1989), shortleaf pine (Mann, 1979), *Quercus ilex* (Aissa, 1983), *Tsuga heterophylla* (Kuser and Ching, 1981) and *P. sylvestris* (Wrzesniewski, 1982a, 1982b and 1982c), no advantages of larger seeds over small seed were found. Accordingly, favouring large seeds over small seeds may not be justified (Wrzesniewski, 1982a, 1982b and 1982c). In addition, there have been other factors which may be associated with large seeds. Mechanical damage often occurs during the operations of hulling and cleaning and large seeds could be bruised or injured this way. Although large sized "hard" seeds may become more permeable this way, there is a danger of damaging embryos of soft seeds (Salunkhe *et al.*, 1987). Damaged seeds would then be more susceptible to fungal and pests during storage, whereas germinating them in water may cause imbibitional damage as observed in pea (Powell, 1985). In cases of no mechanical damage, large seeds would probably have better performance over small seeds owing to greater quantities of stored reserves which are available ready for seedling growth, but there may also be differences in embryo size and the ability of the hypocotyle to elongate (Black, 1959) or in the biochemical composition of the seed (McDaniel, 1969). Thus it is a common practice in *P. radiata* to separate large seeds from the small ones since in the nursery small seeds are not only slow in germination but also produce small seedlings than that of large seeds.

Table 2.1 The effects of size or weight on laboratory and/or field performance of different tree seeds.

Species	The effects of seed size	References
<i>Abies nobilis</i>	- large seeds had higher germination rate and seedling dry weight	Kandya and Ogino (1986)
Eastern white pine	- no effect on seedling growth - large seeds had bigger size and heavier weight of seedling	Ricneke (1942) Demeritt and Hocker (1975)
<i>Cupressus macrocarpa</i>	- large seeds had higher germination rate and seedling dry weight	Kandya and Ogino (1986)
<i>Larix</i> sp.	- no effect on percent germination and germination rate	Logan and Pollard (1979)
<i>Picea abies</i>	- large seeds produced larger seedlings	Mikola (1980)
<i>Pinus densiflora</i>	- large seeds produced higher germination rate and seedling dry weight	Kandya and Ogino (1986)
<i>P. elliottii</i>	- no effect on percent germination, germination rate and seedling size - large seeds had higher percent germination, percent survival and seedling size	Shoulder (1961) Belcher <i>et al.</i> (1984)
<i>P. keysiia</i>	- no effect on percent germination, germination rate and seedling size	Hodgson (1980)
<i>P. oocarpa</i>	- large seeds had higher germination rate and seedling dry weight	Kandya (1978)
<i>P. palustris</i>	- large seeds had higher germination rate and seedling dry weight	Kandya (1986)
<i>P. pinaster</i>	- large seeds had higher germination rate and seedling dry weight	Kandya (1986)
<i>P. ponderosa</i>	- no effect on germination rate - large seeds had higher percent germination and seedling size	Larson (1963) Wang and Patee (1974)
<i>P. roxburghii</i>	- medium sized seeds had higher germination rate and greater biomass - large seeds produced bigger size and higher seedling biomass	Gosh <i>et al.</i> (1976) Chauhan and Raini (1980)

Table 2.1 cont'd

Species	The effects of seed size	References
<i>P. radiata</i>	<ul style="list-style-type: none"> - large seeds had higher germination rate and seedling dry weight - large seeds produced large seedling - large seeds had higher germination rate and seedling dry weight 	Griffin (1975) Chavasse (1979) Kandya and Ogino (1986)
<i>P. strobus</i>	<ul style="list-style-type: none"> - large seeds had higher germination rate and seedling dry weight 	Kandya and Ogino (1986)
<i>P. sylvestris</i>	<ul style="list-style-type: none"> - large seeds produced large seedlings - medium sized seed had faster imbibition, more active respiration and faster germination 	Mikola (1980) Wrzesniewski (1982a,b,c)
<i>P. taeda</i>	<ul style="list-style-type: none"> - large seeds had higher percent germination and bigger seedlings - large seeds produced bigger seedlings - no effect on volume of trees at 25 yr old - seed size was correlated with volume of 5, 10 and 15 yr old trees - no effect on germination and vigour 	Hodgson (1980) Barnett and Dunlop (1983) Sluder (1979) Robinson and van Buijtenen (1979) Struve <i>et al.</i> (1989)
<i>P. thumbergii</i>	<ul style="list-style-type: none"> - large seeds had higher germination rate and seedling dry weight 	Kandya and Ogino (1986)
<i>Pseudotsuga taxiflora</i>	<ul style="list-style-type: none"> - large seeds had higher germination rate and seedling dry weight 	Kandya and Ogino (1986)
<i>P. virginia</i>	<ul style="list-style-type: none"> - large seeds produced higher seedling height 	Mann (1979)
Shortleaf pine	<ul style="list-style-type: none"> - large seeds produced higher seedlings 	Mann (1979)
<i>Quereus ilex</i>	<ul style="list-style-type: none"> - no effect on germination rate 	Aissa (1983)
<i>Tsuga heterophylla</i>	<ul style="list-style-type: none"> - no effect on seedling size 	Kuser and Ching (1981)

2.1.2 Physiological Basis of Poor Vigour and Deterioration

2.1.2.1 General causes

Deterioration is a common phenomenon in living organisms with loss of viability being the ultimate result. However, before this, several deteriorative events occur and thus the purpose of proper storage is to maintain not only high germination but also the vigour of seed by preventing or slowing down deterioration. Unfortunately, it is very difficult to separate out the primary causes of seed deterioration from secondary ones because any critical type of damage in any species or under a given set of ageing conditions may not be very important in another species or set of ageing conditions (Priestly, 1986; Coolbear, 1989). McGee (1983) classified the basic causes of seed deterioration into two broad categories. First, seed tissues may deteriorate due to ageing, and secondary deterioration may also occur because of the invasion of and damage to tissue by micro-organisms, insects or rodents. The former category is a problem to which plant physiologists have paid considerable attention while the latter have usually been addressed by plant pathologists and entomologists.

From the physiological and biochemical viewpoints, plant physiologists have developed three groups of theories explaining the causes of deterioration as follows (Curtis, 1963; McGee, 1983):

(a) The accumulation of deleterious products of metabolism

This theory suspects that toxic metabolites are produced by seeds which could induce intra- and/or inter-molecular cross-linking reactions, inactivation of enzymes and nucleic acids and membranes become non functional. D'Amanto and Hoffman-Ostenhof (1956) reviewed a great literature review where automutogenic substances may accumulate in seeds which cause loss of viability, but much of the evidence is not strongly convincing (Roberts,

1973b). Several compounds like indole acetic acid and its derivatives (Sircar and Biswas, 1960; Sircar and Dey, 1967), phenolics (Dey *et al.*, 1967) and ABA (Sircar, 1967) have been claimed to be responsible for viability losses in rice. However, the hypotheses of toxic metabolites is still inconclusive since in one recent report aged rice seeds, the unknown inhibitors were extracted but their bioassay depended not on germination but on the growth of different species (Chatterjee *et al.*, 1976).

(b) Wear and tear theory

This theory assumes that with increasing use, organelles, cells and organs become ineffective in conducting normal activities. Anderson *et al.* (1970) observed the coalescence of spherosomes or lipid bodies of wheat seeds infected by fungi while others also observed similar findings but in seeds without infections (Harman and Granett, 1972) or it was observed only during imbibition (Hallam *et al.*, 1973). However, Anderson and Baker (1983) found little evidence of such changes in ribosomes and membranes of the nucleus, mitochondria and other organelles. Some changes observed might be due to different fixation techniques, different species and of course different storage regime, since in dry storage, most organelles are largely inactive (Anderson and Baker, 1983).

(c) Somatic mutation theory

It has been shown that the number of mutations increases as the tissues age, and that a corresponding increase in undesirable mutations may lead to metabolic malfunctions.

2.1.2.2 Mechanism of seed deterioration during ageing

As membranes have an important role in regulating sub-cellular activities, it is reasonable to assume that any membrane disruption would impair their functions and

selective permeability characteristics. High moisture content of seed may bring about the hydrolysis of important components, including membranes which then cannot be repaired nor replaced because of the inefficient activity of repair processes (Bewley and Black, 1985). The involvement of molecular O_2 in respiration and other oxidative processes could induce free radical formation. A free radical is an atom or a group of atoms with an unpaired electron which can remove or donate an electron to a neighbouring molecule. Consequently, free radicals are some of the most chemically reactive groups known. They may be formed in various ways, e.g. photolysis (Greenstock, 1986), leakage from the active sites of impaired enzymes, lipoxygenation of polyunsaturated fatty acids (Priestly, 1986) or may be initiated by metal ions (Hendry and Brocklebank, 1985; Sanman and Boger, 1980; Priestley, 1986). The most potent free radicals causing damage are the hydroxyl (OH) and superoxide (O_2^-) radicals. Once a free radical is produced, a chain reaction is initiated which may cause extensive damage. However, free radical chain reactions may be terminated by reactions with other free radicals (Benson, 1990), natural scavengers, e.g. α -tocopherol (vitamin E), ascorbic acid, haemoprotein, isoflavanoids; or quenching with water or superoxide dismutase action (Wilson and McDonald, 1986; Basu and Das Gupta, 1978).

Loss of membrane integrity results in a greater loss of electrolytes, such as amino acids, as well as organic acids and an increase in the conductivity of seed leachate. Accordingly, a higher soak water conductivity indicates a low vigour seed as has been demonstrated in pea (Matthews and Bradnock, 1967; Carver and Matthews, 1975; Scott and Class, 1976), rice (Agrawal, 1977), corn (Gill and Delouche, 1973; Tao, 1983a and 1983b), bean (Matthews and Bradnock, 1967), soybean (Abdulbaki and Anderson, 1973b; Yacklich *et al.*, 1979; Tao, 1983a and 1983b) and in barley (Abdulbaki and Anderson, 1970). Enhanced leakage in many cases can be detected at very early hours in imbibition, prompting suggestions that deficiencies in membranes are present in the dry condition, probably this is due to a changing membrane configuration from the hexagonal phase (Simon, 1978) or gel

phase (Crowe *et al.*, 1989) into much more organised lamellar or liquid crystalline phase and once organisation is completed there would be no leakage from cells. As leakage of organic metabolites could encourage the growth of fungal or micro-organisms on seeds, there have been direct correlations between seed rot and the quantity of carbohydrate exudate from seeds of soybean, peas and garden beans (Keeling, 1974; Matthews and Bradnock, 1968; Schroth and Snyder, 1961). The relationship between imbibition, leakage and ageing, however, cannot be assumed for all species or all types of ageing since Pesis and Ng (1983), as an example, found that a decline in germinability of barley seeds preceded any signs of increasing electrolyte efflux as seeds were subjected to accelerated ageing at 45 °C and 100% RH. Conductivity changes in leachate from cotton seeds subjected to 50 °C and 100% RH were poorly correlated with loss of viability (Halooin, 1975) and in tomato (Coolbear *et al.*, 1984) seeds subjected to controlled deterioration at 45 °C and 75% SMC leaked more sugar and amino acids but did not show increased leachate conductivity.

As phospholipids are the major component of membranes, changes in phospholipids may be associated with viability and vigour losses. A decline in phospholipid levels by up to 50% or more has been reported when peanut and pea seeds were stored at high humidity (Pearce and Abdel-Samad, 1980; Powell and Matthews, 1981b). However, response differences may occur as the consequence of cultivar differences, ageing regimes and/or seed moisture status (Francis and Coolbear, 1984 and 1987; Priestley and Leopold, 1983; Powell and Matthews, 1981b; Yang and Yu, 1982). In addition, contradictory findings have also been reported where ageing soybean and pea seeds seemed to actually increase their phospholipid contents (Chapman and Robertson, 1977; Powell and Harman, 1985). An explanation for the increase of phospholipids at high moisture contents may be the operation of repair processes.

2.1.2.2.1 Enzyme changes

Since many enzymes are involved in the complex metabolism of germination, the loss of ability to synthesise certain enzymes is often to be said to serve as a measure of the aging process (Roberts, 1979). Reduced ability to synthesise ribonuclease (Mierzwinska, 1973), peptidase (Nowak and Mierzwinska, 1978), hydrolases (Samsbery and Banerji, 1979; Saxane and Maheswary, 1980; Agrawal, 1981; Kole and Gupta, 1982) succinic semi-aldehyde dehydrogenase (Gallashi and Floris, 1978), DNA polymerase (Petruzelli *et al.*, 1984b) have all been reported to be associated with loss of viability. However, there have been some conflicting reports on the effects of ageing on enzyme activity. Anderson and Gupta (1986) noted that while some enzyme activities are reported to decline with germinability, some enzymes, e.g. glutamic acid decarboxylase, hexokinase, adenosine kinase, seem to be functional or hardly affected by deterioration. Similar observations were reported by McLeod (1952) where peroxidase and dehydrogenase activity reduced with loss of viability during natural ageing while proteinase, amylase and phosphatase were relatively stable. These findings may lead to a conclusion that inability of deteriorated seeds to germinate is not due to a general decline in enzyme activity, but that there appear to be specific critical enzymes that are affected. Further than this, there is the suggestion that the most vulnerable enzymes during deterioration are involved in anabolic pathways while many hydrolases involved in metabolism are much more stable.

2.1.2.2.2 Respiration changes

Reports of reduced or disrupted respiration during ageing are not surprising given the many changes in enzyme activity and membrane damage. Maize kernels which failed to germinate following storage consumed little oxygen (Woodstock and Grabe, 1967). Several studies have also demonstrated the importance of respiratory metabolism in the expression of seedling vigour, for example in barley (McDaniel, 1969) and corn (Woodstock and Feeley, 1965; Woodstock and Grabe, 1967). Changes that reduced seed quality were associated with

reduced levels of respiration and activities of respiratory enzymes (McDonald, 1975; Woodstock, 1973). Those later observations emphasised the reduction of respiratory enzymes in response to stress conditions that affected seed vigour. Deteriorated barley seeds (Ellerton and Perry, 1983) and low vigour soybean seeds (Woodstock and Taylorson, 1981) were more sensitive to anoxia than undeteriorated or high vigour seeds. Ethanol, which may be toxic to cells, initially accumulated in all seeds but subsequently declined rapidly in high vigour seeds while the high levels persist in low vigour seeds (Powell, 1988). Similarly, the decrease in respiratory quotients which occurs following imbibition decreases after one hour, while high respiratory quotients persist in low vigour seeds. In this respect, Woodstock and Taylorson (1984) proposed that ageing caused a breakdown between glycolysis and Krebs cycle leading to prolonged accumulation of ethanol by alcoholic fermentation during early stages of germination of low vigour seeds, thereby maintaining increased respiratory quotients.

Abu-Shakra and Ching (1967) concluded that mitochondria of aged seeds were fewer and less efficient, probably because of impaired development on imbibition, while the amount of organic phosphate esterified into ATP per unit oxygen consumption was about half of those from untreated seeds. Woodstock *et al.* (1984) also found that after imbibition, the mitochondria of aged seeds displayed depressed rates of oxidation, lower respiratory control and a decrease in ATP production per unit oxygen consumption. These phenomena perhaps suggest that deteriorated seeds are at least partially uncoupled leading to inefficient respiration. Changes in ATP synthesis with ageing have been reported. Increases in ATP control during germination of unaged seeds have been observed in wheat, rape, cauliflower and sugarbeet, while the level of which declined during ageing (Grzesiuk *et al.*, 1983; Lunn and Madsen, 1981). However, a conflicting correlation between ATP content and vigour of seeds has been found by Styer *et al.* (1980). High ATP contents were found in non-germinable seeds of corn, radish and onion. Perl (1986) also found a similar situation of poor

correlation between ATP content and vigour of nine vegetable seeds. In this connection Perl (1986) proposed some important points which should be considered in determining correlations between ATP content and seed vigour, e.g.:

- (a) A positive correlation between ATP and seed vigour may be attributed to the variation in the size of seed used, not to the amount of ATP accumulated.
- (b) Usually 95% of ATP produced is rapidly utilised in ATP requiring systems. Thus an increase or decrease in accumulated ATP even by 100% may be negligible for the evaluation of the rate of ATP synthesis.
- (c) As the accumulated ATP is the result of a balance between processes of synthesis and utilisation, a large amount of ATP accumulated may be either result of active ATP synthesis which reflects high vigour seeds, or probably of impaired ATP utilising systems, indicating low vigour levels.

2.1.2.2.3 Changes in nucleic acids

In rye embryos, Roberts and Osborne (1975) found no loss of total DNA per nucleus during viability loss but the amount of high molecular weight of DNA in non-germinating embryos was only one third of that in high germinating embryos. Although extraction procedures of DNA might result in some DNA breakage (Cherry and Skadsen, 1986), *in vivo* breakage seems important during loss of viability. Reduced DNA synthesis has also been found in aged wheat seeds (Blowers *et al.*, 1980; Dell 'Aquila and Margoita, 1986) and with the increase of severity of storage regime, there was a parallel decrease in the rate of DNA synthesis (Dell 'Aquila and Margoita, 1986). In medium vigour seeds, incorporation of tritiated thymidine was only 70% of that high vigour seeds (Blowers *et al.*, 1980). In cell free and *in vivo* systems, protein synthesis occurring from low vigour wheat seeds was attributed

partly to a significantly low level of poly A⁺-RNA (Blowers *et al.*, 1980). By contrast, Osborne *et al.* (1977) and Sen and Osborne (1977) noted that embryos which have just reached 0% viability synthesise the same amount of RNA as in high germinating seeds, but no protein synthesis was detected. Subsequent investigation of differences in viability and vigour wheat seeds found a good correlation between poly A⁺-RNA level and the rate of protein synthesis (Smith and Bray, 1984).

Chromosomal damage may be the final deteriorative change which is proposed to occur before loss of viability. In barley and pea, a small decrease in viability was associated with an increase in chromosomal aberrations particularly of the chromatid types where the unit of aberration is the chromatid (Durado and Roberts, 1984a). Increases in mutation frequency also occurred with a small fall of viability (Durado and Roberts, 1984b), suggesting that there is no threshold of viability loss without avoiding mutation. Ross and Rincker (1982), however, found no evidence of genetic shift associated with loss of viability during ageing of Pennolate orchardgrass.

2.1.2.2.4 Hormonal changes

Although Skabka (1964) found no difference in auxin levels following deterioration of wheat seeds, Juel (1941/42), Koves *et al.* (1965) and Sremulu (1983c) presented evidence of reduced endogenous auxins in aged wheat seeds which are believed to control growth, assimilate movement and vascularisation (Coolbear, 1989). A similar decline in ethylene production is also characteristic of the germination of aged rape (Takayanagi and Harrington, 1971), rice (Kwun, 1980) and *Phaseolus vulgaris* seeds (Saminy and Taylor, 1983). In durum wheat, application of ethylene decreased the deterioration rate of seeds subjected to 30°C and 19.5% SMC (Petruzelli and Taranto, 1985) while application to aged seeds accelerated the rate of radicle emergence without improving germinability (Takayanagi and Harrington, 1971). These results suggested that the ethylene production

system perhaps is one of many factors responsible for resistance to ageing. In wheat, aged seeds inherently were less efficient in translating the hormonal stimulus since α -amylase production by aleurone tissues in response to added gibberellin decreased with seed age (Aspinal and Paleg, 1971) and similar situation applied to aged rye seeds (Mierzwinska, 1977). However, working with old seeds of wheat Mierzwinska (1977) found promotion of germination following application of gibberellin. Presumably this is an indication that the aged embryo is incapable of producing sufficient endogenous gibberellin.

2.1.3 Current Problems in *P. radiata* Forest Establishment in New Zealand

2.1.3.1 The role of large sized seeds

Seedlings which can easily and rapidly establish contact with water and nutrients in new soil and can also survive under drought and frost conditions seem to be key factors for the success of large scale forest establishment. However, mechanised nursery operations and mass production methods during seedling removal from nursery bed, transportation, storage as well as transplanting even if carefully done, result in damage to the fine roots of seedlings and will reduce or temporarily stop photosynthesis. In this connection plantable seedlings should have certain morphological and physiological specifications for better survival (FRI, 1988; Table 2.2). However, there have been major concerns that small *P. radiata* seeds produce smaller seedlings than that of large seeds (Chavasse, 1973). A study in Australia also demonstrated that small *P. radiata* seeds germinated erratically and at 32 weeks after sowing, seedlings produced by small seeds were 22% shorter, and had 35% less mean shoot diameter and were less sturdier than that produced by large seeds, while their mean dry weights were only 55% of controls (Griffin, 1975). It is not surprising that large seeds are desirable over small seeds. But supply of large sized seeds is limited, therefore seedlings from small seeds should be raised longer in the nursery before transplanting in the field

(Section 2.1.1.2) otherwise small seedlings will not survive, particularly in bad weather (Van Dorsser, 1969).

Table 2.2 Seedling specifications for radiata pine (FRI, 1988).

Indicator	Specification	Measuring technique	How to achieve specification
Root growth potential	4-5 on a 0-5 visual scale	Life, grow, lift, assess new root growth.	Wide spacing, regular conditioning, hand-lifting.
Mineral nutrients*			
Nitrogen	14-16	Analysis of seedling tops.	Judicious application of fertiliser.
Phosphorus	1.2-1.4		
Potassium	c. 3.5		
Magnesium	0.6-0.8		
Calcium	c. 1.0		
Sulphur	c. 1.2		
Boron	c. 0.008		
Copper	0.002-0.003		
Iron	0.025-0.04		
Manganese	0.005-0.014		
Zinc	0.005-0.01		
Water potential	< -0.5 MPa	Measure seedling top on needle fascicle in a pressure bomb.	Adequate watering before and during lifting, wet and cool storage, careful handling.
Height	20-40 cm	From root collar to top.	Careful timing of sowing and undercutting or topping.
Diameter	> 5 mm	At root collar.	Wide spacing, regular conditioning.
Sturdiness	40-60	Height/diameter.	Wide spacing, regular conditioning.
Frost tolerance	-12 °C winter -6 °C summer	Test using artificial frost rooms.	Grow seedling at high-elevation, inland nurseries.

* Quantities are minimum requirements in grams of element per kilogram of seedling top dry matter.

2.1.3.2 Causes of seed size variation

Variation in seed size is known to occur at several levels. At the most basic level, variation in size occurs within clones where seed weight and number of filled seed had been reported to vary between grafts in Scots pine (Hagmann, 1972; Shen and Lindgren, 1981). In European larch, seed size variation was found within and between clones (Hall, 1985). As total number of seed and seed weight were directly related to cone size therefore cone size can be used to predict the number and size of seeds. In *P. radiata* Griffin (1975) found variation in seed size at 3 different levels, e.g. between sites, between trees within sites and between cones within trees or clones. A sample of 12-year-old stands from a fertile soil with high rainfall produced around 32,000 seeds per kg while *P. radiata* stands on the poorest soil produced < 12,000 seeds per kg. One example of clonal variation reported by Griffin (1975) was from seed orchard near Traralgon (Australia) where clonal mean seed weight varied from 23,000 to 47,000 seeds per kg while seed variation between cones from one particular clone planted on one site ranged from 32,000 to 62,000 seeds per kg. Seed size was also reported to vary from year to year of collection in *Abies procera* (Sorensen and Franklin, 1977), European larch (Heiken and Soegaard, 1962), and Scots pine (Strohmeyer, 1938). These results suggest that seed size or weight is considered to be one of some plastic plant characters (Palmlblad, 1968). As a pine seed comprises 3 main parts, e.g. seed coat, megagametophyte and an embryo, which are approximately 44, 50 and 6% respectively, any variation in seed size or weight seems to be due to the seed coat and/or the megagametophyte which is not affected by the male gametophyte (Righter, 1945). Accordingly, any environmental factors affecting seed size are probably mediated via the mother plant. Therefore, improving the compatibility between rootstock and scion which improve movement of nutrients and products of photosynthesis should also increase large cone and large seed production (FRI, 1974). Other procedures which improve the health of trees and also improve large seed production include developing cultural practices such as

pest control, irrigation, fertilisation, soil cultivation or sub-soiling and frequent thinning (FRI, 1975; Schmidfling, 1971; Jett, 1986; Struve *et al.*, 1989).

2.2 Pre-sowing Seed Treatment for the Improvement of Seed Performance

If we realise that the supply of high vigour seeds may not always be readily available (and even high vigour seeds are subject to deterioration), possible approaches to improving seed quality and performance using pre-sowing treatment are most attractive. Comprehensive reviews on seed treatments have been published by Heydecker and Coolbear (1977), Tonkin (1979 and 1984) and Toledo (1988). It is not the aim here to discuss all the methods of seed treatment and this review will be confined to only pre-sowing hydration treatments. Firstly, the need for and types of pre-sowing treatment will be discussed, followed by a discussion of mechanisms of action.

2.2.1 The Need for Pre-Sowing Treatment

As seeds are sown in the field, they are exposed to rapidly changing hostile environments which have profound effects not only on seedling emergence (Khan, 1977; Thomas, 1981) but also on plant survival (Wright, 1989). Rapid emergence and rapid plant establishment to a predetermined stand may be a major limiting factor for successful agriculture. The timing and uniformity of seedling emergence can ultimately affect crop uniformity (Gray, 1978), performance during growth and establishment would be reflected in ultimate plant size (Salter *et al.*, 1981; Wurr and Fellows, 1983) and all those characteristics could have an impact on final yield and quality of the crop produced (Bradford, 1986). Therefore there have been many attempts to devise pre-sowing treatments for improving seed performance in the field. Generally, pre-sowing treatments involve increasing seed

moisture contents. As seeds come into contact with water, they rapidly start to imbibe and after several hours, they enter a lag phase before once again rapidly taking up water as the root begins to grow actively through seedcoat. During the lag phase, considerable biochemical activity associated with respiration and protein biosynthesis occurs and this also enables repair and advancement to take place before seeds are subsequently dried for storage and conventional sowing. Thus any seed treatment designed to shorten the period between sowing and emergence or to allow seeds to complete various stress-sensitive parts of the germination process are likely to be of benefit in terms of emergence and crop establishment (Coolbear *et al.*, 1987). This has been shown for a wide range of vegetable and agricultural seeds (Heydecker and Coolbear, 1977; Bradford, 1986), and has especially proved to be advantageous when direct seeding is used in adverse conditions (e.g. escape from drought or non-optimal temperatures, Heydecker *et al.*, 1975; Cantliffe, 1989). To evaluate the effectiveness of any pre-sowing treatment Coolbear (1989) suggested the following questions should be asked:

- (a) Can the seed be dried back successfully and sown conventionally after treatment?
- (b) If additions have been made to the soak solution, have adequate controls been done to demonstrate real effects for the added chemicals over and above the water or osmotic soak?
- (c) Have the physiological effects been clearly identified (e.g. are there any changes in seedling growth rates or altered physiology or do results depend simply on stress avoidance as a result of earlier emergence)?
- (d) Does the treatment have commercial potential (e.g. are effects reliable, can the treatment be scaled up, can the seeds be stored after treatment)?

2.2.2 Methods of Pre-sowing Hydration Treatment

There are three common pre-sowing hydration treatments, viz.: hydration-dehydration treatment, ("seed hardening") (May *et al.*, 1962), low temperature pre-sowing treatment (LTPST) (Coolbear *et al.*, 1984) and osmotic treatment (Heydecker *et al.*, 1973).

Seed hardening is basically a treatment involving soaking seeds with small quantity of water followed by drying back to their original moisture content. One wetting-drying cycle may suffice for some seeds while others could require two or three cycles for better results (May *et al.*, 1962). However, effects of seed hardening are not always beneficial. While positive results on improved rate of emergence and/or improved yield had been reported in carrot, corn, sunflower, wheat and barley (Austin *et al.*, 1969; Hegarty, 1970; Singh and Wilson, 1974; Choit, 1979; Carleto, 1977). Carleto and Malik (1980) also reported improved drought tolerance and increased seed production following better and quicker seedling emergence of treated wheat seeds. On the contrary, there have been many reports where pre-sowing hardening treatment had negative effects such as reduced seedling emergence in tomato (Bussell and Gray, 1978), an increase in the spread of times of emergence in parsnip (Gray and Steckel, 1977) and reduced shorgum grain yield (Carleto and As-Saqui, 1975). These results demonstrate that seed hardening cannot be used for general pre-sowing treatment in every species, many issues need to be explored comprehensively in order to disclose the mode of action as well as physiological and biochemical changes associated with the treatment. Perhaps negative effects of seed hardening are due primarily to overlong hydration periods resulting in DNA replication and once this has happened, drying back after treatment has damaging effects.

Heydecker (1974) suggested that allowing seeds to imbibe water at a temperature too low for radicle emergence but not for the initial metabolism of germination has promotive

effects as happened in the germination of *Capsicum* seeds upon transfer to 30°C. This method is relatively new but it has been explored by many workers and termed low temperature pre-sowing treatment (Coolbear *et al.*, 1984, 1987, 1990) or psychopriming (Heydecker, 1974). Bussell and Gray (1976) found that upon transfer to 25°C, the uniformity of germination of tomato treated seeds at 8°C for 24 hr was improved while in parsnip (Finch-Savage and Cox, 1982) treatment at 1°C for 6-10 d and transferred to 20°C showed improved rates and uniformity of germination. In tomato, LTPST given either before or after a controlled deteriorative ageing treatment protected or restored the germination rate to that of unaged untreated seeds, but there was no ameliorative effect on the losses of germinability incurred during storage (Coolbear *et al.*, 1984). Compared to osmotic treatment, LTPST was much more effective in improving the uniformity of germination in tomatoes (Coolbear *et al.*, 1987). Although 10°C LTPST increased the germination rates of celery cv. Tall Utah 52-70, neither final germination capacity nor the uniformity were increased while drying back treated seeds to their original moisture content caused a significant loss of germination advancement from the treatment (Toledo and Coolbear, 1988). However, a more recent study using cv. Tall Utah 52-70 and "Green Giant Hybrid" demonstrated that treated seeds of both cvs can be taken down to original moisture content or lower and yet germinate more quickly than surface-dried treated seeds if drying is allowed to proceed slowly (Coolbear *et al.*, 1991). Seeds of cv. "Green Giant Hybrid" which were of relatively low vigour seemed to gain a greater benefit from LTPST than that of cv. Tall Utah 52-70. Coolbear and McGill (1990) showed that LTPST at 10°C for 21 d increased the germination capacity of tomato seeds under stress conditions imposed by high temperatures and osmotica. This treatment brought the uniformity of the poorer lot to levels comparable with, or better than, other lots which responded less well to treatment in this respect.

Osmotic treatment is a pre-sowing treatment where seeds are made to imbibe aerobically under conditions which permit them to pass through the preparatory stages of

germination, but preventing the completion of the process by interposing a physiological barrier to radicle emergence using salt solution or inert osmoticum (Heydecker and Coolbear, 1977; Tonkin, 1984). In other words, seeds are kept at a stage somewhere within the lag phase of the germination process and thus, upon transfer to germination in the field, they will germinate much faster than that of untreated control seeds, due to a shortened period of lag phase. There have been many variables which have to be considered for the success of osmotic treatment, e.g.:

- i. osmotic concentration of soak solution
- ii. soak duration
- iii. temperature
- iv. aeration
- v. light
- vi. drying procedure, e.g. surface drying, air slow-drying or rapid drying
- vii. storage
- viii. initial seed quality
- ix. crop species, cultivars and even seedlot
- x. hygienic conditions
- xi. the type of osmoticum used.

A number of salts as osmotic agents have been used in a wide variety of crops with mixed results. Most of them are potassium salts, e.g. KNO_3 , K_3PO_4 , KH_2PO_4 , while other osmotica with low molecular weights are $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, NaCl , mannitol and dextrose. In contrast, many authors prefer to use high molecular weight osmotica such as: Polyethylene glycol 6000 or 8000.

When the effectiveness of different osmotica are compared, PEG appeared to be more effective than salts in seeds of beet (Khan *et al.*, 1983), leek and onion (Brocklehurst and Dearman, 1984; Haigh and Barlow, 1987a), pepper (Yacklich and Orzolek, 1977) and carrot (Brocklehurst and Dearman, 1984; Globerson and Feder, 1987), while other experiments on tomato (Bussell and Gray, 1976; Haigh and Barlow, 1987), lettuce (Cantliffe, 1981; Guedes and Cantliffe, 1980), pepper (O'Sullivan and Bouw, 1984; Rivas *et al.*, 1984) demonstrated that salts were better than PEG. In carrot and onion some contradictory findings have been found. In carrot, Brocklehurst and Dearman (1984) and Globerson and Feder (1987) found better performance of seed treated with PEG over salt treatment, while other authors (Haigh and Barlow, 1987a; Furutani *et al.*, 1986) found more benefit from salt treatment than PEG. A similar situation of better performance of PEG treated seeds occurred in onion (Haigh and Barlow, 1987a; Haigh *et al.*, 1986).

Although PEG was claimed to be effective in treating many seed crops due to its nature, i.e. it is chemically inert, readily dissolved in water and has a large molecular size which prevents PEG from penetrating cell membranes; this compound is associated with high viscosity, poor oxygen diffusivity (Mexal *et al.*, 1975) and possible toxicity (Heydecker and Coolbear, 1977; Haigh and Barlow, 1987a). In view of this, some workers have devised a method of treating seeds with salts or PEG by which a pump will supply a combination of air and pure oxygen through the bottom of the columns in order to aerate the soaked seeds in solution within the columns (Darby and Slater, 1976; Brede and Brede, 1989). Much work has been done in National Vegetable Research Institute, UK in collaboration with Bristol University in designing a prototype of machine which gives good aeration in PEG solutions.

Meanwhile, Peterson (1976) introduced a technique where onion seeds can be primed in a slurry of polyethylene glycol mixed with vermiculate and stored for a period of time. This method, however, suffered from difficulties in separating seeds from vermiculate when

treatment finished. Progress has been made in the development of the technique for commercial use based on both column and stirred-bioreactor technology which has demonstrated capability of priming up to 10 kg of seed (Bujalski *et al.*, 1989; Gray *et al.*, 1990). Recently an alternative non-osmotic technique, drum priming, has been devised (Rowse, 1987; Rowse and Grey, 1987) which can maintain the water content of seeds within a dose limits achieved in PEG solution and which are necessary for effective priming. This drum priming has capacity of priming large quantities of seeds as in the bioreactor methods. Although this method gave better performance for seeds and seedlings of leek in terms of early emergence, uniformity of emergence, normal seedling and plant dry weight (Gray *et al.*, 1990), more trials need to be carried out for other seed crops.

The benefits of salts over PEG are usually claimed due to low viscosity, higher oxygen solubility and thus ease of aeration which might enhance their suitability for large scale operations (Darby and Slater, 1976; Akers and Holley, 1986). However, ion penetration of salt into cells may account for the deleterious effects of salt treatment (Brocklehurst and Dearman, 1980), and this ion penetration may result in either osmotic imbalance in cells or the disruption of enzymes and membranes (Greenway and Munns, 1980). Thus it is not surprising that Brocklehurst and Dearman (1984) found different moisture contents of onion seeds (PEG: 41% and KH_2PO_4 : 45%) although they were treated at the same water potential. As salt-treated seeds have higher moisture content, it makes them more susceptible to mechanical injury and dehydration stress (Alvarado and Bradford, 1988). If mechanical injury occurs, less protection and increased seed leachate can be expected and this subsequently induces fungal infection. In contrast to reports of harmful effects of ion accumulation, Haigh and Barlow (1987a) argued that ions absorbed by salt treated seeds have beneficial effects on germination. However, more investigations on this aspect need to be made in order to answer whether salt uptake allows any nutritional (or specific chemical) benefit during treatment (Durrant *et al.*, 1983).

2.2.3 Mechanisms of Action of Osmotic Treatment

2.2.3.1 Water relation in osmotic treatment

In seeds the water potential of cells (φ) is the algebraic total of solute potential (φ_{π}), matrix potential (φ_m) and turgor (φ_p) components which can be expressed as

$$\varphi = \varphi_{\pi} + \varphi_m + \varphi_p$$

and this φ is a negative number, unless all cells within seed are very turgid where φ approaches zero (Bewley and Black, 1985). Since water uptake during germination is triphasic, water uptake during imbibition (phase I) is usually rapid owing to differential of water potential between dry seed (dry seed φ_m can be as low as -100 MPa) and imbibant (pure water, $\varphi_o = 0$). As φ increases, there is a transition to a greater dependence of water uptake on φ_{π} and φ_p of hydrated cells with φ_m becoming negligible, thus during the lag phase $\varphi = \varphi_{\pi} + \varphi_p$ and probably in this phase φ is in the range of -1.0 to -1.5 MPa for many seeds (Bewley and Black, 1985). In osmotic treatment external water potential (φ_o) is set sufficiently low that radicle emergence or expansion cannot occur, or the duration of osmotic treatment at higher φ_o is shortened to be within the plateau period (phase II). Theoretically, therefore, there is no net water movement into the seed when it has equilibrated with the treatment medium since $\varphi = \varphi_o$.

Bradford (1986) suggested that solute generated within cells lowers φ_{π} and thus provides a driving force for water uptake in this relatively high φ_o range. The resistance of cell walls to expansion as water taken up results in turgor pressure which simultaneously increases φ and reduces driving force for water uptake. The benefit of osmotic treatment may be preserved during drying-back where such treated seeds have low φ_{π} and high φ_p upon rehydration. This idea seems to be supported by Akers *et al.* (1987) who suggested that osmotic adjustment which occurred during priming of parsley seed at low φ_o resulted in increases in germination response. Similarly, Carpita *et al.* (1979) and Takeba (1980a,

1980b) working with lettuce found that osmotic pre-incubation prior to radicle emergence caused an increase in amino-nitrogen compounds and K^+ ion levels in the embryos in a more negative φ_{π} . In contrast, other workers have disputed this hypothesis of osmotic adjustment. In cabbage seeds, Liou (1987) found two distinct changes associated with osmotic treatment. An increase in water uptake would cause a faster rise of seed φ , but this change was not due to the osmotic concentration of the cell since untreated seeds had a higher solute concentration than treated seeds. A progressive increase in seed φ_{π} might be due to extra water uptake and reduction of solutes; while a reduction might result from leakage or active hydrolysis of substrates where active hydrolysis of food reserves is a common characteristic in early germination. Consequently, a rise in the mean molecular weight of cell constituents is not likely to happen and thus the proportion of osmotic adjustment could not be accepted. However, supposition of active hydrolysis of food reserves during early germination (Liou, 1987) is not compatible with the general notion that this is actually a post germination event, while osmotic treatment works before germination is completed (Bewley and Black, 1985). In tomato, Haigh and Barlow (1987b) observed that during the lag phase, the seed φ was in equilibrium with φ_o , the seed φ_{π} and φ_p were about -0.4 and 0.5 MPa respectively and there was no change throughout the lag phase until radicle emergence. While seed φ was in equilibrium with φ_o , the embryo φ and φ_{π} remained constant below that of the whole seed of about -0.5 and -1.7 MPa each, and there was no lowering of embryo φ_{π} nor build up embryo φ_p during the lag phase. It concluded that the initial stages of seed activation can occur at φ lower than that allowing radicle protrusion without altering osmotic regulation within the seed (Haigh and Barlow, 1987b) and this is in agreement with Simon and Wiebe (1975) theory where physiological activity starts at about -8.0 MPa.

It was also concluded that the limiting step of germination lies in the mechanism which either increases cell wall plasticity or leads to a weakening of enclosing tissues that restrict embryo water uptake and embryo expansion. This was in support of Karssen *et al.* (1989)

who found that in tomato, endo- β -mannanase activity from germinating primed seeds was higher than that of controls where this enzyme is believed to be responsible for weakening endosperm cell walls by hydrolysing galactomannan components. Similarly, Haigh (1988) observed rapid expansion of embryos from primed tomato seeds associated with changes in radicle cell wall extensibility during osmotic treatment while mechanical resistance of the enclosing tissues decreased to the same extent.

2.2.3.2 Physiological and biochemical changes in osmotic treatment

Many effects have been directed to a better understanding of physiological and biochemical changes associated with the advantages gained from the treatment. These aspects have been reviewed recently by Seetagos (1989) and Ranganarasimhiah (1989) and include changes in the synthesis of ATP, DNA, RNA, proteins and enzymes.

2.2.3.2.1 ATP changes

Adenosine triphosphate (ATP) is biological energy needed for biosynthesis pathways as well as biological work, e.g. transport, assembly of organelles and operation of repair systems. In dry seeds the ATP level is very low (Osborne, 1983) but production of ATP begins very early after the start of imbibition. Sufficient ATP is produced within a short time to allow metabolic processes to commence (Bewley and Black, 1985). Working with spinach, kohlrabi, pepper and eggplant, Mazor *et al.* (1984b) found an increase in ATP content during 24 hr of PEG treatment, regardless of the temperature. However, drying back after treatment appreciably reduced the ATP content of PEG treated seeds although at the early stage of germination there was a higher ATP content in PEG treated seeds than in untreated controls. Since the initiation of ATP synthesis only requires a single ATP molecule, each molecule will yield two molecules of ATP by the action of adenyl kinase and phosphoenolpyruvate-kinase, thus resulting in a logarithmic increase in its synthesis (Perl, 1984). Therefore a small amount of ATP may speed up the conversion of pre-existing AMP

to ATP which in turn increase ATP-dependent activities, including synthesis of protein, membrane and nucleic acids. Those activities accompanied by the accumulated components resulting from synthesis seem to have a contribution for seed invigoration from PEG or salt treatments (Mazor *et al.*, 1984b).

2.2.3.2.2 Changes in DNA

DNA synthesis is usually associated with cell division, thus in tomato (Coolbear and Grierson, 1979) and lettuce (Khan *et al.*, 1980), no DNA synthesis was found during osmotic treatment, but following transfer to water DNA synthesis occurred much earlier than in untreated controls. However, in leek (Bray *et al.*, 1989) DNA synthesis was detectable from embryos during treatment in the absence of cell division, followed by a five-fold increase in the rate of DNA synthesis (from the levels detected during treatment), at just 18 h of germination. Similarly, Fu *et al.* (1988) found ^{32}P incorporation into DNA during 0-3 h of imbibition and significant incorporating following this imbibition period (0-3 d). This result suggested that early incorporation of ^{32}P into DNA might represent DNA repair rather than DNA replication, accordingly the completion of any DNA repair occurring during priming could be a contributing factor for rapid emergence as suggested by Burgass and Powell (1984).

2.2.3.2.3 RNA changes

In tomato (Koehler, 1967; Coolbear and Grierson, 1979) and lettuce (Khan *et al.*, 1980), there was an increase in the accumulation of RNA during treatment, in particular rRNA. In low vigour leek seeds, osmotic treatment permitted replacement of damaged rRNA while 6 d germinating low vigour untreated controls still exhibited evidence of damaged rRNA (Davidson *et al.*, 1991). The use of cordycepin, an inhibitor of RNA synthesis incorporated during treatment or subsequent germination, failed to inhibit radicle protrusion (Khan *et al.*, 1980). Therefore the newly synthesised RNA probably is not

involved in the germination advancement of treated seeds, thus in *C. bonus-henricus*, Khan *et al.* (1980) suggested that promotion of germination by osmotic treatment may be operated via stored mRNA which may be functionally different from the newly synthesised poly A⁺-RNA. Although germination was not under rigid control of new synthesised rRNA (Khan *et al.*, 1980), RNA synthesis seems to be required for repair and replacement of fragmented RNA, and this is an important event which is completed during treatment.

2.2.3.2.4 Changes in protein and enzymes

In lettuce seeds (Khan *et al.*, 1978), the rate of synthesis and quality of synthesised proteins were found to be significantly higher in osmotically treated seeds than in untreated controls. This newly synthesised protein was not due to alternation of amino acid pool size but perhaps due to removal or production of inhibitory and promotive factors necessary for translation of existing or new mRNA. These promotive factors may include early polyribosome formation (Khan *et al.*, 1978), availability of ATP (Mazor *et al.*, 1984), and nucleic acids (Coolbear and Grierson, 1979; Coolbear *et al.*, 1980).

There have been many reports claiming an increase in the activity and/or synthesis of different enzymes during osmotic treatment including: α -amylase in oat and wheat (Berrie and Drennan, 1971; Hanson, 1973), acid phosphatase and esterase in lettuce (Khan *et al.*, 1978), malate dehydrogenase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, isocitrate lyase and aldolase, all in capsicum seeds (Smith and Greg Cobb, 1989). While in lettuce, carrot and okra (Kundu, 1980), mustard (Dey and Mukherje, 1988), radish and eggplant (Rudrapal and Nakamura, 1988a, 1988b) and in onions (Choudhuri and Basu, 1988), increased total dehydrogenase activity was reported following hydration-dehydration treatments. In similar course, an increase in the activity of endo- β -mananase was observed during osmotic treatment of tomato seeds (Karssen *et al.*, 1989). Therefore it is possible that osmotic treatment induces some key metabolic activities including hydrolysis

of protein and lipid reserves and turnover of phosphate esters (Khan *et al.*, 1980) as well as the hydrolysis of galactomanan from endosperm cells (Karszen *et al.*, 1989).

2.2.3.2.5 Hormonal changes

As inhibitors may be related to germination, it is appropriate to examine the levels of inhibitors such as ABA within seeds. In lettuce, osmotic treatment can reduce or eliminate abscisic acid to undetectable levels compared to relatively high levels of free and trace amounts of bound ABA in untreated controls (Khan *et al.*, 1978). Even 1 d osmotic treatment led to a complete disappearance of ABA (Borkowska *et al.*, 1978). However, when seeds were imbibed at 35°C followed by germinating them at 25°C, the time taken for germination was independent of the ABA content of the seed. Thus it would be probable that changes in ABA (during osmotic treatment or imbibition at 35°C) may be a function of hydration itself and not related to germination *per se*.

2.2.3.2.6 Respiration changes

In tomato Koehler (1967) found a significant stimulation of respiration due to seed priming. Similar findings were also presented by Malnassy (1971) for pepper seeds where osmotically treated seeds showed a larger initial rate of respiration during imbibitional phase and after the completion of germination. It was suggested that such differences between osmotically treated and untreated controls could result in a greater availability of respiratory energy in treated seeds.

2.2.4 Osmotic Treatment of Forest Tree Seeds

Data on the promotive effect of osmotic treatments for different tree seeds probably can be summarised in Table 2.3. In general, water potentials required for osmotic treatment ranged from -0.58 MPa (Paci, 1987) to -1.5 MPa (Fleming and Lister, 1984). Longer

Table 2.3 The effectiveness of osmotic treatment in different tree seeds.

Species	Osmotic treatment	Drying back	Results	Reference
<i>Larix decidua</i>	*-0.58 MPa, PEG, at 15 °C	DB	Improved speed of germination	Paci, 1987
<i>Larix gmelinii</i>	-0.62 MPa, PEG, 10 °C for 2 d	DB	Improved percentage, speed and uniformity of germination	Zou and Huang, 1987
<i>Larix gmelinii</i>	-0.62 MPa, PEG, 10 °C for 2 d	DB	Improved percentage, speed and uniformity of germination	Huang and Zou, 1987
<i>Picea glauca</i>	-0.8 MPa, PEG, 15 °C for 5 d	DB	Improved uniformity of germination, restored vigour of aged seed, prevented loss of vigour during ageing at 45 °C 100% RH germination.	Huang, 1989
<i>Picea mariana</i>	-1.25 MPa, PEG, 20 °C for 14 d	DB	Improved speed and uniformity of germination.	Fleming and Lister, 1984
<i>Pseudotsuga menziesii</i>	*-0.58 MPa, PEG, at 15 °C	DB	Improved percentage and speed of germination.	Paci, 1987
<i>Pinus contorta</i>	-0.8 MPa, PEG, 15 °C for 5 d	DB	Improved uniformity, restored vigour of aged seed, prevented loss of vigour during ageing at 45 °C, 100% RH.	Huang, 1989
<i>Pinus elliottii</i>	-1.5 MPa, PEG, 25 °C for 5 d	No drying	Improved percentage, speed and uniformity of germination.	Haridi, 1985
<i>Pinus nigra</i>	*-0.58 MPa, PEG, 15 °C	DB	Improved percentage and speed of germination.	Paci, 1987
<i>Pinus sylvestris</i>	-0.58 MPa, PEG, 15 °C for 11 d	DB	Improved percentage, speed and uniformity of germination.	Simak, 1976a

continued over

Table 2.3 cont'd

Species	Osmotic treatment	Drying back	Results	Reference
<i>Pinus sylvestris</i>	-0.62 MPa, PEG, 10°C for 2 d	DB	Improved percentage, speed and uniformity of germination.	Zou and Huang, 1987
<i>Pinus sylvestris</i>	-0.62 MPa, PEG, 10°C for 2 d	DB	Improved percentage, speed and uniformity of germination.	Haung and Zou, 1989

* Reference in Italian, summary did not mention a complete osmotic regime.

DB = Drying back after treatment

treatment duration (14 d) was necessary if treatment was at low water potential (-1.25 MPa) otherwise a high water potential (-0.62 MPa) needs a short treatment duration (2 d). This seems in agreement with Bewley and Black's (1985) concept where at any given temperature, longer treatment is required for optimum benefit of treatment at low water potential. None of the authors used salts or other solutions except PEG as an osmoticum.

With a view to improving the performance of tree seeds, Simak (1976) seems to have been the first person to use osmotic treatment particularly for Scots pine. His results showed that osmotically treated Scots pine seeds germinated faster, uniformly and had a higher percentage germination than untreated controls. Although water treated seeds also germinated faster than that of untreated controls, at 37 d after sowing their germination remained 30% lower than the best PEG-treated seeds. Higher percentage germination of treated seeds was dependent upon the PEG concentration being around -5.8 bars as optimum water potential. At 21 d after sowing, only about 40% of control seeds germinated and germination continued very irregularly and slowly up to 200 d when 80% of seeds had germinated, whereas the same germination percentage was reached after only 10 d following

PEG treatment (Simak, 1976a). Without doubt this is a very considerable improvement. Continuing Simak's (1976a) work in 14-year old Scots pine seeds, he demonstrated that PEG treatment resulted in higher percentage germination compared with untreated controls. Apparently this treatment helped to stimulate the germination of an increased number of weak abnormal seedlings which occurred frequently in aged seed samples. Therefore any increase in such morphologically, physiologically or genetically abnormal seedling by any treatment seems to be useless since abnormal seedlings would hardly survive in the field, while if they did survive they would not produce good plantation.

In *Picea mariana* osmotic treatment improved speed, vigour and sometimes the uniformity of germination (Fleming and Lister, 1984). At best treatment at either -1.25 MPa for 14 d at 20°C or -1.25 MPa for 21 d at 15°C reduced T_{50} by 14 d at 10°C, by 3 d at 21°C and by 5 d at 32°C (Fleming and Lister, 1984). This work indicated that treatment benefits were considerable when treated seeds subjected to low temperature as might be likely to be the case in Canada, particularly in upland sites where cool spring temperatures are followed by periods of drought, and on lowland sites where cold temperatures limit seed germination and seedling growth (Kenety, 1971 cited by Fleming and Lister, 1984). In *Pinus elliottii*, increased percentage germination and vigour measured as peak value, which is a resultant of maximum value of cumulative germination percentage divided by days of test (Czabator, 1962), were only achieved if seeds treated at -1.5 MPa and 25°C while treatment at 35°C resulted in lower percentage germination and lower vigour than from untreated controls (Haridi, 1985). This indicated that temperature during treatment determined treatment benefits. Since seeds used in this experiment were low in quality, having an average percentage germination < 40%, seeds would probably have low tolerance to suboptimal conditions (Larsen, 1965), thus 35°C was probably too high for this seed lot, although for *P. elliottii* optimum germination temperatures were just around 25-30°C (ISTA, 1985). However, one limitation of Haridi's (1985) experiment was no treatment was carried out at

> -1.5 MPa, since in other experiments such as in *Pinus nigra*, *Larix Larix* and *Pseudotsuga menzeisii* (Paci, 1987), *Pinus sylvestris* (Simak, 1976; Zou and Huang, 1987) and *Larix gmelinii* (Huang and Zou, 1989), the optimum benefits of osmotic treatment were at higher water potentials (Table 2.3).

Apart from direct sowing after osmotic treatment, treated seeds of *Picea mariana* which were dried back to 6% SMC can be stored for 56 d at 5°C without any reduction in germinability or vigour (Fleming and Lister, 1984). In *Pinus contorta* and *Picea glauca*, osmotic treatment has been claimed to impart resistance to either high temperature and humidity (45°C, 100% RH) or low temperature and humidity (-5°C and 20% RH) (Huang, 1989). In this case, artificially deteriorated seeds could recover to nearly the same germination level as that of the untreated controls (unaged seeds) after the aged seeds were treated with PEG. In addition, all osmotic treatment improved the vigour level of deteriorated seed of both species (Huang, 1989). Accordingly, he concluded that osmotic treatment could be an effective measure for improving seed performance under stressful environmental conditions in the field.

2.3 Conclusion

It is obvious from this review that successful crop establishment relies heavily on the performance of the seed planted. Good planting practice requires rapid, uniform and complete germination which are typical of high vigour seeds, while slow and erratic germination is undesirable.

Pre-sowing seed treatments are one means by which seed deficiencies in vigour may be remedied and seeds are rendered more tolerant of adverse effects of the environment, and

more competitive. Accordingly, pre-sowing seed treatments will then have commercial value, particularly for seeds where germination is slow and erratic and when uniformity of establishment is important. This is the case for *Pinus radiata* where small seeds usually germinate slowly, produce small seedlings and need more time in the nursery bed before being transplanted to the field (Section 2.1.3.1). Thus improved germination rates presumably would reduced nursery operation costs.

Apart from improvement for sowing purposes, storage is another consideration where pre-sowing treatment might be of use to protect or improve the vigour of aged seeds. Generally it seems that seeds should be sown as soon as possible after treatment, however, when storage is necessary, treated seeds should be stored at low temperatures and low moisture contents.

For physiological studies, pre-sowing treatment can provide a potential investigative tool to evaluate the complicated mechanisms governing seed performance, for example, in association with aged seeds (Coolbear *et al.*, 1984). As the ultimate success of any treatment can only be justified when the crop is harvested, the study of pre-sowing treatments needs a multi-disciplinary approach by integrating different aspects, not only the technological, physiological, agronomical and pathological, but also by taking a commercial viewpoint where the cost-benefit ratios of any proposed treatment must be assessed on an economic scale.

III. MATERIALS AND METHODS

This study used *Pinus radiata* seeds donated by ProSeed N.Z. Limited from the Forest Research Institute Rotorua, stock No. 88/103 collected in 1988 from 8 year old clones of mixed genotypes grown at the Amberley 850 seed orchard, Canterbury.

Seeds were graded by FRI into four different grades and the second largest grade was then split again so that there were five different grades, as shown in Table 3.1.

Table 3.1 The diameter and weight of seed from different grades.

Seed grade	Diameter (mm)	1000 Seed Weight (g)
I	> 4 mm	49.91 ± 0.47
II	3.7 mm - 4 mm	41.02 ± 0.55
III	3.3 mm - 3.7 mm	33.33 ± 0.39
IV	2.8 mm - 3.3 mm	25.87 ± 0.24
V	< 2.8 mm	18.53 ± 0.15

Seed moisture contents were adjusted to 7.3%.

3.1 Osmotic Pre-sowing Treatments

The osmotic treatment method was adopted from that carried out by Coolbear and Grierson (1979) using lidded plastic boxes which were fitted with horizontal glass platforms supporting filter paper kept moistened via a paper wick from a reservoir of osmoticum below. The osmotica used was either mixture, weight for weight, of KNO_3 + KH_2PO_4 solution or Polyethylene glycol (PEG 6000). The water potentials of the salt solutions were calculated from freezing point depression data using the formula as follows:

$$\varphi = \frac{\frac{A + B}{1.86} \cdot R \cdot T}{C_{WA} + C_{WB} - 1}$$

where:

φ = water potential of mixed solution A + solution B, in atmospheres, atms.

A = freezing point depression of solution A at any given concentration, in °C.

B = freezing point depression of solution B at any given concentration, in °C.

R = a constant, 0.08206 litre atm. mol⁻¹. deg⁻¹

T = absolute temperature, °K

C_{WA} = water concentration in 1 litre of solution A

C_{WB} = water concentration in 1 litre of solution B

1 atm = 101,325 Pascal

A sample calculation is presented in Appendix I.

Meanwhile, the water potential of the PEG 6000 solution was calculated according to Michel and Kaufman (1973) where a worked example is shown in Appendix II.

Before osmotic treatment was carried out, boxes with osmoticum and filter paper fitted were equilibrated overnight at 20°C. Seeds were then sown on top of these filter papers and subsequently the boxes were tightly closed and wrapped with two layers of aluminium foil and placed in the dark at 20°C for different periods of time. A temperature of 20°C was chosen on the assumption that osmotic treatment conditions should be essentially the same conditions that are optimal for germination, but the final event of germination (radicle protrusion) is prevented during the treatment by a low solution water potential (Heydecker *et al.*, 1975; Akers and Holley, 1986). Twenty degrees centigrade is the temperature recommended by ISTA (1985) for germinating *Pinus radiata* seeds. Thus if

germination processes are more favourable at warm temperatures, the use of shorter periods of treatment at warm temperatures would be more desirable than using longer periods of treatment at lower temperatures (Heydecker and Gibbins, 1978).

3.2 Drying Seed After Treatment

3.2.1 Surface drying

Here seeds were dried on top of filter paper in a laminar flow cabinet at ambient temperatures for a short period of time until they flowed freely (2-4 h).

3.2.2 Drying back to low moisture content

Seeds were left on top of filter paper in the laminar flow cabinet under ambient conditions (22-27°C, 50-60% RH) for 48-96 h. In Experiment III, drying for 48 h in ambient conditions was then followed by further drying for 12 h over silica gel within desiccators (0% RH).

3.2.3 Slow drying

This drying was carried out on top of filter papers at a constant 20°C and high relative humidity, 80-85%, until seed moisture content was in equilibrium with its relative humidity. This humidity was a result of keeping close the constant temperature room. In some studies (Experiment 4), seeds were then subjected to other methods of drying as described above. Seed moisture contents were determined at every stage of drying.

3.3 General Testing Procedures

3.3.1 Germination and seedling growth measurements

Seed germination was conducted in plastic boxes with translucent lids. Twenty-five seeds were placed on top of 9 x 20 cm blotters over kimpak towelling, moistened with 50 ml of distilled water. Three replicates were used. Germination was carried out at 20°C under continuous low light and daily observations were made to count radicle emergence (when radicle length is about 1 mm) and normal seedlings. In this case seedlings with all their essential structures including cotyledons well developed, complete, in proportion and healthy, were recognised as normal seedlings. Such seedlings like that would have potential for continued development into satisfactory plants when grown in good quality soil and under favourable conditions of moisture content (ISTA, 1985).

The seedling growth test in the glasshouse (Experiment 5) was also done using Plix bedding root trainers filled with a mixture (1:1) of sand and a commercial potting mix containing spagnum peat moss and pumice (Section 3.4.5). In order to keep the substrate moist, watering was supplied twice a day. No pesticides or additional fertiliser were given, while the temperature of the glasshouse was maintained at 25°C. This glasshouse experiment was terminated at 45 d after sowing and following the end of observations, all normal seedlings were carefully removed from the media by washing them with water. Subsequently, seedlings were dried at 60°C for 4 d to determine seedling dry weights.

3.3.2 Moisture content determination

The low constant temperature oven method was used in determining the moisture content in the experiments where seeds were dried back in the oven at $103 \pm 2^\circ\text{C}$ for $17\text{ h} \pm 1\text{ h}$ (ISTA, 1985). The moisture content as a percentage by weight was calculated according to the following formula:

$$(M_2 - M_3) \times \frac{100}{(M_2 - M_1)}$$

where:

M_1 = the weight in grams of the container and its cover

M_2 = the weight in grams of the container, its cover and its contents before drying

M_3 = the weight in grams of the container, its cover and its contents after drying

3.3.3 Data collection and statistical analysis

The data gathered were as follows:

(a) Final percentage radicle emergence and normal seedling production.

(b) Daily count of radicle emergence and normal seedling production to count:

- Median times (T_{50}) of radicle emergence, normal seedling and cotyledon emergence as follows:

$$T_{50} = t_i + \frac{(N + 1)/2 - n_i}{n_j - n_i} \times (t_j - t_i)$$

where N = final number of seeds showing radicle emergence, producing normal seedlings or showing cotyledon emergence

n_i, n_j = total number of either emergents, normal seedlings or cotyledons produced by adjacent counts at t_i and t_j where:

$$n_i < \frac{N + 1}{2} < n_j$$

- The uniformity or mean spread of radicle emergence times, normal seedling production or cotyledon emergence (T_{90} - T_{10}) was calculated as follows:

$$T_{10} = t_i + \frac{(N + 1)/10 - n_i}{n_j - n_i} \times (t_j - t_i)$$

where $n_i < \frac{N + 1}{10} < n_j$ and t_i, t_j are adjacent counting times.

$$T_{90} = t_i + \frac{9(N + 1)/10 - n_i}{n_j - n_i} \times (t_j - t_i)$$

where $n_i < \frac{9(N + 1)}{10} < n_j$ and t_i, t_j are adjacent counting times.

- (c) Mean dry weight of seedlings which were values gained from normal seedlings dried at 60°C for 4 d.

Unless otherwise stated, the experiments were usually carried out in a randomised complete block design (RCBD) and percentage data of germination parameters were statistically analysed without transformation.

3.4 Organisation of Experiments

Although there is much evidence that osmotic pre-sowing treatment improved the germination performance of many different crop seeds (cf. Heydecker and Coolbear, 1977;

Bradford, 1986), no work has been done previously in *Pinus radiata*. Thus in this study each experiment conducted will be described individually, its objective explained and the general procedures used in each experiment outlined.

3.4.1 **Experiment 1.** Preliminary Study on the Effects of Salt Treatment and Subsequent Drying on Grade IV *Pinus radiata* Seeds

This preliminary experiment was intended to determine the effect of three different water potentials of mixed $\text{KNO}_3 + \text{KH}_2\text{PO}_4$ (1:1 w/w) solutions, i.e. -0.5, -1.0 and -1.5 MPa with treatment periods of 7 and 14 d. Following treatment, seeds were surface dried for 4 h or dried back for 48 or 96 h at ambient temperatures. Seeds used in this preliminary experiment were of grade IV.

3.4.2 **Experiment 2.** The Effects of Salt Treatment Duration and Fungicide Applications on the Germination Performance of Grade IV *Pinus radiata* Seeds

It has long been realised that the effectiveness of osmotic treatment can be reduced by fungi (Heydecker and Coolbear, 1977; Coolbear, 1989). This second experiment was therefore designed to investigate the effect of fungicide applications during osmotic treatment. Also the effect of drying following osmotic treatment was also evaluated. Based on the findings in Experiment 1, osmotic treatment was carried out at -1.0 MPa of mixed $\text{KNO}_3 + \text{KH}_2\text{PO}_4$ at 20°C for 4, 7 and 10 d. At 20°C, this water potential was achieved by mixing up equal amounts of $\text{KNO}_3 + \text{KH}_2\text{PO}_4$; for 1 l solution, it would require 500 ml of 1.32% $\text{KNO}_3 + 500$ ml of 1.32% KH_2PO_4 . In some treatments, the fungicide Thiram was applied at a rate of 1% seed weight before and/or after osmotic treatment. When applied before osmotic treatment, the fungicide was dusted on the seeds by mixing them up with seeds in a flask. If the fungicide is applied following osmotic treatment, the fungicide was

initially suspended in distilled water (around 0.6 g Thiram in 50 ml water) and this solution was then used to moisten the germination substrate (see Section 3.3.1). Following osmotic treatment, seeds were washed four times with distilled water to remove any residual osmoticum and thereafter the seeds were either surface dried for 2 h or dried back for 48 h at ambient temperatures. The seeds used in this experiment were of grade IV.

3.4.3 Experiment 3. Comparison of Polyethylene glycol and Salt Treatments and Interactions with Drying Back

Occasionally it has been reported in the literature that salts were less effective than other osmotica such as polyethylene glycol (PEG 6000) because the latter is inert and non-toxic (Heydecker and Coolbear, 1977; Brocklehurst and Dearman, 1984). Therefore an experiment was conducted to compare the effectiveness of PEG 6000 and mixed $\text{KNO}_3 + \text{KH}_2\text{PO}_4$ for treating *Pinus radiata* seeds of grade IV. Interactions with drying back were also studied.

Seeds were dusted with Thiram at a rate of 1% seed weight before osmotic treatment which was carried out using a -1.0 MPa solution of either mixed $\text{KNO}_3 + \text{KH}_2\text{PO}_4$ or PEG solution (284 g/kg H_2O) at 20°C for 10 days in the dark. Following treatment seeds were washed four times as described in the previous experiment and immediately thereafter the seeds were dried back on top of filter paper under ambient conditions for 48 h. Half of the seeds were then dried over silica gel for another 12 h. During germination, no Thiram was applied.

3.4.4 Experiment 4. The Effect of Slow Drying After Salt Treatment

In order to keep the seeds easily, and for sowing by conventional methods, treated

seeds need to be dried back to low moisture contents of around 7%. The work of Toledo (1988) and Seetagos (1989) showed that if drying of celery seeds is allowed to proceed slowly, such as under regulated relative humidity conditions, treated seeds retain low median germination times and avoid some of the deleterious effects of drying back. Since it was found in earlier experiments that treatment benefits were lost when treated seeds were dried continuously for 4 d at ambient temperatures and 50-60% RH (Experiment 1) or when they were dried for 12 h over silica gel after 2 d drying at ambient temperatures (Experiment 3), this fourth experiment was then intended to investigate the effect of slow drying under relatively high humidity (80-85% RH) at 20°C followed by further drying under ambient conditions or over silica gel. Two separate sub experiments (A and B) were established in Experiment 4. Experiment A was started early where after slow drying at 80-85% RH for 10 d seeds were further dried over silica gel for different periods of time while in Experiment B (osmotic treatment was carried out 1 month after Experiment A) initial drying was similar to Experiment A but further drying was done at ambient temperature for 12-36 h and also followed by 7 h drying over silica gel (Table 4.4.1 for more information). Osmotic treatment followed procedures described in Experiment 3 using a mixed solution of $\text{KNO}_3 + \text{KH}_2\text{PO}_4$ while seeds used were of grade V.

3.4.5 **Experiment 5.** Germination and Seedling Emergence from Different Grades of Salt Treated *Pinus radiata* Seeds

In this experiment the effects of optimal osmotic treatment conditions on germination and seedling emergence were determined on all five grades of *Pinus radiata* seeds. Osmotic treatment was carried out as described in Experiment 3 using mixed $\text{KNO}_3 + \text{KH}_2\text{PO}_4$ solutions for 10 d at 20°C while drying after treatment to 7-8% SMC was carried out slowly for 9 d at 20°C, RH 80-85%, followed by drying at ambient temperature for 12 h. Germination trials were done in the laboratory while seedling emergence and seedling

growth trials were established in the glasshouse by sowing seeds at a depth of 5 mm in Plix bedding root trainers previously filled with a 1:1 mixture of fine sand and commercial potting mix containing sphagnum, peatmoss and pumice. This potting mix is considered to be a suitable substrate for seedlings since it has very high organic materials as well as having high porosity. Seedlings were categorised as emerged when hypocotyles were 1 cm above the ground and the observations were completed 45 d after sowing. The normal seedlings were then dried at 60°C for 4 d after being carefully removed from the nursery bed (Section 3.3.1).

3.4.6 Experiment 6. Evaluation of Osmotic Treatment for Improving the Germination Performance of Artificially Aged Seeds

Up until now there has been mixed success in using pre-sowing treatments for protecting and/or improving the germination performance of aged seeds (cf. literature review, Section 2.2.2). The purpose of this study was then to evaluate whether osmotic treatment applied before or after the ageing could maintain vigour in store or improve performance after ageing.

For this reason, ageing was carried out using a controlled deterioration technique (CD) at 45°C and a nominal seed moisture content of 20% for period of 0 to 8 d. Grade V of *Pinus radiata* seeds were used in this study. The procedures of CD were adopted from Matthews and Powell (1987) as follows:

1. Moisture content of seeds was determined according to ISTA (1985) with three replicates of 25 seeds.
2. Seeds were put in waterproof pouches made of polyester-aluminium foil-polyethylene laminate (12 µm/20µm/50µm).

3. The calculated amount of water added to seeds in the pouches to get 20% of seed moisture content followed the formula:

$$Y = \frac{SMC_1 - SMC_2}{100 - SMC_1} \times Fwt_0$$

where: Y = the amount of water to be added

SMC₁ = initial moisture content of seeds (%)

SMC₂ = required moisture content of seeds (%)

Fwt₀ = initial fresh seed weight (g)

4. The pouches were then tightly sealed and placed at 5°C overnight for equilibration and subsequently transferred to 45°C for 1, 2, 3, 4, 6 and 8 d and the moisture contents were checked again after the ageing period.

Osmotic treatment was carried out as previously described with mixed salts at -1.0 MPa and the treatment was either established before or after ageing. Following osmotic treatment or ageing, seeds were dried back to their original moisture contents of 7-8% as described in Section 3.4.4.

3.4.7 Experiment 7. Respiration Studies in the Early Germination of Osmotically Treated Seeds

Seed germination and seedling growth are energy-requiring processes and they are therefore dependent on respiration. A decrease in the rate of respiration of germinating seeds has been shown to precede a decrease in the rate of seedling growth (Woodstock, 1968) and thus significant positive correlations have been observed between oxygen uptake during early germination and seedling growth (Woodstock and Grabe, 1967). The objectives of this

study were therefore designed to investigate any differences in the respiration rate between osmotically treated seeds and untreated seeds and, secondly, to evaluate whether respiratory changes in intact seeds differ from decoated seeds, since the presence of seed coat in some way prevents gaseous exchange, thus influencing respiration (Mayer and Poljakoff-Mayber, 1982; Rimbawanto, 1987).

This experiment used seeds of grade V and respiration studies were done manometrically using the Gilson Differential Respirometer with 12 Warburg flasks. Osmotic treatment and drying after treatment were done as in Section 3.4.4 where subsequent drying was at ambient temperatures for 12 h. Before imbibition, both intact and decoated seeds were firstly surface sterilised using 30% Janola (NaOCl) for 10 min to minimise any fungi which may develop during time course of imbibition and could interfere with respiration measurements. After surface sterilisation, the seeds were washed with distilled water and transferred to 0.01 N of HCl for 10 min to remove any residual chlorine since this substance affects metabolism (Abdulbaki, 1974). Finally, seeds were washed again with distilled water and imbibed on top of Whatman filter paper moistened with distilled water. At various times during 5 d of germination the seeds were taken for respiration measurements. The seeds were then put into Warburg flasks (15 seeds each flask) either with or without 0.2 ml of 20% KOH in the centre well and the seeds were either intact or decoated. From 12 available flasks, only 8 were randomly selected and these were arranged in pairs, 1 v 9, 5 vs 12, 2 vs 11 and 3 vs 6, where the first two flasks were decided to be used for treated intact and treated decoated seeds respectively, while the rest were for untreated control intact and untreated control decoated seeds respectively. In each pair, a flask containing KOH would measure oxygen (O_2) uptake while those without KOH measured net gas evolved ($CO_2 - O_2$); thus CO_2 evolved could be calculated. The respiratory activity was measured 6 times from different seeds (6 replicates) where measurements for replicates 1, 2 and 3 were 8 d earlier than measurements in replicates 4, 5 and 6. Respiration measurements were at 20°C after

20-25 min equilibration in the Gilson Differential Respirometer where the flask containing seeds were shaken at 130 oscillations per min. Respiratory quotients were calculated on the basis of ratio between CO₂ evolved and O₂ uptake.

3.4.8 Experiment 8. Measurements of Dehydrogenase Activity in Osmotically Treated Seeds

Measurements of biochemical changes within seeds can be used for a better understanding of the advantages from any treatment and up until now there are many pieces of evidence that osmotic pre-sowing treatments are associated with many metabolic changes in seeds (see Section 2.3.6). The importance of dehydrogenase enzyme assays for testing viability or seed vigour generally is based on the assumption that dehydrogenase enzymes are known to be involved in a number of metabolic events. Many of them are known as mitochondrial dehydrogenase enzymes which could be linked to respiration, an energy producing system which is required by any living organism. Accordingly, higher activity of dehydrogenase is perhaps associated with energy production. This experiment was then decided to investigate any differences in the activity of total dehydrogenase between osmotically treated and untreated controls. This was determined by the capacity of dehydrogenase enzyme to reduce Tetrazolium solution entering embryo tissues to a red water insoluble formazan where the extracted formazan can be measured spectrophotometrically (Harman and Gorecki, 1987; Johnston *et al.*, 1986; Kittock and Law, 1968; Sung and Chen, 1988). For this purpose, the method of Johnston *et al.* (1986) was adopted where osmotically treated and untreated control seeds of grade III were firstly imbibed for 24 h in distilled water. Subsequently they were excised where testa and megagametophyte tissues were removed carefully. An equal number of embryos (10) were then placed in vials containing 2 ml of 0.02% of 2,3,5 triphenyl tetrazolium chloride (TTC) for 3 to 35 h in the dark at 35°C. Excess TTC was then decanted off and the embryos were rinsed with distilled

water to remove any tetrazolium on the surface of embryos and subsequently they were surface dried for 2 h on top of filter papers at ambient temperatures. In order to extract the formazan produced, 5 ml of methylcellosolve (2-methoxyethanol) were added to 10 embryos in glass tubes for 12 h and the optical density reading of formazan extracted were taken using Phillips spectrophotometer (Pye Univam Pu 8600 UV/vis) at 480 nm. A second assay was also done using the same embryos which had previously been extracted in an attempt to evaluate whether higher activity of dehydrogenase in first assay, if any, is simply due to easy penetration of tetrazolium reagent.

IV. OPTIMISING THE PRESOWING TREATMENT

The early experiments in this study were conducted to characterise the optimum conditions for pre-sowing treatment. These included the determination of optimum water potential and duration of treatment, while a comparison of polyethylene glycol and salts as osmotic media was also made. Methods of fungicide applications and drying-back following treatment were also investigated.

4.1 Experiment 1. Preliminary Study on the Effects of Osmotic Treatment and Drying Back Treated *Pinus radiata* Seeds

This experiment surveyed the effect of different water potentials of mixed $\text{KNO}_3 + \text{KH}_2\text{PO}_4$ solutions as a treatment medium. Suitable durations of treatment and effects of drying back for different times following treatment were also studied. Seeds used were of grade IV and all treatments were conducted at 20°C .

4.1.1 Pre-germination and Moisture Content of Seeds During Osmotic Treatment

The relatively high water potential of -0.5 MPa was not sufficient to inhibit the radicle emergence of grade IV *Pinus radiata* seeds during treatment while -1.5 MPa effectively inhibited any visible germination (Table 4.1.1). The seed moisture content (SMC) after osmotic treatment was correlated with the number of pre-germinants, since seeds osmotically treated at high water potential for 2 weeks had an SMC of 42% after surface drying whereas those treated at -1.5 MPa had an SMC of around 23% (Table 4.1.2).

Table 4.1.1 The percentage pre-germinants of grade IV *Pinus radiata* seeds during treatment in mixed salt solution at different water potentials and treatment durations.

Duration of treatment (week)	Water potential of salt solution (MPa)		
	-0.5	-1.0	-1.5
1	13.1 (± 1.80)	0.88 (± 0.48)	0
2	39.8 (± 2.09)	6.44 (± 1.09)	0

Data presented are means of 9 replicates, numbers in brackets are s.e.s of individual means.

Table 4.1.2 The moisture contents (% fresh weight basis) of grade IV *Pinus radiata* seeds after salt treatment at different water potentials and treatment durations followed by drying back at ambient temperatures.

	Treatment conditions					
	-0.5 MPa		-1.0 MPa		-1.5 MPa	
	1 wk	2 wk	1 wk	2 wk	1 wk	2 wk
surface drying	30.1 (± 0.43)	42.2 (± 0.75)	23.8 (± 0.21)	29.3 (± 0.35)	20.5 (± 0.35)	22.6 (± 0.28)
48 h	14.1 (± 0.30)	20.3 (± 0.38)	11.7 (± 0.23)	13.1 (± 0.21)	9.7 (± 0.26)	11.1 (± 0.20)
96 h	8.2 (± 0.23)	9.7 (± 0.26)	7.0 (± 0.14)	7.9 (± 0.18)	6.9 (± 0.17)	7.5 (± 0.13)

1. Data presented are means of 3 replicates, numbers of brackets are s.e.s of individual means.
2. Drying was carried out at ambient temperatures, 20-27°C and RH 50-60%.

4.1.2 Final Percentage Radicle Emergence and Normal Seedling Production

As shown in Appendix Table A.1 and A.4 osmotic treatment duration had significant effects on both final germination parameters. Compared with untreated control, osmotic treatment in general reduced final percentage radicle emergence since treatment for 2 weeks significantly reduced the value of radicle emergence although treatment for 1 week was no different from untreated control (Figure 4.1.1). While the final numbers of normal seedlings from untreated controls were similar to the level of radicle emergence, a proportion of osmotically treated seeds showing radicle emergence failed to produce normal seedlings (compare Figure 4.1.1 and 4.1.2). The ungerminated seeds and abnormal seedlings were mainly seeds and seedlings heavily infected by fungi. Although attempts to minimise the effect of fungi during germination were made using surface sterilisation with NaOCl before germination testing, seeds still became infected.

4.1.3 Median Times (T_{50}) and Spread Times (T_{90} - T_{10}) of Germination

There were significant but independent effects of duration of treatment and drying back following treatment on the median times (T_{50}) of both radicle emergence and normal seedling production, whereas water potential significantly affected median times of normal seedling production (Appendix Tables A.2 and A.5). Median emergence times of untreated controls were of 7.1 and 16.4 d for radicle emergence and normal seedling production respectively. The effect of different durations of osmotic treatment on median times of radicle emergence are presented in Figure 4.1.3 where osmotic treatment for 1 week caused a greater reduction of median radicle emergence times (average 18%) than 2 weeks (average 13%). Similar findings were also obtained for median normal seedling production times (data not shown).

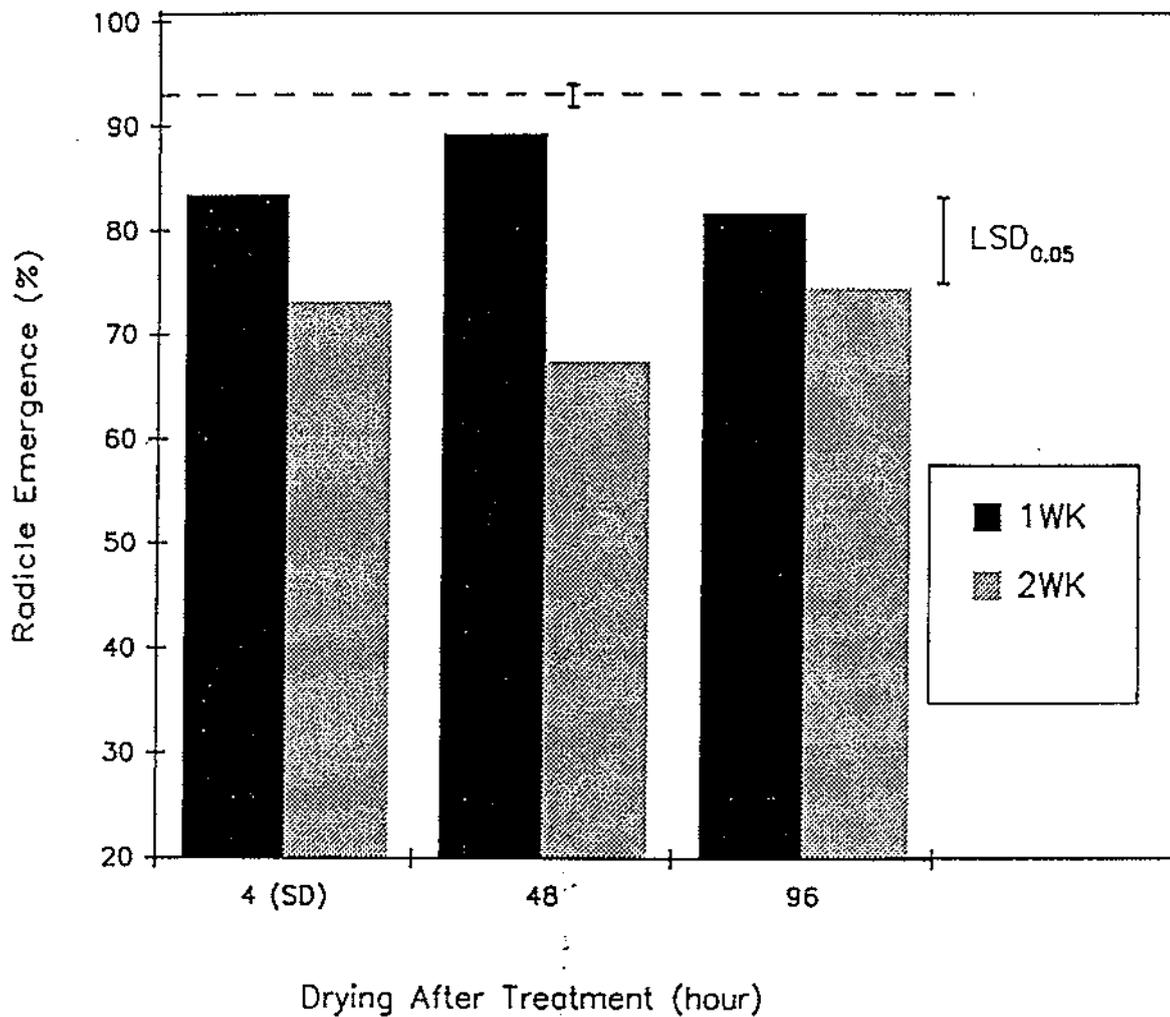


Figure 4.1.1 The final percentage radicle emergence of grade IV *Pinus radiata* seeds which were osmotically treated for either 1 week or 2 weeks and dried back at ambient temperatures. The data presented for treated seeds are averaged over different water potentials of treatment. The horizontal line with a vertical bar is the mean of untreated control seeds with \pm s.e. (3 replications).

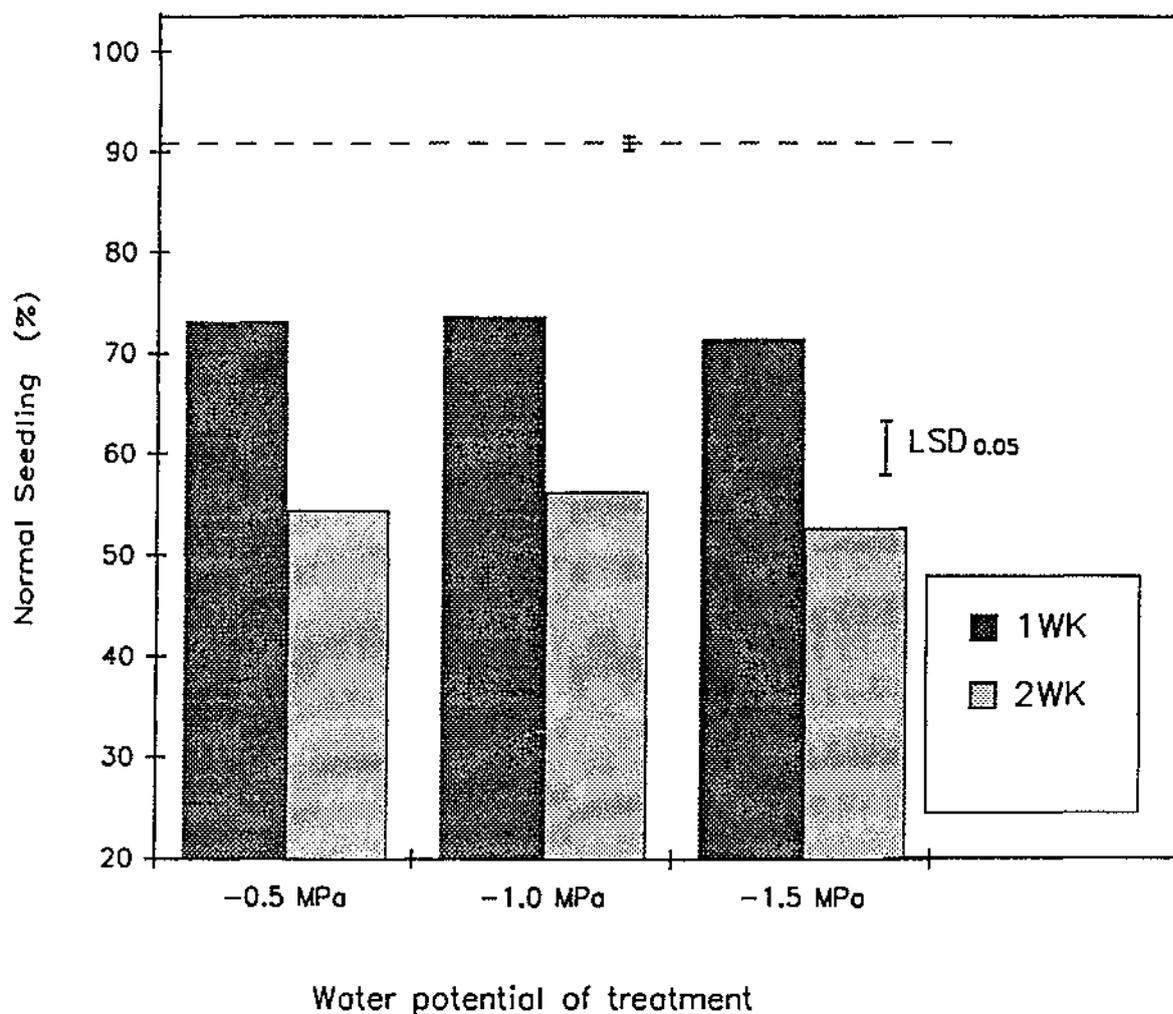


Figure 4.1.2 The final percentage normal seedling of grade IV *Pinus radiata* seeds after salt treatment at different water potentials and treatment durations for either 1 week or 2 weeks. The data presented for treated seeds are overall means over drying treatments. The horizontal line with a vertical bar is the mean of untreated control seeds with \pm s.e. (3 replications).

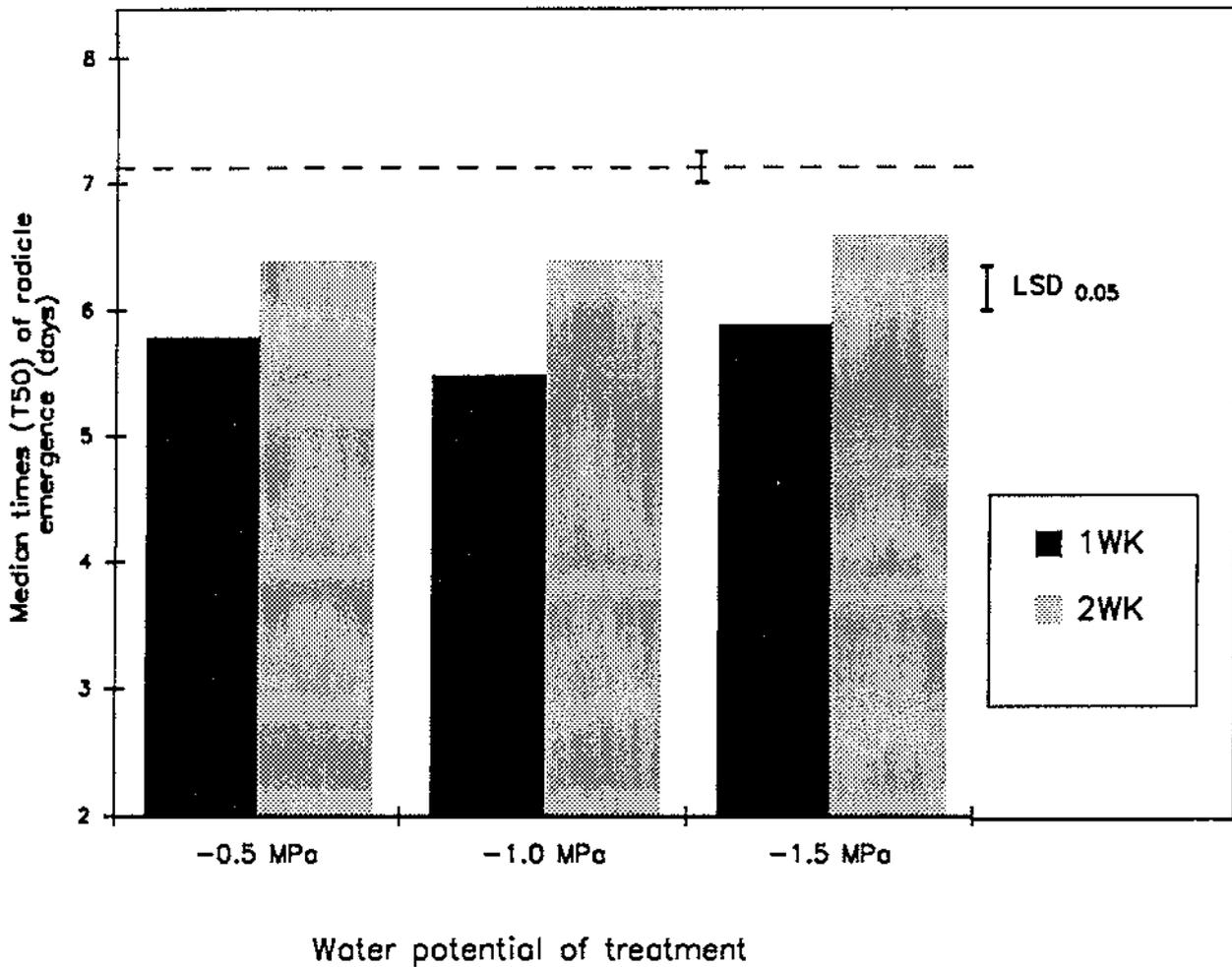


Figure 4.1.3 The median times (T50) of radicle emergence of grade IV *Pinus radiata* seeds after salt-treatment at different water potentials and treatment durations for either 1 week or 2 weeks. The data presented are overall means of different drying treatments. The horizontal line with a vertical bar is the mean of untreated control seeds \pm s.e. of 3 replications

The maximum benefit gained from osmotic treatment was a 25 to 28% reduction in median radicle emergence times after -0.5 or -1.0 MPa salt treatment for 1 week. This was only found in surface dried seeds while drying back to original seed moisture contents resulted in loss of treatment benefit (Figure 4.1.4).

Different water potentials, durations of treatment and drying back following treatment of all had no significant effects on any aspect of uniformity of germination (data not shown, analyses are shown in Appendix Tables A.3 and A.6). The overall mean spread of both parameters of germination was around 7.5 days.

4.2 Experiment 2. The Effects of Salt Treatment Durations and Fungicide Applications on the Germination Performance of Grade IV *Pinus radiata* Seeds

Based on the results of Experiment 1, a mixed salt solution of -1.0 MPa potential was chosen for further investigation in this second experiment. Seeds were treated for 4, 7 and 10 d. The fungicide Thiram at a rate of 1% seed weight was applied either before, after or both before and after osmotic treatment since there were fungal attacks during treatment and subsequent germination in the previous experiment. In addition, osmotically treated seeds in this experiment were dried back for either 2 h (surface drying) or 48 h at ambient temperatures.

4.2.1 Pre-germination and Moisture Content

Table 4.2.1 shows that there were just over 1% of pre-germinants after 10 d osmotic treatment at which time treated seeds attained a moisture content of around 26% (measured

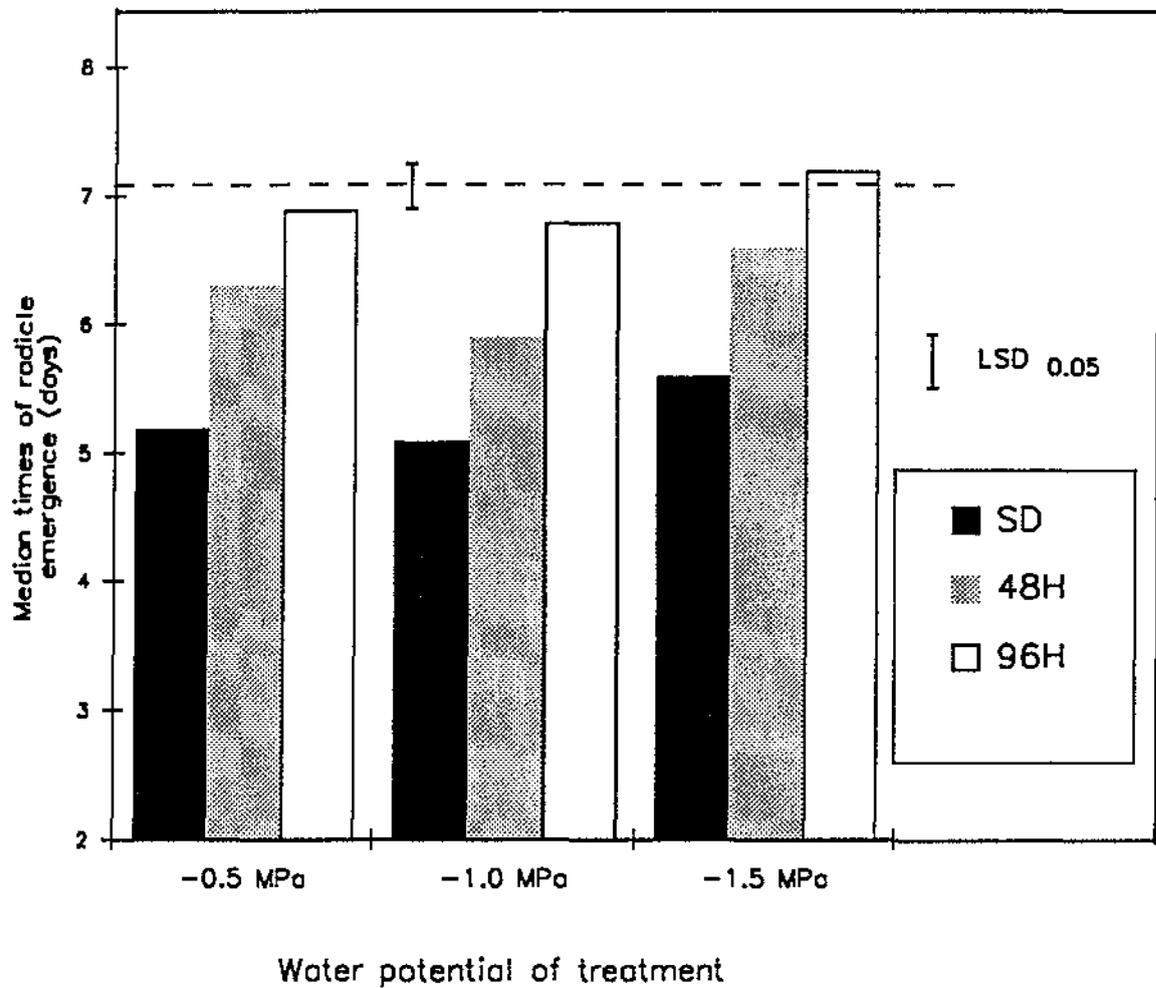


Figure 4.1.4 The effects of drying back on the median times (T50) radicle emergence of grade IV *Pinus radiata* seeds after salt treatment at different water potentials. Seed moisture content attained are presented in Table 4.1.2.
 SD : surface drying for 4h at ambient temperatures
 48H : drying back for 48h at ambient temperatures
 96H : drying back for 96h at ambient temperatures
 The data presented are means over treatments with different durations. The horizontal line with a vertical bar is the mean of untreated control seeds with \pm s.e. (3 replications).

after surface drying, Table 4.2.2). Subsequent drying for 48 h reduced the moisture content to values between 12 and 14%.

Table 4.2.1 The number of pre-germinants of grade IV *Pinus radiata* seeds during treatment at -1.0 MPa for different durations.

	Treatment duration (days)		
	4	7	10
Percentage pre-germinants	0	0.42 (\pm 0.21)	1.16 (\pm 0.36)

Data presented are means of 24 replicates.
Numbers in brackets are s.e.s of individual means.

Table 4.2.2 The moisture contents of grade IV *Pinus radiata* seeds after different durations of salt treatment at -1.0 MPa followed by drying at ambient temperature (percentage fresh weight basis).

	Treatment duration (days)		
	4	7	10
Drying back			
Surface drying	21.8 (\pm 0.15)	24.6 (\pm 0.17)	26.3 (\pm 0.19)
48 h	12.4 (\pm 0.13)	13.2 (\pm 0.10)	13.9 (\pm 0.13)

Data presented are means of 12 replicates, number in brackets are s.e.s of individual means.
Untreated controls had seed moisture contents of 7.3%.
Drying was carried out under ambient conditions, 22-27°C and 50-60% RH.

4.2.2 Final Percentage of Radicle Emergence and Normal Seedling Production

The benefit of using fungicide was very significant during osmotic treatment where seeds with no fungicide (C_1) or seeds treated with fungicide but after the osmotic treatment

(C₃; thus no fungicide during osmotic treatment) were heavily covered by fungal spread (data not shown). However, the percentage radicle emergence and normal seedling production for seeds treated with fungicide (C₂, C₃ and C₄) or not treated with fungicide (C₁) were not different. Overall means for final percentage radicle emergence and normal seedling of osmotically treated seeds were around 90% and were not different from untreated control (Analysis Appendix B1). Following 2-3 weeks after sowing there were very high incidences of fungal infection in developed seedlings from osmotically treated seeds with no fungicide (C₁) or from seeds treated with fungicide after osmotic treatment (C₃). Seeds treated with fungicide before osmotic treatment (C₂) and those treated with fungicide before and after osmotic treatment (C₄) had very little incidence of fungal infection in the developed seedling (Figure 4.2.1).

4.2.3 Median Times (T₅₀) of Germination

Fungicide applications did not affect median times of both radicle emergence and normal seedling production, but the durations of osmotic treatment, drying back of the treatment and interaction between these two factors did have significant effects (Appendix Tables B.2 and B.5). These effects on median radicle emergence time are shown in Figure 4.2.2 which demonstrates that the effect of drying seems to be unequal at different treatment durations as drying for 48 h in osmotically treated seeds for 10 d did not reduce the benefit of osmotic treatment. A similar situation also occurred for median normal seedling times (data not shown). Figure 4.2.2 clearly shows that 10 d is the best treatment duration resulting in a reduction of median times of radicle emergence of around 36%.

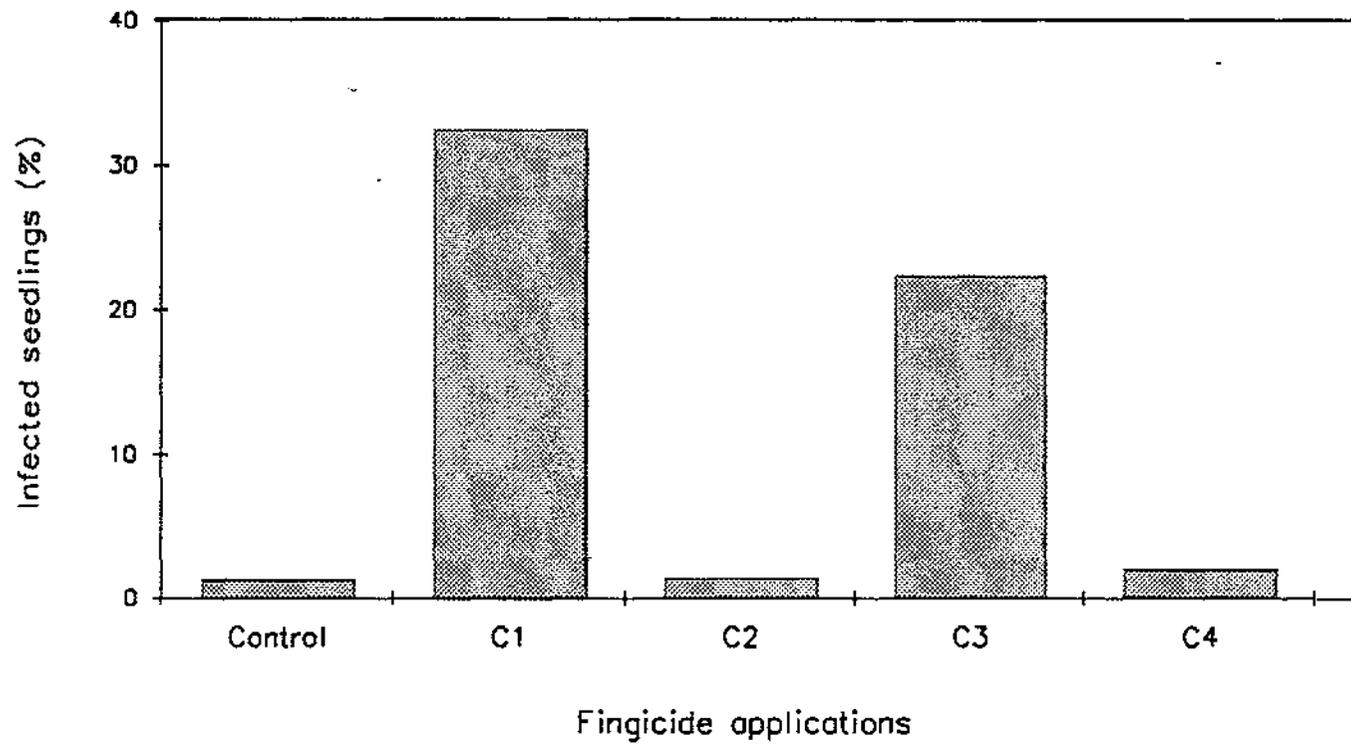


Figure 4.2.1 The effects of fungicide Thiram applications on the incidence of infected seedlings from grade IV *Pinus radiata* seeds after salt treatment

Control : no osmotic treatment (control seeds)

C1 : osmotically treated seeds without fungicide applications

C2 : fungicide applied before osmotic treatment

C3 : fungicide applied after osmotic treatment

C4 : fungicide applied before and after osmotic treatment

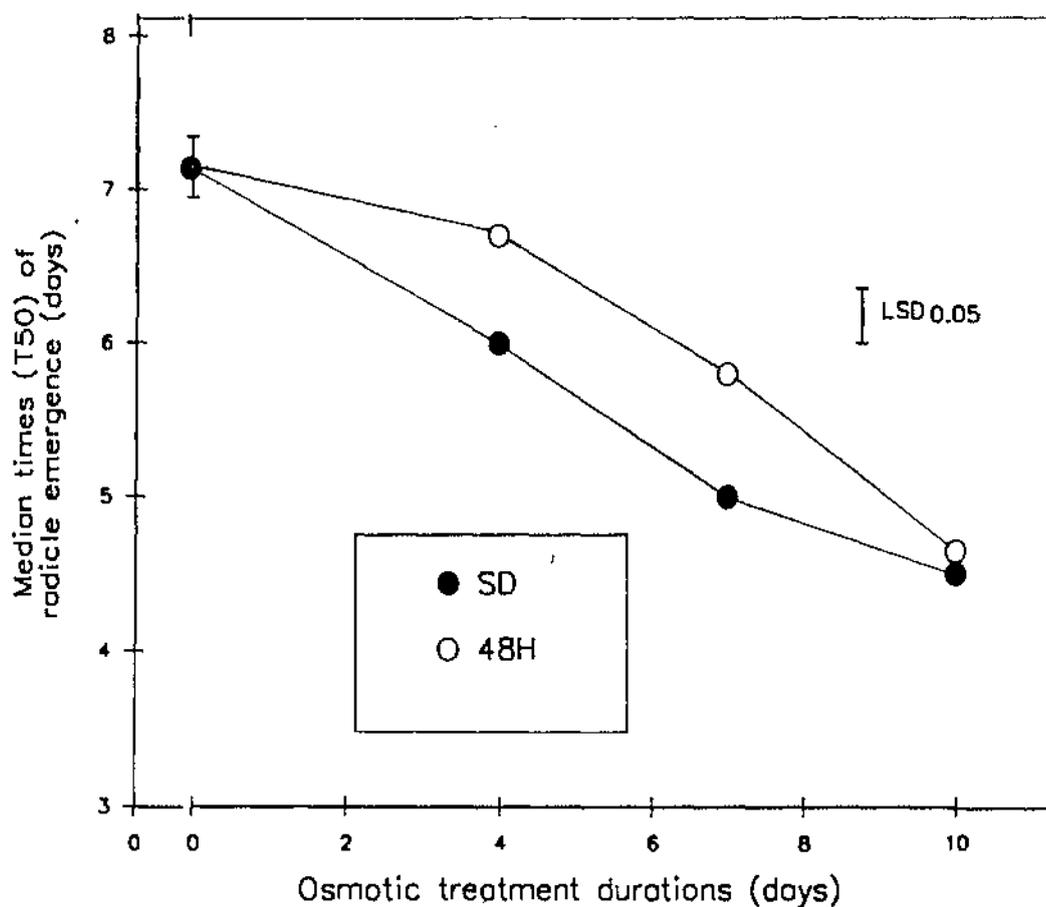


Figure 4.2.2 The effects of different durations of salt treatment and drying after treatment on the median times (T50) of radicle emergence of grade IV *Pinus radiata* seeds. The attained seed moisture contents are presented in Table 4.2.2

SD : surface drying for 2h at ambient temperatures
 48H : drying back for 48h at ambient temperatures

The data for treated seeds are overall means over different fungicide applications. $LSD_{0.05}$ is used for comparison among treatments without involving untreated control. The vertical bar in untreated control is \pm standard error of 3 replications

4.2.4 Mean Spread of Times ($T_{90} - T_{10}$) of Germination

Application of fungicide both before and after pre-sowing osmotic treatment (treatment C₄) and drying-back both significantly ($P < 0.05$) decreased uniformity of radicle emergence (Tables 4.2.4 and 4.2.5). Extending the duration of pre-sowing treatment from seven to ten days caused a significant reduction in spread of radicle emergence times (Table 4.2.3). There were no significant interactions between the three treatments (Appendix B.3). The response pattern for uniformity of normal seedling production was broadly similar except that there was no significant effect of treatment duration (Tables 4.2.4, 4.2.5 and Appendix Table B.6).

Table 4.2.3 Mean spread of times ($T_{90} - T_{10}$) of radicle emergence of grade IV *Pinus radiata* seeds which were treated at -1.0 MPa of mixed $KNO_3 + KH_2PO_4$ solution (1:1 w/w) for different treatment durations.

Mean spread times of	Treatment duration (days)			Untreated control
	4	7	10	
Radicle emergence	6.2	6.0	5.1	6.0 (± 0.25)

Data presented are means of 24 replicates
 $LSD_{0.05} = 0.88$ (see Appendix Table B.3).
 Value in brackets is s.e. of 3 replications

Table 4.2.4 Mean spread of times (T_{90} - T_{10}) of radicle emergence and normal seedling production of grade IV *Pinus radiata* seeds after salt treatment and fungicide applications.

Spread of times	Fungicide applications				LSD _{0.05}
	C ₁	C ₂	C ₃	C ₄	
Radicle emergence	5.1	6.0	5.4	6.3	1.01
Normal seedling	6.3	7.0	6.7	7.8	0.95

Data presented are means of 18 replicates.

C₁ = osmotically treated with no fungicide application; C₂ = fungicide applied before osmotic treatment; C₃ = fungicide applied after osmotic treatment; and C₄ = fungicide applied both before and after osmotic treatment.

Table 4.2.5 Mean spread of times (T_{90} - T_{10}) of radicle emergence and normal seedling production of grade IV salt-treated *Pinus radiata* seeds after drying at ambient temperatures.

Spread of times	Drying back		LSD _{0.05}
	SD	48	
Radicle emergence	5.3	6.2	0.73
Normal seedling	6.7	7.3	0.57

Data presented are means of 36 replicates.

SD = surface drying for 2 h; 48 = drying for 48 h at ambient temperatures.

4.3 Experiment 3. Comparison of Polyethylene glycol and Salt Treatments and Their Interactions with Drying

In this experiment the best mixed KNO₃ + KH₂PO₄ treatment (-1.0 MPa for 10 d at 20°C) was compared with a similar potential of Polyethylene glycol as an osmoticum. After

treatments the effects of drying back were further investigated. Seeds of grade IV were used in this study.

4.3.1 Seed Moisture Contents Following Drying

As treated seeds need to be washed after treatment for removing any osmoticum which may be toxic to seeds, a supplementary control was included which consisted of seeds washed without prior treatment, but on drying this washed untreated control came up with similar levels of moisture content to dried back unwashed untreated control (Table 4.3.1). Meanwhile drying seeds treated with different osmotica resulted in different levels of moisture content although treatment was carried out at similar water potential (-1.0 MPa). No pre-germination occurred during PEG treatment while around 1.46% was observed during salt treatment.

Table 4.3.1 The moisture content of grade IV *Pinus radiata* seeds after 10 d treatment at -1.0 MPa of different osmotica following by drying back.

Drying back	Osmotica		Control	
	PEG	Salt	Unwashed	Washed
48 h	7.8 (± 0.11)	13.1 (± 0.22)	6.0 (± 0.14)	6.2 (± 0.11)
48 h + 12 h over silica gel	4.5 (± 0.10)	6.6 (± 0.19)	4.5 (± 0.12)	4.4 (± 0.09)

Data are means of 3 replicates, numbers in brackets are s.e.s of individual means. Drying for 48 h was carried out at ambient temperatures.

4.3.2 The Final Number of Germination

There were no significant differences between the different osmotica on final percentage germination (Appendix Tables C.1 and C.4), overall mean numbers of seed showing radicle emergence and normal seedling being 88% and 84% respectively. Ungerminated seeds were mostly fresh ungerminated (10%), less than 2% being dead seeds.

4.3.3 Median Times (T_{50}) of Germination

Washing of untreated controls produced similar median germination times to unwashed untreated controls (data not presented). In untreated control seeds drying for either 48 h at ambient temperatures or 48 h followed by further drying over silica gel for 12 h did not increase median germination times; in osmotically treated seeds drying for 48 h still gave early emergence having 5.4 d for salt and 6.1 d for PEG treated seeds, whereas further drying over silica gel resulted in losses of treatment advantages (Tables 4.3.2 and 4.3.3).

Table 4.3.2 Median times (T_{50}) of radicle emergence of grade IV *Pinus radiata* seeds after osmotic treatment with different osmotica and drying back after treatment.

Drying back	Osmotica		Untreated control
	PEG	Salt	
48 h	6.1	5.4	7.3
48 h + 12 h over silica gel	7.1	6.9	7.5

Drying for 48 h was carried out at ambient temperatures, 22-27 °C and 50-60% RH; 12 h drying was over silica gel.

Osmotic treatments were carried out with similar water potential (-1.0 MPa for 10 d).

LSD_{0.05} = 0.6 d.

Table 4.3.3 Median times (T_{50}) of normal seedling (days) of grade IV *Pinus radiata* seeds after osmotic treatment with different osmotica and drying back after treatment.

Drying back	Osmotica		Untreated control
	PEG	Salt	
48 h	14.8	13.7	16.4
48 h + 12 h over silica gel	16.0	15.8	16.7

Drying for 48 h was carried out at ambient temperatures, 22-27°C and 50-60% RH; 12 h drying was over silica gel.

Osmotic treatments were carried out with similar water potential (-1.0 MPa).

LSD_{0.05} = 1.0 d.

4.3.4 Mean Spread Times (T_{90} - T_{10}) of Germination

The spread of both radicle emergence and normal seedling times was not affected by any aspect of treatment with different osmotica or subsequent drying. Overall mean spread times of radicle emergence and normal seedling production were around 6.2 d and 7 d respectively.

4.4 Experiment 4. Effect of Slow Drying After Salt Treatment

Previous results clearly showed that salt or PEG-treated seeds lost their treatment benefits when they were dried back to original moisture contents, either by continuous drying at ambient temperatures (Experiment 1) or drying over silica gel (Experiment 3). In celery the work of Toledo (1988) and Seetagosos (1989) demonstrated that if drying is properly controlled at a slow rate, treated seeds can be dried back without losses of treatment effects

(see Literature Review 2.2.2). In this present study two experiments (A and B) were established to investigate the effects of slow drying at 20°C and 80-85% RH followed by subsequent drying over silica gel or at ambient temperatures (22-27°C and 50-60% RH).

4.4.1 Moisture Losses During Drying

Moisture losses of treated seeds during drying at 20°C, 80-85% RH were curvilinear and equilibrium seed moisture contents at this regime were around 11% which were achieved after 9 or 10 d drying in Experiment A and B respectively (Figure 4.4.1). The calculated drying curve based on an exponential model had a high correlation coefficient ($r = -0.969$, $P < 0.001$). Meanwhile subsequent drying over silica gel or at ambient temperatures reduced seed moisture content still further, but the reduction of seed moisture contents was much higher during drying over silica gel compared to drying at ambient temperatures (Figure 4.4.1).

4.4.2 Final Percentage Radicle Emergence and Normal Seedling

In general, drying back treated seeds did not cause a reduction in the final number of germination percentages, but drying to very low moisture content induced some loss of germination as seeds with 3.1% moisture content (Experiment A) had 12% ungerminated seeds while those with 4.2% moisture content (Experiment B) produced 26% (Figure 4.4.2). Verification that these seeds were dormant was done by Tetrazolium testing where embryo and megagametophyte tissues of all fresh ungerminated seeds showed a clear red colour (viable) (Figure 4.4.3). In all cases percentage normal seedlings was similar to those of final percentage radicle emergence (data not shown).

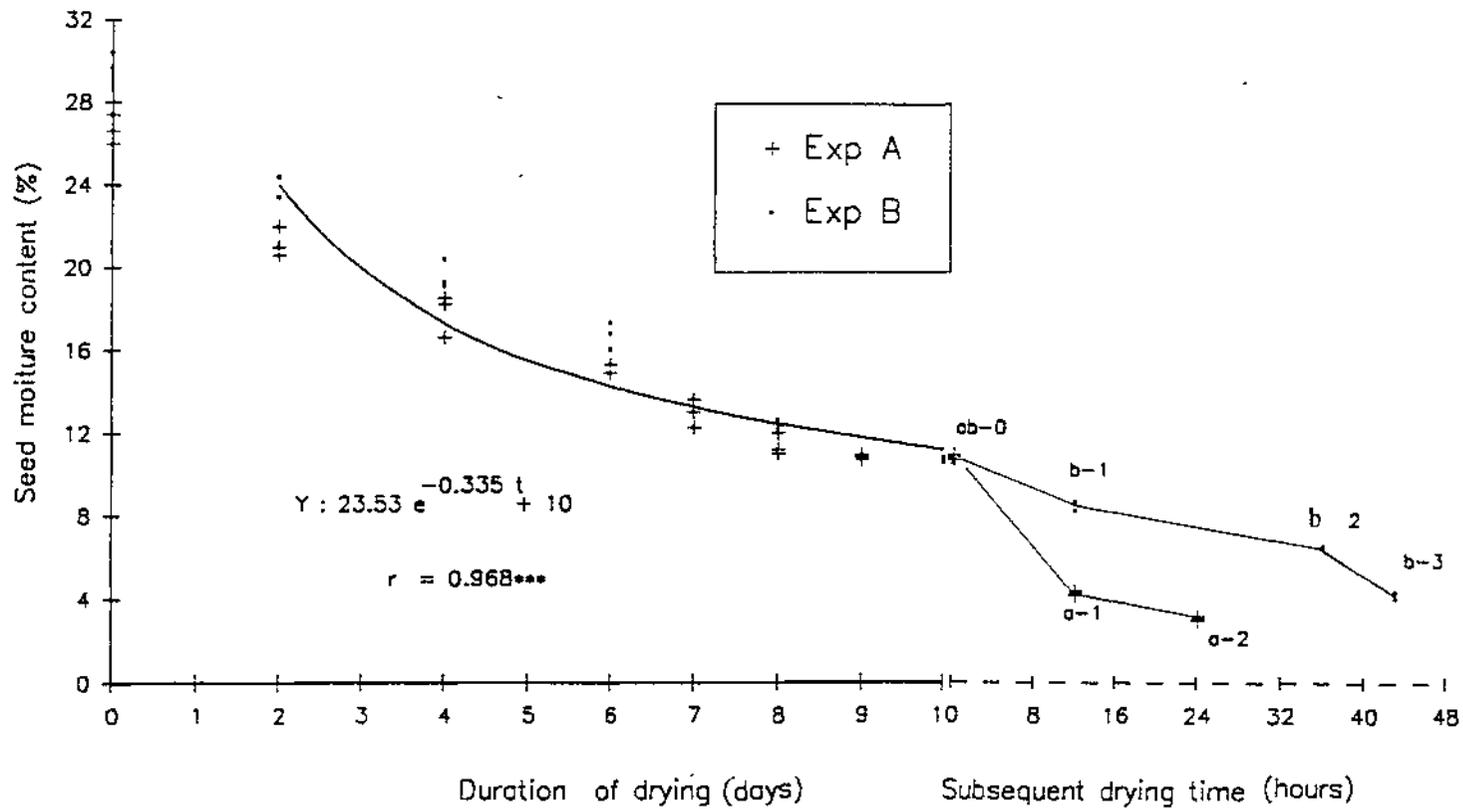


Figure 4.4.1

The moisture losses of salt-treated grade V *Pinus radiata* seeds during drying at 20°C and 80-85% relative humidity (RH) and subsequent drying over silica gel or at ambient temperatures.

- ab-0: equilibrium SMC at 20°C and 80-85% RH
- a-1 and a-2: SMC after subsequent drying over silica gel (0% RH) for either 12h or 24h (Experiment A)
- b-1, b-2 and b-3: SMC after subsequent drying for 12h, 38h at ambient temperatures or 38h at ambient temperatures followed by 7h over silica gel (Experiment B).

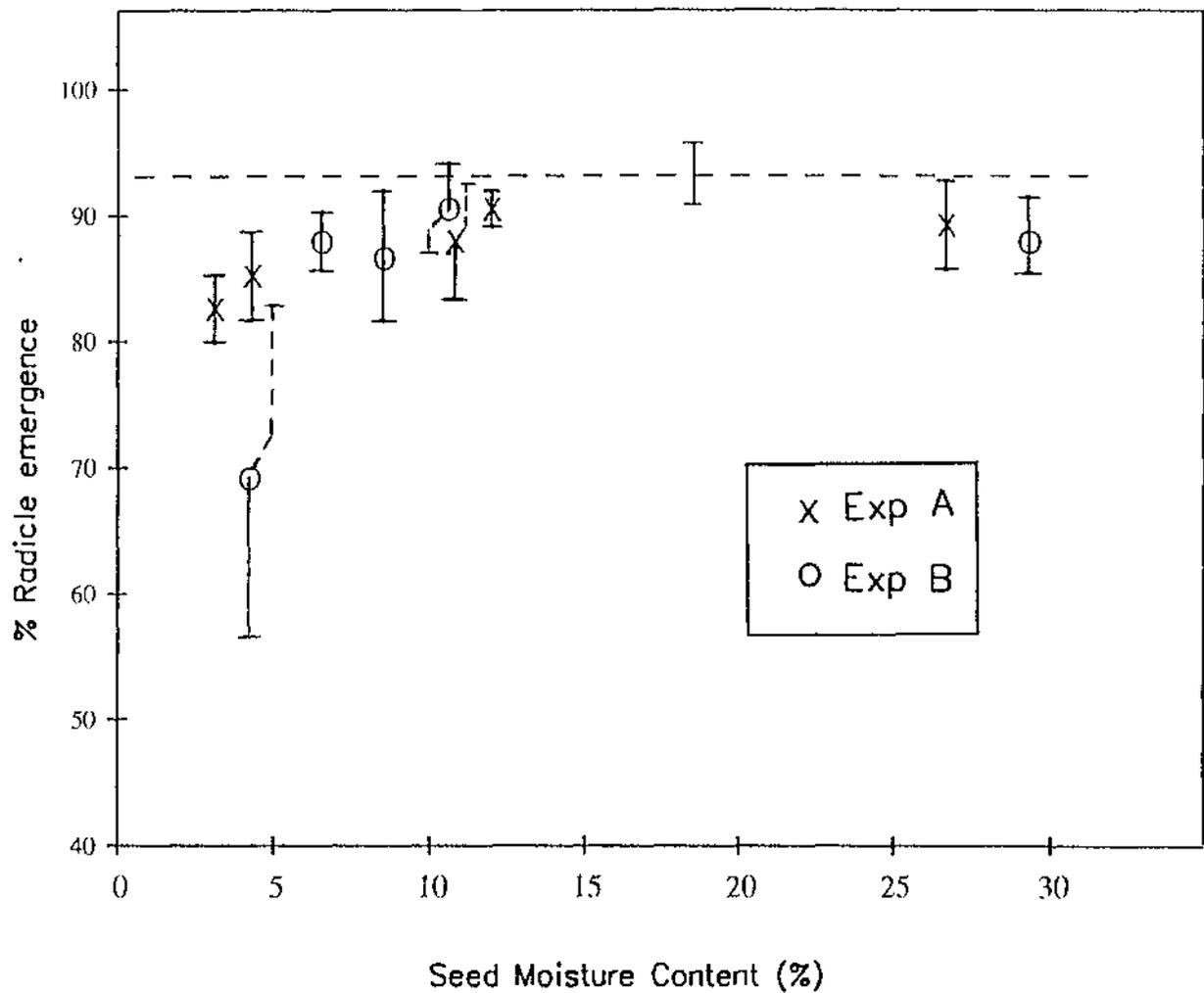


Figure 4.4.2 The final percentage radicle emergence of salt-treated grade V *Pinus radiata* seeds after drying back to different seed moisture contents. Moisture contents > 10% were attained from drying at 20°C, 80-85% RH while moisture contents < 10% were attained following subsequent drying over silica gel or at ambient temperatures (see Figure 4.1.1). The horizontal line with a vertical bar is the untreated control mean \pm s.e. of 3 replications



Figure 4.4.3 Red coloured formazan developed from megagametophyte and embryo tissues of secondary dormant salt-treated *Pinus radiata* seeds which were dried to < 5% moisture content. Both tips of seeds were cut off during preparation prior imbibing them into Tetrazolium solution

4.4.3 Median Times (T₅₀) and Spread Times (T₉₀-T₁₀) of Germination

As shown in Figure 4.4.4, slow drying at 20 °C and 80-85% RH retained the benefits of osmotic treatment. Osmotically treated seeds dried slowly for 9-10 d followed by further drying at ambient temperatures which resulted in 6-8% moisture content still had 25-30% reduction in median radicle emergence times. Meanwhile, when seeds with equilibrium moisture content at 20 °C and 80-85% RH were subsequently dried back over silica gels, large variations of median radicle emergence were found, although these mean values were still lower than that of untreated control. A similar benefit of slow drying was also observed in terms of median normal seedling germination times (2.7 - 3.1 d earlier than those from untreated controls, data not shown). Attempts to devise a best-fit curve for relationship between seed moisture content after drying and the median radicle emergence times was established, resulted a mathematical model of:

$$Y = 3.983 e^{-0.3905 \text{ SMC}} + 4.811$$

with a correlation coefficient (r) of 0.605 ($P < 0.05$). Another alternative model of $Y = a\{(e)^{-b \text{ SMC}} + 1\}$ was also developed which produced quite similar formula but a reduced coefficient correlation ($r = -0.589$) where $a = 4.826$ and $b = 0.444$. As treated seeds should have a similar level of moisture to untreated controls (7-8%), the reduction of median times of radicle emergence of treated seeds after drying might be expected to be around 30% by applying the first model. Even when treated seeds were dried back to < 7% moisture contents, low values of median radicle emergence times could still be expected.

In terms of the mean spread germination times, osmotic treatment did not change either spread times of radicle emergence or normal seedling but seeds which were dried back to low moisture levels resulted an increase in mean spread radicle emergence (Table 4.4.1).

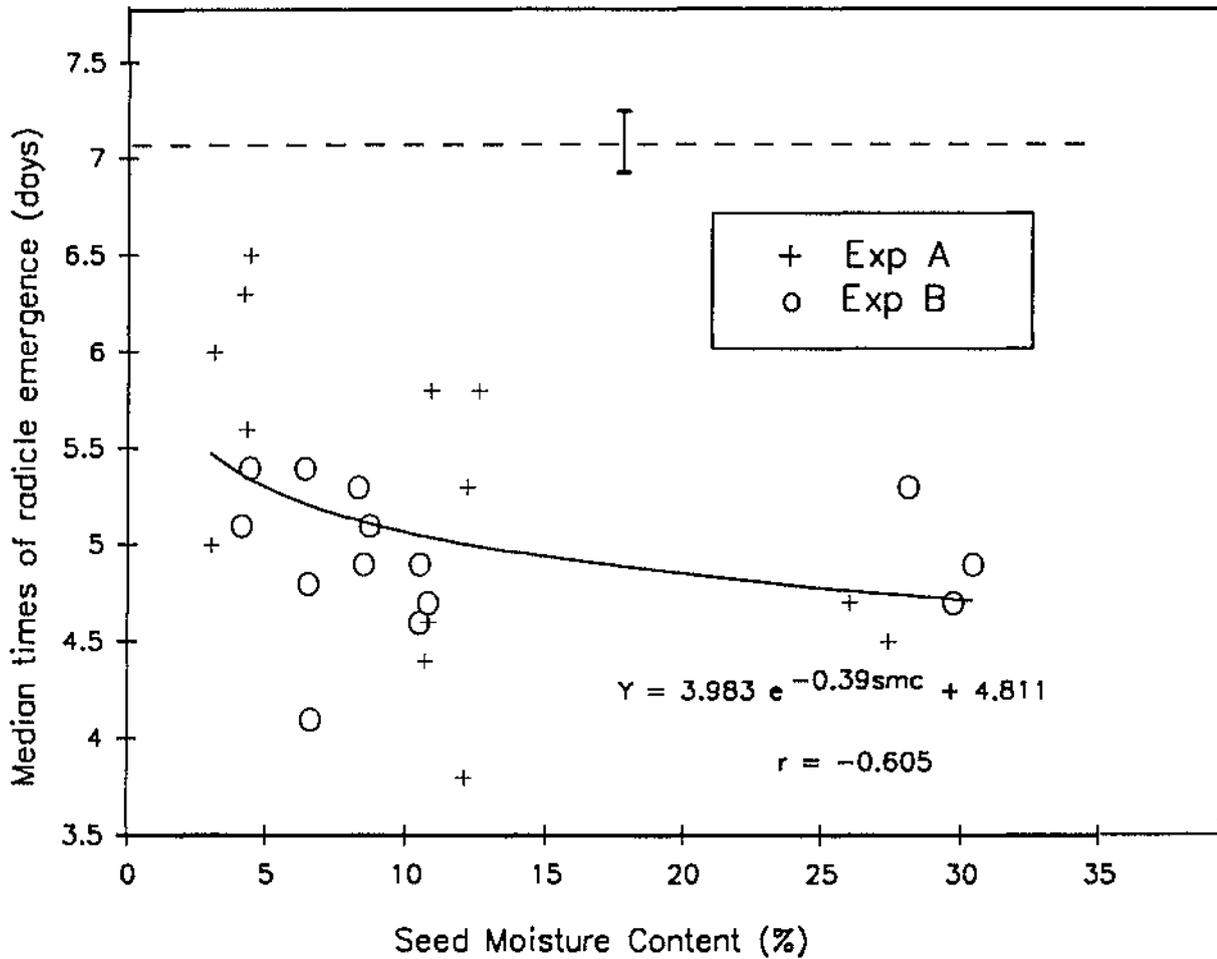


Figure 4.4.4 The relationship between moisture contents after drying and the median times (T_{50}) of radicle emergence of grade V *Pinus radiata* seeds which were salt treated at -1.0 MPa at 20°C for 10d. Moisture contents $> 10\%$ were attained after drying at 80 - 85% of RH while moisture contents $< 10\%$ were attained after subsequent drying over silica gel or drying at ambient temperatures from Experiment A and B. The horizontal line with a vertical bar is the untreated control mean \pm s.e. of 3 replications.

Table 4.4.1 Mean spread of times ($T_{90} - T_{10}$) of radicle emergence of salt treated grade V *Pinus radiata* seeds after drying to different moisture contents.

Drying treatment	Final SMC (%)	$T_{90} - T_{10}$ (days)	Remark
Moist seed, no drying	26.76 (± 0.51)	6.18 (± 0.32)	Experiment A
Slow drying for 9 d	10.82 (± 0.05)	5.90 (± 0.49)	Experiment A
Slow drying for 9 d followed by 12 h drying over silica gel	4.30 (± 0.05)	6.20 (± 0.28)	Experiment A
Slow drying for 9 d followed by 24 h drying over silica gel	3.10 (± 0.06)	6.90 (± 0.14)	Experiment A
Moist seed, no drying	29.4 (± 0.68)	6.0 (± 0.31)	Experiment B
Slow drying for 10 d	10.62 (± 0.09)	5.42 (± 0.33)	Experiment B
Slow drying for 10 d followed by 12 h drying at ambient temperatures	8.26 (± 0.12)	5.20 (± 0.39)	Experiment B
Slow drying for 10 d followed by 38 h drying at ambient temperatures	6.49 (± 0.04)	6.60 (± 0.33)	Experiment B
Slow drying for 10 d followed by 38 h drying at ambient temperatures and 7 h over silica gel	4.20 (± 0.06)	7.10 (± 0.18)	Experiment B
Untreated control	7.6 (± 0.04)	6.20 (± 0.18)	Experiment A

Data presented are means of 3 replicates.
Numbers in brackets are s.e.s of individual means.

4.5 Discussion

4.5.1 The Optimum Osmotic Treatment

A starting point of this study was a common conception that osmotic treatment has been claimed to be effective in accelerating germination, seedling emergence and the performance of many different crops in the field (Heydecker and Coolbear, 1977; Khan *et al.*, 1980; Thomas, 1981). Incubation period of treatment, a precise control of water supply

to each individual seed in order to achieve and maintain the desired water potential and no colonisation by a hostile microflora seem to be major determinants for successful osmotic treatment.

The results of this present study also indicated that osmotic treatment had the desired effect of enhancing the germination of seed in the laboratory irrespective of water potential, duration of treatment and type of osmotica used. However, the best osmotic treatment conditions were when seeds were treated at -1.0 MPa for 10 d at 20°C (Figure 4.2.3). Although rate of radicle emergence at this water potential was similar to that of -0.5 MPa treated seeds, the latter suffered from the reasons of large losses of seed due to pre-germination during treatment (Table 4.1.1). At water potential of -1.5 MPa, however, as this water potential effectively inhibited any visible sign of radicle protrusion, this high concentration would not allow seeds to take up enough water, thus they might not have completed enough of the preparatory processes of germination, expected to occur during treatment.

The greater benefit of longer treatment durations at -1.0 MPa potential (10 d rather than 7 d) seems in agreement with Heydecker's (1974) idea that seeds should be allowed to progress as far as possible through their initial stages of germination, but without permitting radicle emergence. Usually there are two general rules of osmotic treatment to apply: (i) for any given osmotic or water potential potential, a longer duration of treatment is required at lower temperatures, and (ii) at any given temperature, increasing concentration of osmoticum prolongs the priming time (Bewley and Black, 1985). Thus high water potentials would shorten treatment duration accordingly. But the -0.5 MPa potential was too high for the *P. radiata* seeds used in this experiment since preparatory process of germination progressed too far resulting in considerable pregerminants during 7 d of treatment, while relative merits of germination advancement was not different from -1.0 MPa treatments

(Appendix Table A.2). Thus optimum conditions for osmotic treatment should comprise treatments that produce substantial improvements in germination rate and vigour, without affecting final percentage germination and no premature germination during treatment. But optimum treatment varies with species, even seed lots used. Working with coniferous tree seeds of *Picea mariana*, Fleming and Lister (1984) found that -1.25 MPa for 14 to 21 d at 15°C seemed to be optimum conditions as germination, seed coat shed (cotyledon emergence), and the primary needle stage occurred 3-5 d earlier than untreated control, whereas those treated at higher water potential for shorter durations had less advancement. This result was not similar to Huang's (1989) experiment which presented evidence of optimum treatment at -1.1 MPa for 3 d or -0.8 MPa for 5 d at 15°C for *Pinus contorta* and *Picea glauca*. Although Haridi (1985) recommended -1.5 MPa for 5-6 d at 25°C as the best regime for *Pinus elliottii* in enhancing and synchronising germination, he did not, in fact, examine the higher ranges of water potential between 0 to -1.5 MPa. A more dilute solution of Polyethylene glycol at -0.58 MPa has also been used successfully for improving the performance of other tree seeds, e.g. *Pinus nigra* Arn., *Larix decidua* Mill E., *Pseudotsuga menziesii* and *Pinus sylvestris* (Paci, 1987; Simak, 1976a).

It should be noted that the present study only used a single temperature of 20°C for seed treatment. Up until now research on tree seeds using warm temperatures has only been done by Haridi (1985) and Fleming and Lister (1984). In *Pinus elliottii* (Haridi, 1985), treatment at 25°C and -1.5 MPa gave the best results, e.g. increased peak value and higher percentage germination compared to treatments carried out at supra-optimal temperature (30-35°C) while Fleming and Lister (1984) found a combination of low water potential for longer periods at higher temperature (20-21°C) as best treatment for *Picea* sp. These results were compatible with the opinion of Akers and Holley (1986) based on a screening procedure of osmotic treatment. Their assumptions were that treatment temperatures and duration of treatment should meet the germination requirements of untreated controls, the

water potential of osmoticum should be as high as possible but without allowing any radicle protrusion. Therefore the use of 20°C for *Pinus radiata* seeds can be justified since this temperature is considered to be optimum for germination of this type of seed (ISTA, 1985).

In terms of the percentage of radicle emergence, osmotic treatments at -1.0 MPa (Experiment 2 and thereafter) did not change final percentage but following seedling growth there were some considerable incidences of infection in the developing seedlings (Figure 4.2.1). Although this result was similar to Fleming and Lister's (1984) results, it was very different from findings in *Pinus elliottii* (Haridi, 1985) and *Pinus sylvestris* (Simak, 1976a) where this treatment was claimed to increase germination counts as also happened in Douglas fir (Paci, 1987). In the present experiment final percentage radicle emergence was > 90%, thus in evaluating ideal osmotic treatment, it should be borne in mind that this response was due essentially to the already high germinability of seeds. Haridi's (1985) experiment showed that seeds were of low quality where percentage germination was less than 50%. Similarly, Simak (1976a) used low quality or poorly developed seeds of *Pinus sylvestris* from circumpolar regions of Sweden where collected seeds usually comprise seeds with widely varying embryo development. Thus osmotic treatment very likely improves percentage germination of low quality seeds.

There are some pieces of work reporting the effect of pre-moistening pine seeds before sowing. As an example, steeping pine seeds 24 h in the water or pre-moistening for 11 d at 15°C gave higher germination rates and percentage germination than those of untreated control, but osmotically treated seeds at -0.5 MPa potential gave much better performance since percentage germination of osmotically treated seed were 30% higher than water pre-moistened seed (Simak, 1976a). One might expect that pre-moistened or soaked seeds will always perform better than air-dried control seeds. But this is not the case since Kartiko's (1990) experiment in *P. radiata* showed that 2 d soaked in water at 5°C resulted in poorer

performance than unsoaked seeds. Similar results have been presented by Larson and Schubert (1969) that soaking *P. ponderosa* seeds in water gave less germination and poorer vigour (measured by germination rate; Czabator, 1962). Although it was not established the reasons for poor performance of water soaked seeds in the latter, we might suggest that soaking might induce some sort of stress such as oxygen deficiency and/or weakening membrane integrity (Barla-Szabo and Dolinka, 1988; Laidman *et al.*, 1979; Kartiko, 1990). In many grain legumes reduced seed quality also occurs due to rapid uptake of water during imbibition (Powell *et al.*, 1984b; Powell, 1985) which is said to cause physical membrane disruption, preventing normal cell organisation and leading to reduced vigour, therefore the relative advantage of PEG treatment over water treatment in pine (Simak, 1976a) was possibly due to slower water movement into the seed, allowing normal cell organisation while the preparatory process of germination were then regulated properly.

4.5.2 The Effects of Fungicide Application

Attempts to overcome fungal infection were successful in the second experiment by using the fungicide Thiram at a rate of 1% seed weight. Interestingly, seeds heavily infected by *Aspergillus* during osmotic treatment (C₁: seed without fungicide application and C₃: fungicide applied after osmotic treatment) produced high radicle emergence similar to C₂ (fungicide applied before osmotic treatment) and C₄ (fungicide applied both before and after osmotic treatment). However, following seedling growth C₁ and C₄ had high incidence of infected seedlings apparently caused by field fungi and very little incidence occurred in C₂ and C₃ (Figure 4.2.1). This suggests that the use of fungicide is an obligatory step required in the standard protocol of osmotic treatment for *P. radiata* since Thiram applied at this time at a rate of 1% seed weight did not have any negative effect on rate of either radicle emergence or normal seedling germination. This is very important since osmotic treatment regimes are usually favourable not only for growth of storage fungi but also for other

microflora. Most of *Aspergillus* and *Penicillium* are able to grow without free water, and on media with a high osmotic concentration (Neergaard, 1977). The harmful effects may include: loss of germinability, seed discolouration or decay, increase of acidity and heating and mustiness. For example, in peas when such fungi were present and the seeds kept at 85% RH and 30°C infected seeds lost their germination capacity while uninfected seeds still had 95% germinability (Fields and King, 1962). Similarly, *Aspergillus* sp could cause mortality in groundnut seed (Christensen, 1973). In case of field fungi it was not known whether these fungi occurred superficially or from inside seed as Thiram is usually used for surface sterilisation.

4.5.3 Effect of Different Osmotica

The response of *Pinus radiata* to salts and PEG treatment showed that salt was better than PEG in terms of germination rates while no differences were found in terms of final germination counts (section 4.3). This result was in accordance with findings in carrot (Haigh and Barlow, 1987a; Furutani *et al.*, 1986), tomato (Bussell and Gray, 1976; Globerson and Feder, 1987; Alvarado *et al.*, 1987), pepper (O'Sullivan and Bower, 1984; Rivas *et al.*, 1984; Globerson and Feder, 1987), celery (Slater and Danbry, 1976), asparagus (Laksana, 1991) and lettuce (Cantliffe, 1981; Guedes and Cantliffe, 1980).

The reason of better performance of salt treated seeds compared PEG treated seed is not known, but probably involves differences in the moisture content of seed attained during treatment. Lack of moisture content data following osmotic treatment may be a weakness of this experiment but Table 4.3.1 clearly showed salt treated seeds following drying had higher moisture values. Similarly, the fact that no pre-germination occurred during PEG treatment compared to 1.43% of salt treatment indicated that the preparatory processes of germination were completed much faster in salt treated seeds.

Apart from differences in seed-water relationships, there is also much evidence that KNO_3 increases seed germination as it is also recommended by ISTA (1985) for breaking dormancy. It might be possible that the benefit of salt treatment could be due to the availability of nitrate as additional substrate which could be reduced through nitrite, hyponitrite and hydroxylammonium intermediates to the ammonium ions which were then assimilated into amino acids and/or for protein synthesis (Hendrick and Taylorson, 1972). Other plausible explanations of better performance of salt treated seeds may be due to the supply of O_2 during treatment as the high viscosity of PEG could cause insufficient of O_2 and this leads to anaerobic respiration (Furutani *et al.*, 1986). Products of anaerobic respiration, e.g. ethanol, are undesirable because of their toxicity (Mayer and Poljakoff-Mayber, 1982). Meanwhile promotive effects of KNO_3 on germination of various weed seeds were associated with increases in oxygen uptake (Hilton and Thomas, 1986). Higher O_2 uptake may reflect higher ATP production which indicates energy status of seeds. However, these aspects of nitrate metabolism (Hendrick and Taylorson, 1972) and ATP status of salts and PEG treated seeds were not explored in any detail in this study. Further experiments are perhaps required to clarify different performances between salts and PEG treated seed in these areas.

4.5.4 The Effects of Drying After Treatment

Figure 4.1.4 shows that drying continuously at ambient temperatures resulted in loss of treatment advantages when attained moisture contents were around 8%. Similarly, Tables 4.3.1 and 4.3.2 present evidence that if drying salt-treated seeds was carried out at ambient temperatures for 2 d followed by 12 h over silica gel, the germination rates of treated seeds were no longer better than untreated control. A similar situation was found by Brocklehurst and Dearman (1983a, 1983b) in certain carrot and celery lots that drying back treated seeds reduced germination rate and the spread of emergence became larger than from treated

seeds without drying. Celery is particularly vulnerable to drying back since drying to near or lower than original moisture content almost completely disposed the advancement gained of presowing treatment (Toledo, 1988). Previous work in tree seeds also showed reduced germination rates or vigour as a result of drying treated seeds under ambient humidity (Huang and Zou, 1989; Zou and Huang, 1987; Fleming and Lister, 1984). Negative effects of drying correlated with higher conductivity of seed leakage (Huang and Zou, 1989; McKersie and Tomes, 1980; Biddington *et al.*, 1980) which suggests that drying induces membrane damage.

As osmotically treated seeds had 26-28% moisture content (Figure 4.4.1) drying rate at ambient temperature during the first 2 days was around 6-8% a day or 0.25-0.33% an hour (Table 4.1.1) while drying rate over silica gel was much higher around 0.5% an hour (Table 4.3.1). This drying rate was probably too high for *P. radiata* seeds since Seetagos and Coolbear (1992) found no loss of treatment effect when drying is regulated at a slow rate. Thus Experiment 4 was set to test this idea. Drying treated seeds under 80-85% RH clearly showed a low rate having 0.04-0.05% an hour at 5 d of drying while 10% SMC was attained after 10 d (Figure 4.4.1). The rate of germination at this moisture level (10% SMC) was similar to treated seeds without drying back; being 2.5-3 d earlier than that of control seeds (Figure 4.4.4). Subsequent drying over silica gel which resulted in high moisture losses (Figure 4.4.1) increased the variation of germination rate (Figure 4.4.4). However, if subsequent drying was at a slow rate, the benefit of osmotic treatment was preserved. The benefit of slow drying here coincided with arguments addressed by Bewley and Pacey (1978) and Krochko *et al.* (1978) where speed of water loss has a determining consequence upon ultrastructural changes. Rehydration of the gametophyte of *C. filicinum* following drying, the cytoplasm of phyllidia cells is disorganised, mitochondria are swollen and have ill-defined structures, the outer chloroplast membrane is lost and lamellae are dispersed. In contrast, at the same time of rehydration following very slow desiccation, the number of disrupted cells is

only one-fifth of that when rapid drying has occurred. Damage to membrane has been a subject addressed by Crowe *et al.* (1989). If membranes (e.g. membrane phospholipids) are maintained in liquid crystalline phase even under low water content, lateral phase separation between phospholipids may be avoided and this preserves structural integrity of membrane. It is not known in the present study whether slow drying maintained membrane phospholipids in a more fluid state or not.

V. APPLICATIONS OF SEED TREATMENT

Previous results (Chapter 4) showed the advantages of osmotic treatment in advancing the germination of small grade *Pinus radiata* seeds. Osmotically treated seeds can be dried back to their original moisture content without major losses of treatment effects if drying proceeds slowly. In this Chapter the effects of osmotic treatment on different seed grades are investigated (Experiment 5). In addition, the value of osmotic treatment for protecting seeds in store or repairing aged seeds was also studied (Experiment 6).

5.1 Experiment 5. Germination and Seedling Emergence from Different Grades of Salt Treated *Pinus radiata* Seeds

It was the aim of this experiment to investigate the effect of osmotic treatment on different grades with respect to:

- i. germination performance in the laboratory, and
- ii. seedling emergence from soil.

Seeds of grades I to V were osmotically treated using -1.0 MPa of mixed $\text{KNO}_3 + \text{KH}_2\text{PO}_4$ solutions for 10 d at 20°C. Before treatment, seeds were dusted with the fungicide Thiram at 1% seed weight, and after treatment seeds were slow dried to around 8% moisture content (holding seeds for 9 d at 20°C, 80-85% RH, following by 1 d at ambient temperature, 22-27°C and 50-60% RH).

5.1.1 Laboratory Experiment

5.1.1.1 Final percentage radicle emergence and normal seedling production

The result of laboratory testing showed a small but significant difference ($P < 0.05$) in the final radicle emergence between grade I and other grades (Figure 5.1.1A). Seeds from different grades responded differently to osmotic treatment as in grade II this treatment increased final percentage radicle emergence ($P < 0.05$) but in other grades there was no change in this type of response (Figure 5.1.1A). A similar pattern of results was also found for normal seedling production where grade I seeds had lower percentage normal seedling production (Appendix Table E.4 and Figure 5.1.1B).

5.1.1.2 Median time (T_{50}) of germination

Osmotic treatment advanced radicle emergence by 3.1 d, 40% earlier than untreated controls and there was no significant differences in response between seed grades (Figure 5.1.2). For median times of normal seedling germination, however, the advancement of normal seedling germination due to osmotic treatment was around 5.2 and 5.9 d in grades I and II seeds compared to 3.5 to 4.2 d in other grades (data not shown; analysis of variance presented in Appendix Table E.5).

5.1.1.3 Mean spread of times ($T_{90}-T_{10}$) of germination

Although seed grade, osmotic treatment and interaction between these factors had no effect on the uniformity of radicle emergence (Appendix Table E.3), Figure 5.1.3 shows a decrease ($P < 0.05$) in the uniformity of normal seedling production due to osmotic treatment.

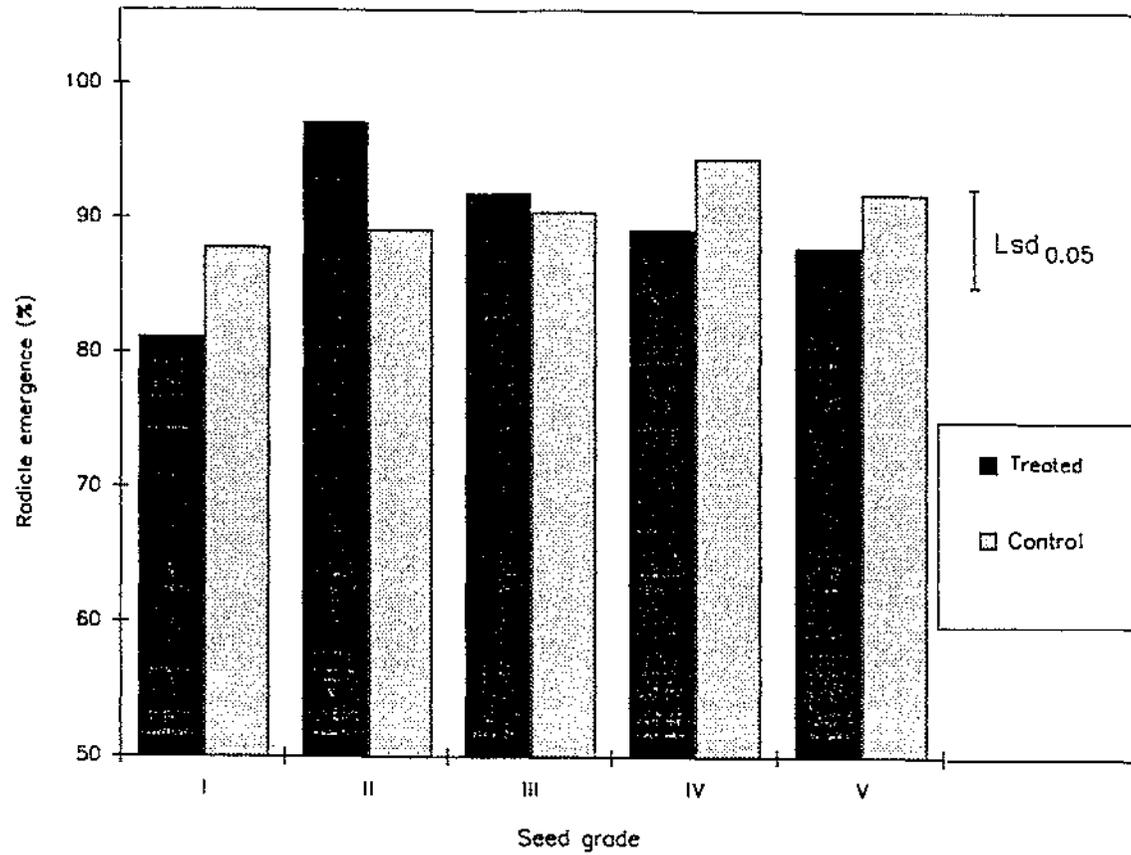


Figure 5.1.1A Percentage radicle emergence from different grades (I - V) of *Pinus radiata* seeds which were previously treated or untreated with a -1.0 MPa solution of mixed $\text{KNO}_3 + \text{KH}_2\text{PO}_4$ for 10d at 20°C and dried back slowly to around 8% seed moisture content. Data presented are means of three replicates, grade represents different weights or sizes of seed (see Chapter Three for details).

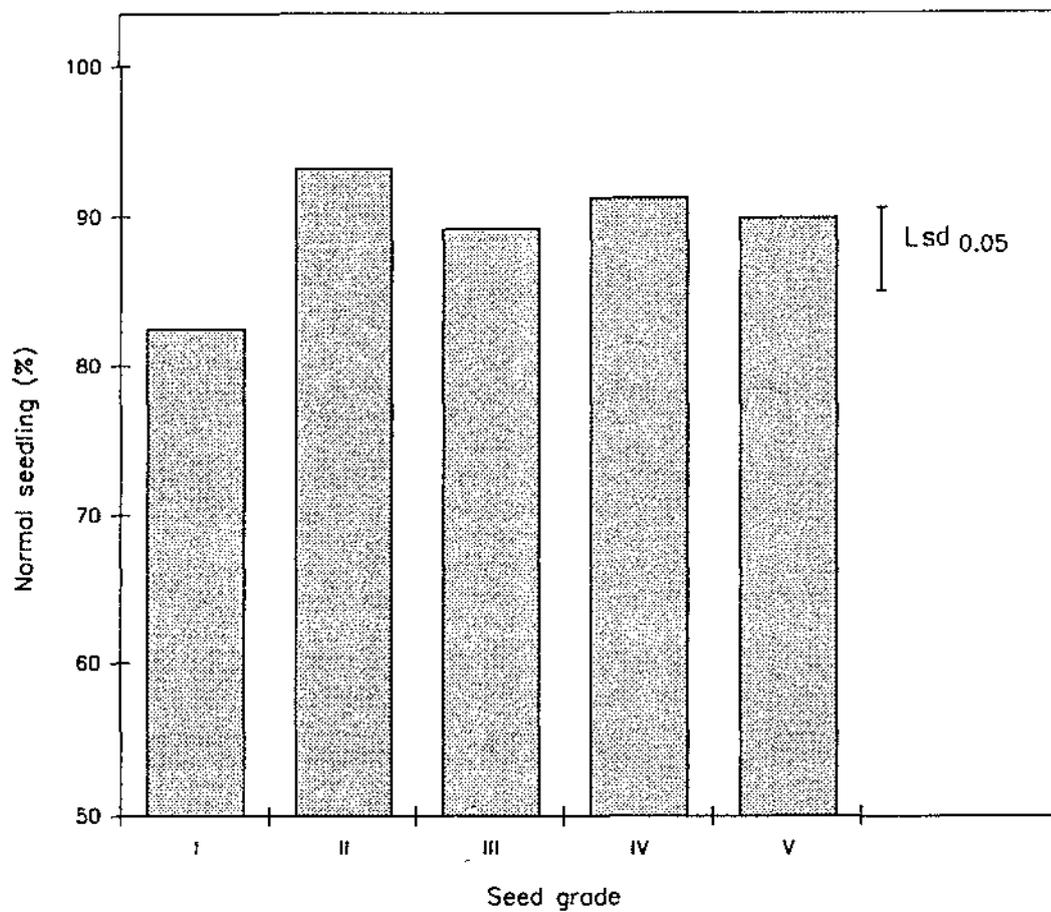


Figure 5.1.1B The effects of seed grade on mean final percentage normal seedling of *Pinus radiata* seeds. Data presented are values averaged over treated and untreated seeds; treatment effects were not significant (Appendix E.4)

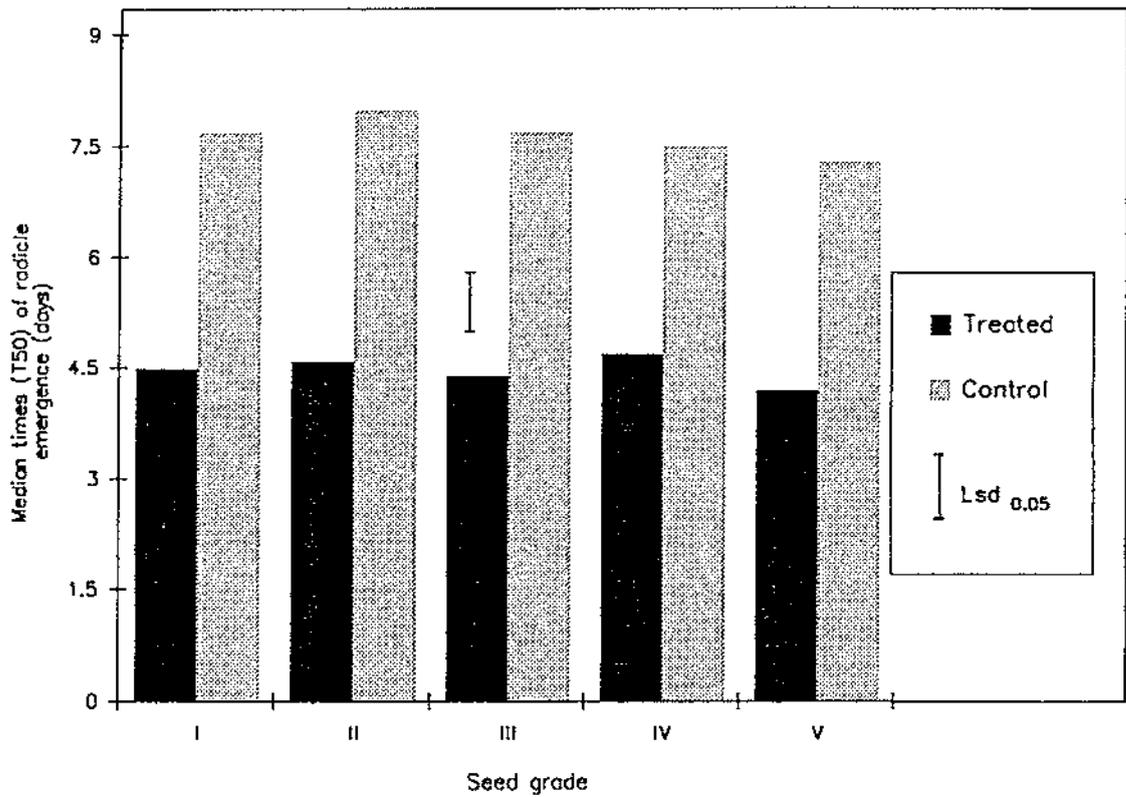


Figure 5.1.2 Changes in median times (T_{50}) of radicle emergence from different grades (I - V) of *Pinus radiata* seeds due to osmotic presowing treatment with a -1.0 MPa solution of mixed $\text{KNO}_3 + \text{KH}_2\text{PO}_4$ (1:1 w/w) for 10d at 20°C and dried back slowly to around 8% seed moisture content. Data presented are means of three replicates.

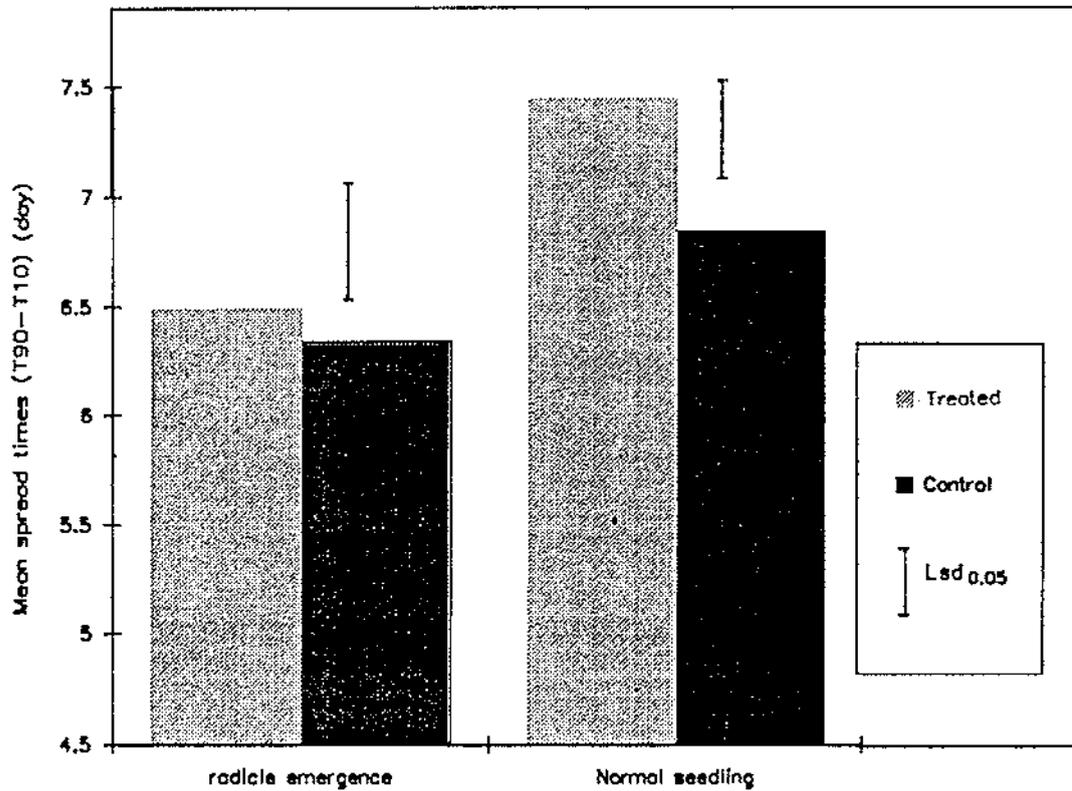


Figure 5.1.3 Changes in mean spread times ($T_{90} - T_{10}$) of radicle emergence and normal seedling production from different grades (I - V) of *Pinus radiata* seeds due to osmotic presowing treatment with -1.0 MPa solution of mixed $KNO_3 + KH_2PO_4$ for 10d at 20°C and dried back slowly to around 8% seed moisture content. Data presented are means over different grades of seed.

5.1.2 Glasshouse Experiment

5.1.2.1 Seedling emergence

The glasshouse experiment did not confirm any significant effect of either seed grade, osmotic treatment or these associated interactions on final numbers of emerged seedlings (Appendix Table A.8). Overall mean seedling emergence was 84%, a little lower than the 89% normal seedlings produced in the laboratory experiment.

5.1.2.2 Median times (T_{50}) and spread times ($T_{90}-T_{10}$) of seedling emergence

Median times of seedling emergence were significantly reduced by osmotic treatment equally in all grades (Appendix Table E.9) from around 22 d in untreated controls to around 17 d after treatment (Figure 5.1.4). The uniformity of emergence from the soil was unaffected by both seed grade and treatment. The spread of emergence being about 12 d (Appendix Table E.10).

5.1.2.3 Mean seedling dry weight

In the laboratory, seedling dry weight was not changed by osmotic treatment, but in the glasshouse experiment, very highly significant effects were found as a result of osmotic treatment, seed grade and interaction between osmotic treatment and seed grade on the dry weights of seedling grown for 40 d (Appendix Table E.11). As shown in Figure 5.1.5 osmotic treatment increased mean seedling dry weights but the increase was much more evident in larger or heavier seeds over the smaller or low vigour seeds. Apart from evidence that calculated best-fit line for the relationship between seed weight and seedling dry weight had highly positive coefficient correlation (r) for both osmotically treated and untreated controls (Figure 5.1.5), T-tests for s.e.s of constants of those regression lines were also highly significant (Appendix III). Homogeneity testing of the two slopes (Steel and Torrie, 1980) was also established in an attempt to answer whether those two independent lines could be

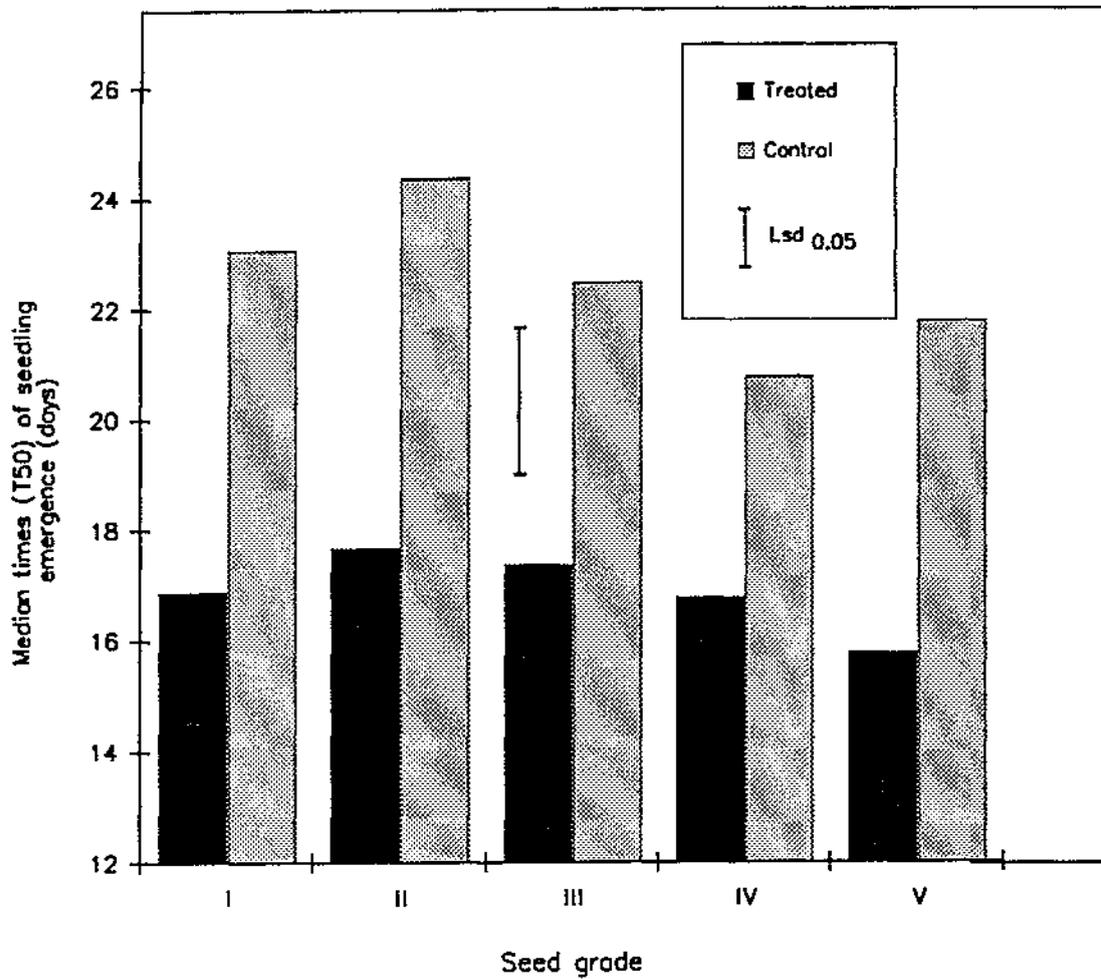


Figure 5.1.4 Changes in median times (T_{50}) of seedling emergence in the glasshouse from different grades of *Pinus radiata* seeds due to osmotic pre-sowing treatment with a -1.0 MPa solution of mixed $KNO_3 + KH_2PO_4$ for 10d at 20°C and dried back slowly to around 8% seed moisture content. Data presented are means of 3 replications

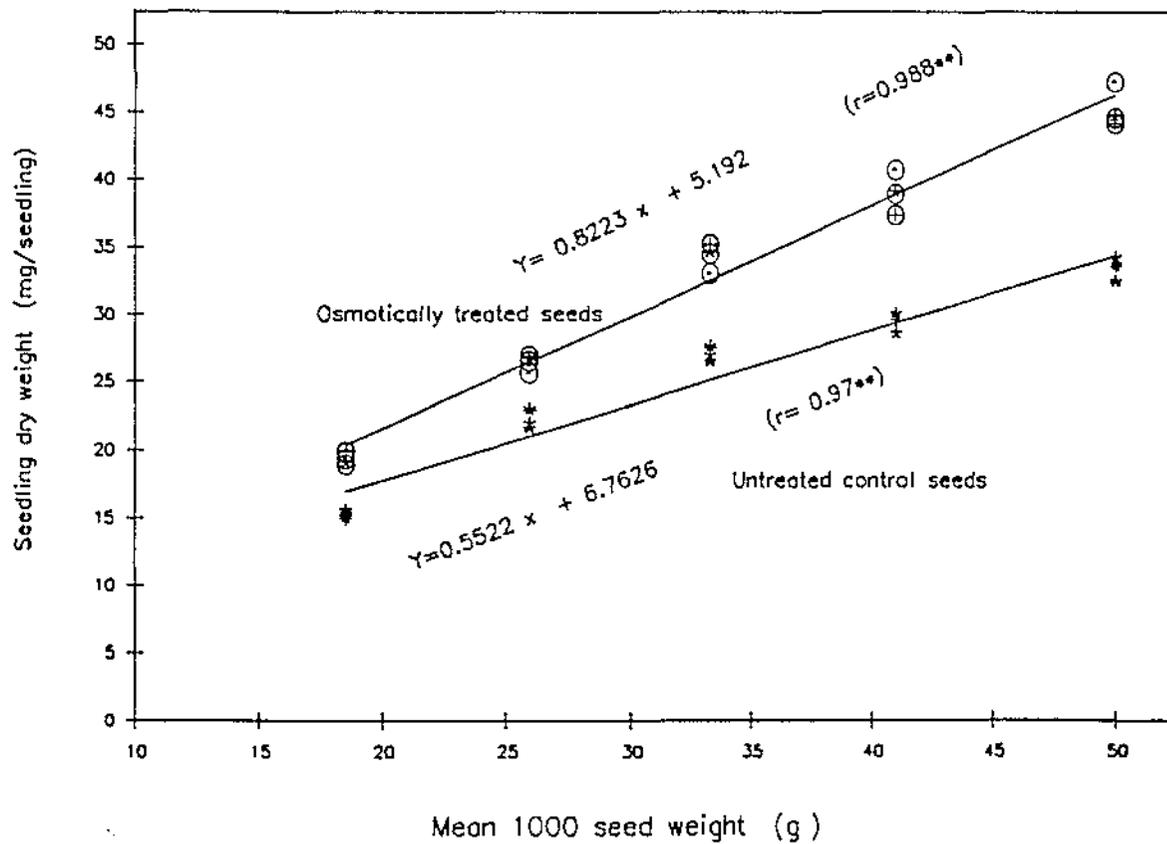


Figure 5.1.5

The relationship between mean dry weight of seedlings grown 45d in the glasshouse and mean 1000 seed weight of *Pinus radiata* seeds which were treated or untreated at -1.0 MPa of mixed $KNO_3 + KH_2PO_4$ solutions for 10d at 20°C and dried back slowly to around 8% seed moisture content.

considered to be one estimate of a common slope but the result indicated no reason to establish a common slope of two independent lines ($P < 0.001$).

5.2 Experiment 6. Effect of Salt Treatment Applied Before or After Ageing

In this study salt treatment at -1.0 MPa using mixed $\text{KNO}_3 + \text{KH}_2\text{PO}_4$ solutions for 10 d at 20°C was carried out either before or after a controlled deterioration ageing treatment at 45°C and a nominal 20% of seed moisture content for different periods of time. Seeds used in this experiment were of grade V.

5.2.1 Moisture Content After Ageing

The actual moisture content of grade V *Pinus radiata* seeds after different periods of artificial ageing treatment averaged around 19.1% with a range of $\pm 1.3\%$ (Appendix Table F.1).

5.2.2 Final Percentage Radicle Emergence and Normal Seedling Production

In untreated controls, artificial ageing treatment at 45°C and a nominal 20% moisture content, resulted in complete loss of viability of grade V *Pinus radiata* seeds after 4 d of ageing (Figure 5.2.1). Ageing for 1 or 2 d did not significantly reduce the final percentage radicle emergence of untreated control seeds but 2 d of ageing significantly reduced ($P < 0.05$) the final number of normal seedlings produced, a 10% reduction over radicle emergence (compare Figures 5.2.1 to 5.2.2). Following ageing for 3 d, both radicle emergence and normal seedling production reduced still further.

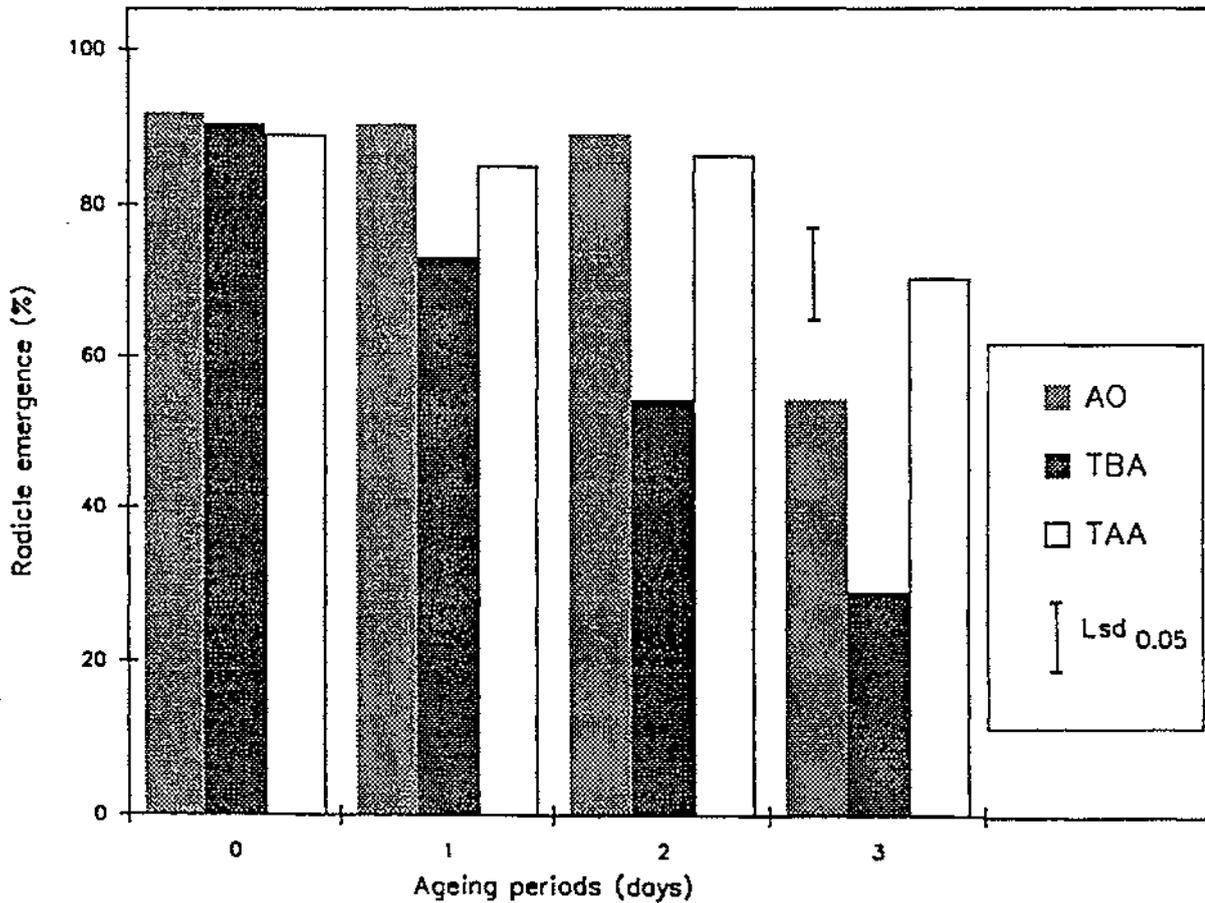


Figure 5.2.1 Percentage radicle emergence of grade V *Pinus radiata* seeds which were osmotically treated before ageing (TBA) or after ageing (TAA) compared to untreated aged controls (AO). Osmotic treatments were carried out at a -1.0 MPa solution of mixed $\text{KNO}_3 + \text{KH}_2\text{PO}_4$ for 10d at 20°C and dried back slowly to around 8% SMC whereas ageing treatments were at 45°C and 20% SMC. Data presented are means of 3 replicates, values for 4d onward were zero and are omitted.

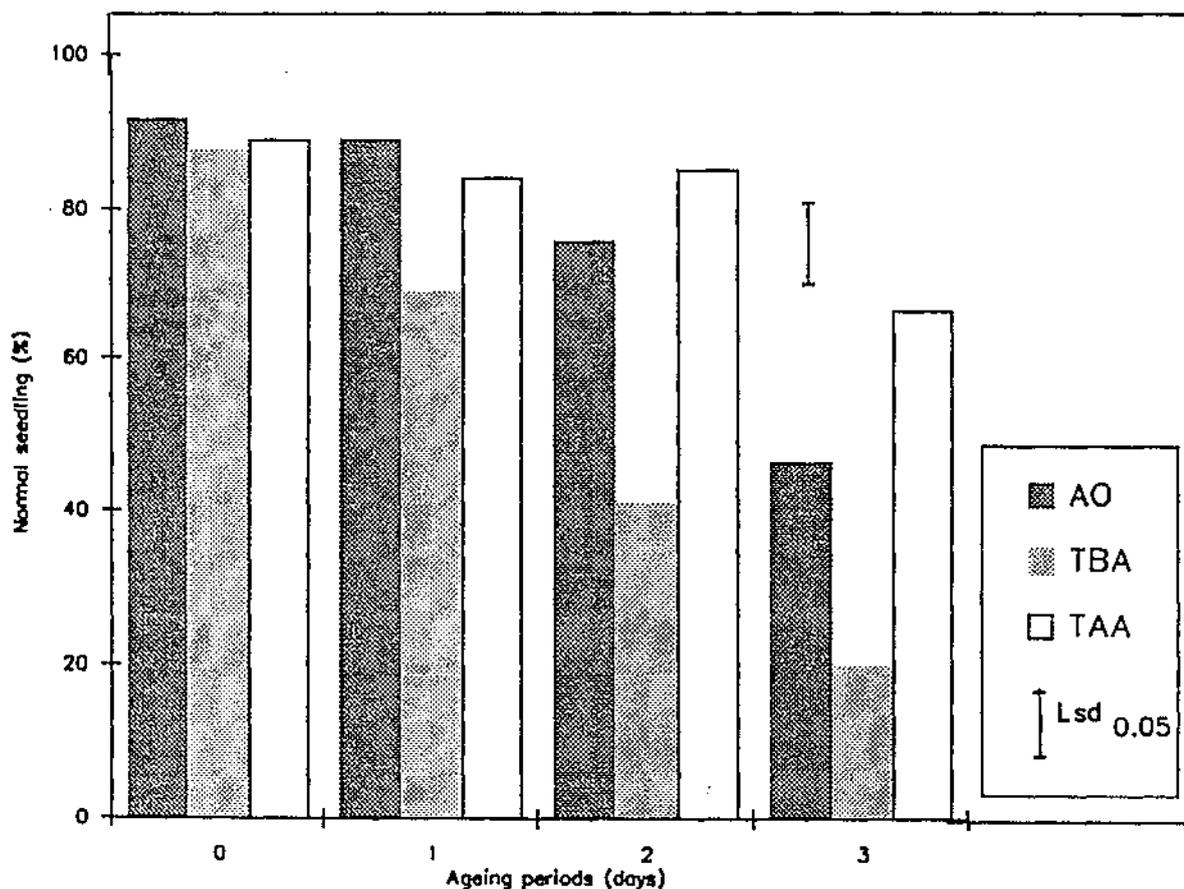


Figure 5.2.2

Percentage normal seedlings produced by grade V *Pinus radiata* seeds which were osmotically treated before ageing (TBA) or after ageing (TAA) compared to untreated aged controls (AO). Osmotic treatments were carried out using a -1.0 MPa solution of mixed $\text{KNO}_3 + \text{KH}_2\text{PO}_4$ for 10d at 20°C whereas ageing treatments were at 45°C and 20% seed moisture content and seeds were dried back slowly to around 8% moisture content after either osmotic or ageing treatments. Data presented are means of 3 replicates, values for 4d onward were zero and are omitted.

Osmotic treatment applied before ageing (TBA) had no benefit and actually increased the rate of deterioration causing a more rapid loss of germinability. In contrast, osmotic treatment applied after ageing (TAA) offered potential benefits since the numbers of either radicle emergence or normal seedling of 3 d aged seeds were improved significantly. However, this osmotic treatment failed to restore seeds which were previously subjected to artificial ageing treatment for 4 d or longer. It should be mentioned here that although seeds were dusted with the fungicide Thiram before ageing, seeds still became infected by fungi. When aged seeds were then osmotically treated (TAA, treatment after ageing), fungi grew rapidly, particularly those from longer durations of ageing treatment (Figure 5.2.3). Attempts to isolate fungi on agar media identified only two types of storage fungi present on infected seeds, mainly *Aspergillus glaucus* with very small levels of *Aspergillus flavus*.

5.2.3 Median Times (T₅₀) of Germination

Significant effects of osmotic treatment, duration of ageing and interaction between osmotic treatment and the duration of ageing on median times of radicle emergence and normal seedling production are noted in Appendix Table F.3 and F.6. As illustrated in Figure 5.2.4, increases in median times of radicle emergence occurred progressively with the increase of ageing duration in untreated controls where as early as 2 d of ageing significant differences had been observed ($P < 0.05$). Meanwhile, osmotic treatment reduced median radicle emergence times by 35% when seeds were not aged. When osmotically treated seeds were then subjected to ageing treatment (TBA, treatment before ageing), increases in T₅₀ happened as early as 1 d of ageing and this increase continued progressively with longer duration of ageing. Only osmotic treatment applied after ageing (TAA) preserved low median radicle emergence times (Figure 5.2.4).



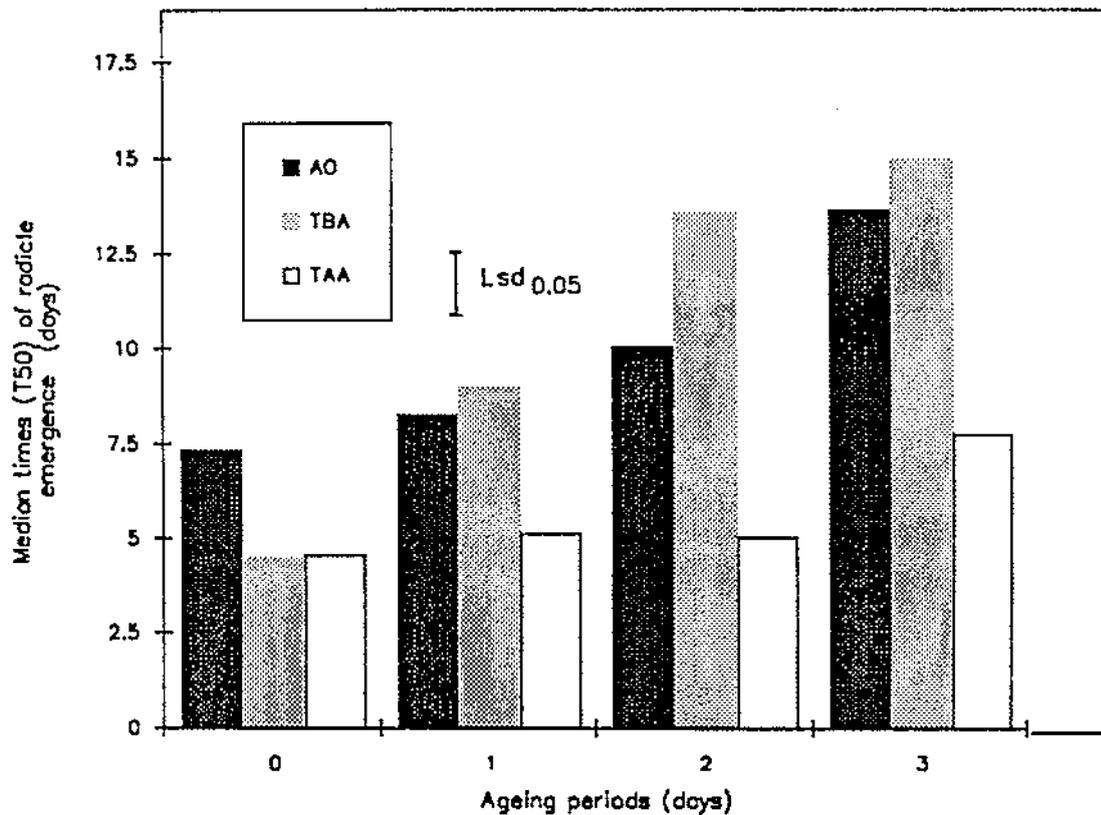


Figure 5.2.4. Median times (T_{50}) of radicle emergence of grade V *Pinus radiata* seeds which were osmotically treated before ageing (TBA) or after ageing (TAA) compared to untreated aged controls (AO). Osmotic treatments were carried out at -1.0 MPa of mixed $\text{KNO}_3 + \text{KH}_2\text{PO}_4$ solutions for 10d at 20°C , ageing was at 45°C and 20% seed moisture content and seeds were dried back slowly either after osmotic or ageing treatments. Data presented are means of 3 replicates

There were significant linear correlations between either final percentage radicle emergence or final normal seedling production and their median times of germination (Figures 5.2.5A and 5.2.5B).

5.2.4 Uniformity (T_{90} - T_{10}) of Germination

There were significant but independent effects of osmotic treatment and duration of ageing on the mean spread of time of germination (Appendix Tables F.4 and F.7). As illustrated in Figure 5.2.6 and 5.2.7, small increases in mean spread of radicle emergence times were mainly caused by ageing for > 2 d as well as by osmotic treatment applied before ageing (TBA). Increases in mean spread of normal seedling production times were similarly observed as a result of either ageing duration or osmotic treatment applied before ageing (TBA), having an increase around 1.3 d when seeds were aged for 2 or 3 d or increases around 0.7 d when they were osmotically treated before ageing (TBA) (data not shown).

5.2.5 Mean Seedling Dry Weight

It was decided to complete this experiment 12 d after median normal seedling production in each set of replications. Only ageing duration had significant effects on mean dry weight of seedlings grown in the laboratory (Appendix Table F.8). Figure 5.2.8 illustrates a gradual decrease in mean dry weight of seedlings with increased ageing duration. Plotting the data of mean seedling dry weight against final percentage normal seedling produced of aged seeds resulted in a straight line relationship ($r = 0.905$, $P < 0.01$; Figure 5.2.9A). Similarly, as ageing increased median germination times (t_{50}), there was good relationship between median times of radicle emergence and mean dry weights of seedlings (Figure 5.2.9B).

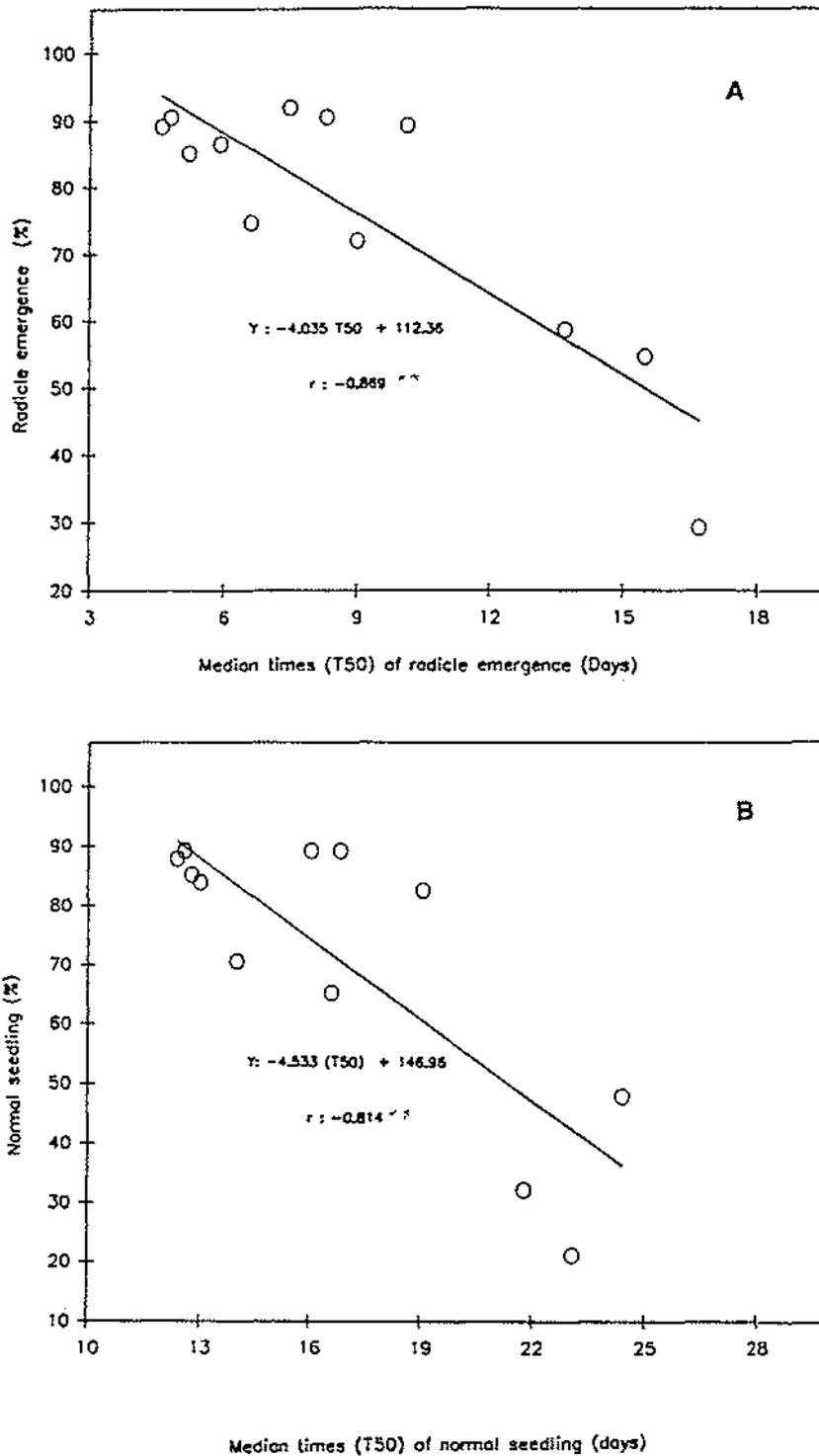


Figure 5.2.5

Relationships between the percentage of radicle emergence (A) and normal seedling production (B) and their median times (T50) of germination of grade V *Pinus radiata* seeds.

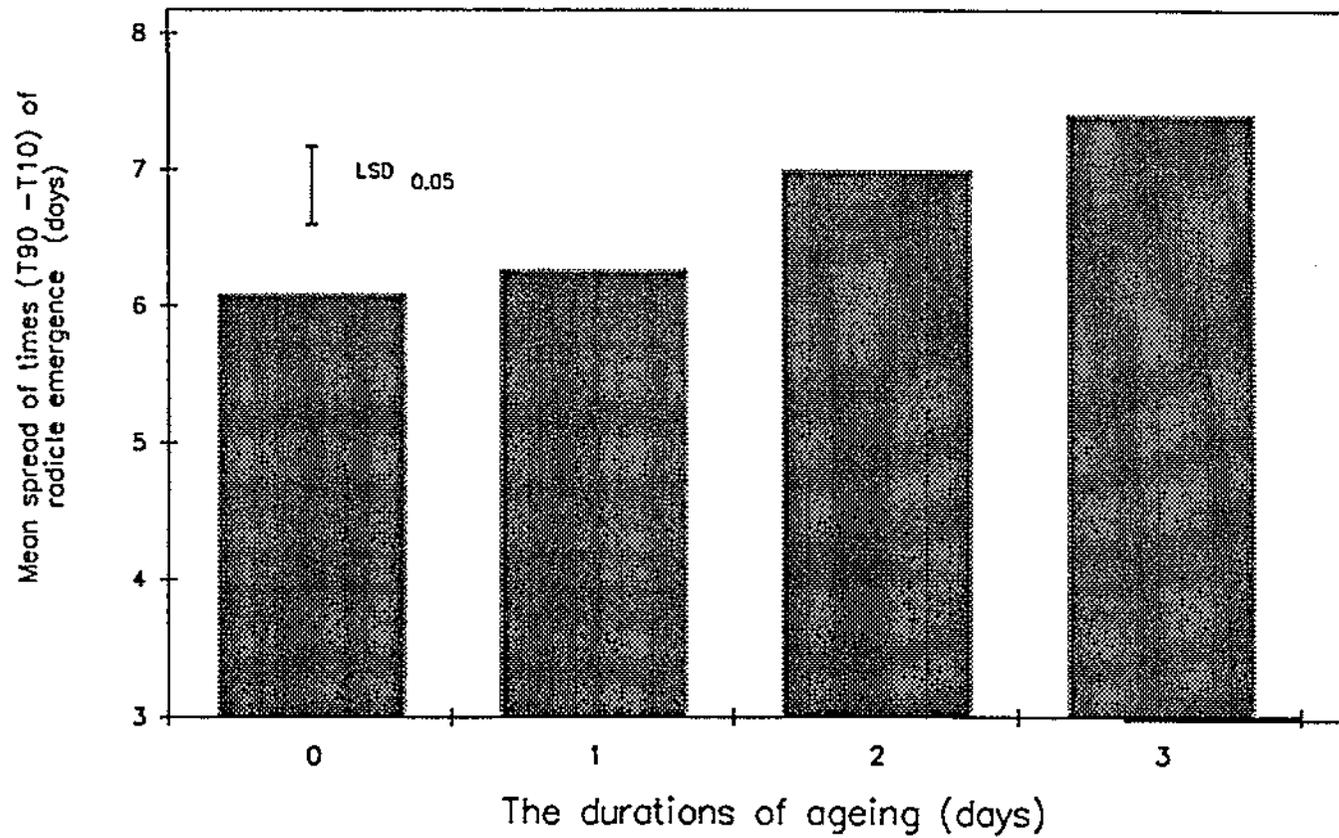


Figure 5.2.6. Mean spread times (T₉₀ - T₁₀) of radicle emergence of grade V *Pinus radiata* seeds which were aged at 45°C and 20% seed moisture content for different periods of time. Data presented are average values of osmotically treated and untreated control seeds.

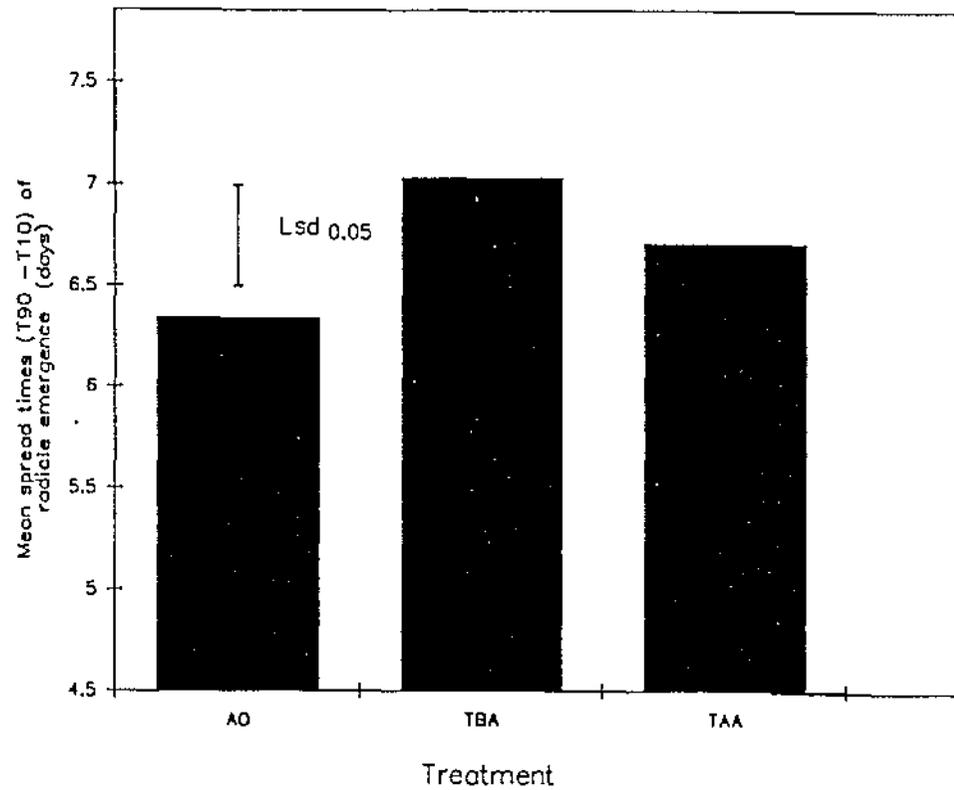


Figure 5.2.7. Mean spread times ($T_{90} - T_{10}$) of radicle emergence of grade V *Pinus radiata* seeds which were osmotically treated before ageing (TBA) or after ageing (TAA), compared to untreated aged controls (AO). Data presented are averaged over ageing times. Osmotic treatments were carried out at -1.0 MPa of mixed $KNO_3 + KH_2PO_4$ solutions for 10d at 20°C. Data presented are averaged over ageing times.

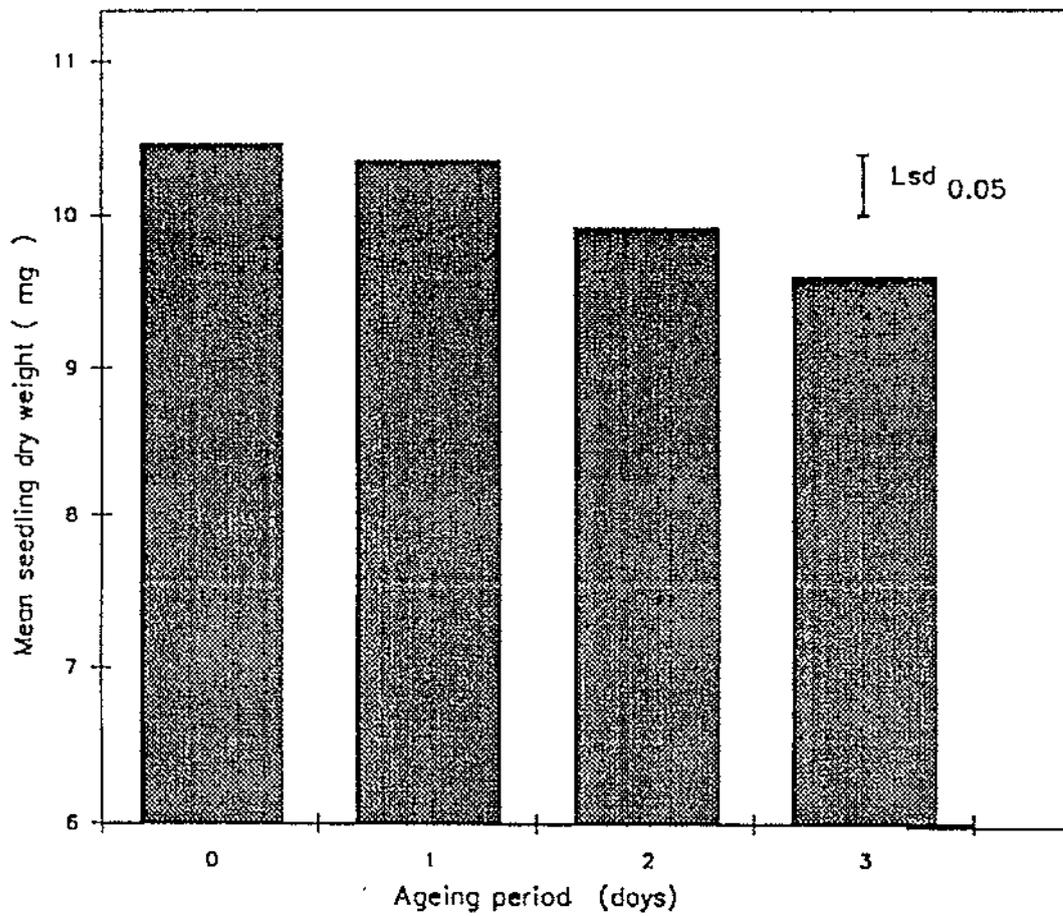


Figure 5.2.8 Mean dry weight of seedlings from grade V *Pinus radiata* seeds grown for up to 12d after T_{50} of normal seedling at 20°C in the laboratory. Seeds were aged at 45°C and 20% seed moisture content for different periods of time. Data presented are average values of osmotically treated and untreated seeds.

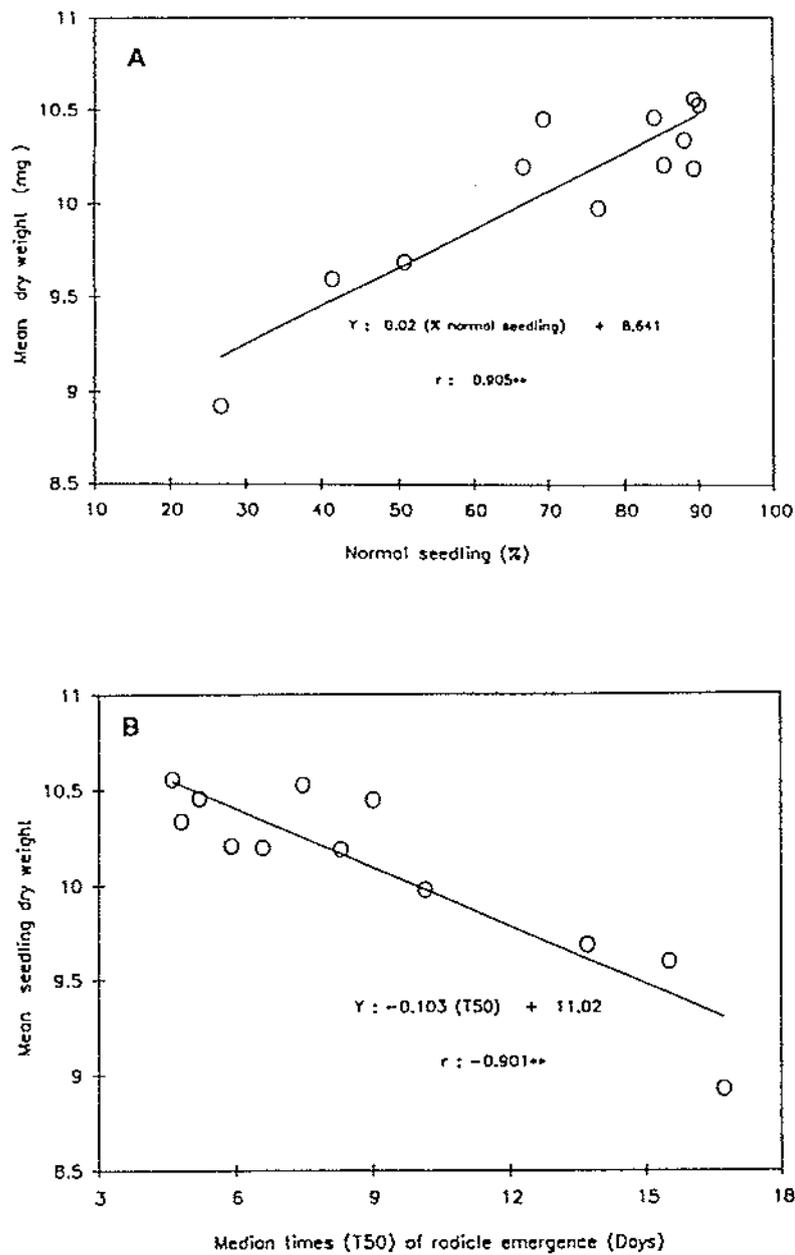


Figure 5.2.9 Relationships between either percentage normal seedlings (A) or median times (T₅₀) of radicle emergence (B) of grade V *Pinus radiata* seeds and their mean dry weight of seedlings grown at 20°C in the laboratory. Seedlings were harvested at 12d after T₅₀ of normal seedlings.

5.3 Discussion

5.3.1 Osmotic Treatment in Different Grades of Seed

In tree species, variation of individual seed size or weight has often been reported (Section 2.2.3). In *Pinus radiata*, seed size or weight variation occurs at several levels: amongst different production sites or provinces, between trees within one production site area and also amongst seeds within a single tree (Griffin, 1975). Variation in seed size of a species may have ecological significance in a number of ways, for instance, large seeds may have higher germination percentages, or higher rates of germination or seedling growth. In the present study seed grade had a significant effect on percentage germination where grade I seeds produced a somewhat lower percentage of radicle emergence than any other grades ($P < 0.05$, Figure 5.1.1A). This result seems an exception to other findings, usually showing a distinct advantage of large seeds over small seeds on this type of response as happened in cabbage (Hanumaiah and Andrew, 1973), *Pinus radiata* (Griffin, 1975), *P. elliottii* (Belcher *et al.*, 1984), onion and carrot (Bedford and Mackay, 1973), radish (Kubka *et al.*, 1974), *Mirabilis hirsuta* (Weis, 1982) and soybean (Hoy and Gamble, 1985), and in cowpea (Singh and Rai, 1988), or all different seed grades equally germinated well as shown by Shoulder (1961) in *Pinus elliottii*, Struve *et al.* (1989 in *P. taeda*, Liou (1987) in cabbage and by Zhang and Maun (1990) in *Agropyron* sp. Meanwhile osmotic treatment generally had no effect on final percentage emergence in the laboratory with one exception being that the treatment increased percentage radicle emergence of grade II seeds (Figure 5.1.1A). This is probably an anomalous result in that there were no effect of osmotic treatment on final normal seedling production or percentage soil emergence in any grades (Appendix Tables E.4 and E.7).

Large seeds reportedly have more energy as reflected by large food reserves (Belcher *et al.*, 1984; Kandya and Ogino, 1986), therefore, large seeds are expected to germinate first and get a faster start than from smaller seeds. In the present experiment there was no difference in the germination rate amongst different grades of seed (Figure 5.1.2). Although this finding was in agreement with results reported in *P. elliotii*, *P. ponderosa* and *P. taeda* (Larson, 1963; Shoulder, 1961; Struve *et al.*, 1989), it was in contrast to other pine species (Cannel *et al.*, 1978; Belcher *et al.*, 1984; Dunlap and Barnet, 1982; Kandya and Ogino, 1986), including *P. radiata* (Griffin, 1975) where large seeds were found to have higher germination rates. As high germination rates will eventually followed by heavier or superior seedling size (Kandya and Ogino, 1986; Logan and Pollard, 1979; Mexal and Fisher, 1987), germination rate thus represents one dimension of vigour. In the present study untreated control seeds from different grades had similar germination rates (Figures 5.1.2 and 5.1.4) but seedling dry weight produced by large or heavy seeds was much higher against those produced by small seeds (Figure 5.1.5). Heavier seedlings from large seeds are most likely a function of a large embryo, more abundant reserve food materials and probably larger dimensions of the cotyledons where such conditions would favour a more intensive process of photosynthesis and better growth (Burriss *et al.*, 1971; Carleton and Cooper, 1972; Kandya and Ogino, 1986; Righter, 1945). Thus separating seeds into different grades in commercial nursery operations can be justified since it would increase seedling uniformity and minimise cull numbers (unacceptable seedlings due to undersized or any other defects). Osmotic treatment increased germination rate in all different grades by reduced T_{50} of seedling emergence around 3.5 to 5.9 d (Figure 5.1.4). Early emergence as a consequence of osmotic treatment was followed by increases in seedling dry weight but the increases were much higher in large seeds than that in small seeds as illustrated in Figure 5.1.5 where the effects of seed grade on seedling dry weight of osmotically treated and untreated control were very different (see section 5.1.2.3 for explanation). Acceleration of germination or emergence and improved seedling growth resulted from osmotic treatment reinforces

previous claims on invigoration effect of pre-sowing treatments which had been reported in several species. In celery, Brocklehurst and Dearman (1983b) and Brocklehurst *et al.* (1987) claimed increases in seedling weight by 182% when seeds were osmotically treated with PEG. Increases in plant weight due to PEG treatment were also noticed in carrot, onion and leek (Brocklehurst and Dearman, 1983b; Brocklehurst *et al.*, 1987), parsley (Elly and Heydecker, 1981; Pill, 1986), cabbage (Liou, 1987), while salt treatments increased plant dry weight in tomato (Alvarado *et al.*, 1987; Barlow and Haigh, 1987). In this present experiment, however, the advantages of treatment may have little value for small seeds (grades IV and V) since increases in seedling dry weight of these two smallest seeds could not compete with seedlings from untreated control of grades I and II (Figure 5.1.5). If these differences persist during seedling development for 12-18 months, variability of seedling size will inevitably occur in the nursery. Thus grading of seeds should be continued for sowing purposes. Small seeds perhaps need to be sown far earlier than do larger seeds in order to catch-up to the size of seedlings produced by large seeds from late sowing. Apart from this, the invigoration effects of osmotic treatment which might cause interesting increases in seedling growth need also to be tested operationally in the nursery.

5.3.2 Effect of Osmotic Treatment and Ageing

In the present study artificial controlled deterioration treatment at 45°C and a nominal 20% SMC severely reduced viability and germinability, 4 d of ageing resulting in total loss of viability. This finding was in contrast to Kartiko's (1990) experiment where quite similar conditions of ageing for either 2 d or 4 d did not reduce viability. Individual clones were used in Kartiko's (1990) experiment, while the present study used seeds gained from mixed genotypes and differences in genotype might be responsible for the poorer performance of *Pinus radiata* seeds used here.

In untreated seeds controlled deterioration at 45°C and 20% SMC started to reduce viability and germinability for 2 d onward, while median times for either radicle emergence or normal seedling germination changed after just 1 d of ageing (Figure 5.2.4). This result seems to be in agreement with the general concept of losses in vigour preceding losses of viability during storage (AOSA, 1983; Perry, 1981).

While osmotic treatment increased seed vigour in unaged seeds measured by decreases in T_{50} , a drastic increase in deterioration occurred when pre-treated seeds were then subjected to ageing, suggesting that osmotically treated seeds would have poor performance when they are stored under unfavourable storage regimes. The same results have also been reported in black spruce (Fleming and Lister, 1985) and in many agricultural seeds (e.g. Dearman *et al.*, 1987; Alvarado and Bradford, 1988; Argerich and Bradford, 1989; Argerich *et al.*, 1989; Nath *et al.*, 1991), where osmotic treatment applied before ageing had no benefit at all. These findings thus dispute the generally accepted view that vigorous seeds will survive better than less vigorous seeds under any storage conditions (Matthews, 1980; Powell and Matthews, 1981) or with reports that osmotic treatment extended seed longevity and maintained seed quality under adverse storage conditions (e.g. Priestly, 1986; Dearman, 1986). In *Pinus radiata*, osmotic treatment increases that part of seed vigour measured by reduction in median times of either radicle emergence or normal seedling production, but simultaneously reduces the resistance of seed to storage, and this effect becomes very evident when storage regimes are very unfavourable. The work of Nath *et al.* (1991) and Nath (1991) suggests that longer hydration treatment increased levels of solutes and/or damage to membranes when treated seeds were subjected to unfavourable storage regimes and increased solute or membrane damage may be mediated by hydrolytic activity which is considered to be involved in seed deterioration (Perl *et al.*, 1978; Francis and Coolbear, 1988). This supports results obtained in wheat (Hanson, 1973) and oat (Berrie and Drenan, 1971) where α -amylase and protease activity increased following osmotic or hydration

treatments. These enzymes are responsible for reserve mobilisation and/or synthetic processes which are essential for rapid germination of treated seeds, however, they are highly undesirable in stored seeds.

Osmotic treatment applied following ageing improved final radicle emergence despite reduced median times of radicle emergence of 3 d aged seeds (Figures 5.2.2 and 5.2.3). Although improved percentage radicle emergence due to osmotic or other pre-sowing treatment has also been demonstrated in 14-year old Scots pine seeds (Simak, 1976b) or in some agricultural seeds (Dell Aquilla *et al.*, 1984; Rao *et al.*, 1987), in the majority of cases improved germination percentage was not found (e.g. Brocklehurst and Dearman, 1983a; Heydecker *et al.*, 1975; Francis and Coolbear, 1984, 1988; Coolbear *et al.*, 1984). Improved normal germination percentage of aged seeds after treatment has been investigated in some detail in lettuce where Rao *et al.* (1987) found that post-storage hydration treatments including osmotic treatment reduced the frequency of chromosomal aberrations, increased the rate of root growth and decreased the frequency of morphologically abnormal seedlings. The same recovery from damage caused by chemical mutagens has been investigated in barley where storing seeds at 30% SMC following mutagen treatments resulted in recovery as indicated by increased seedling height, decreased the frequency of chromosomal aberrations in radicle tips and by a decrease in chlorophyll-deficiency mutations from the next generation.

Since there was no interaction between osmotic treatment and ageing period but highly significant effect of ageing period on mean seedling dry weight, it was likely that osmotic treatment applied after ageing (TAA) could not completely restore the damaging effect of artificial ageing. As illustrated in Figures 5.2.1 and 5.2.4, osmotic treatment applied to 3 d of aged seeds restored germinability and T_{50} of radicle emergence comparable to untreated and unaged controls, but their seedling dry weight was lower than that of unaged

controls (Figure 5.2.8). This probably indicates that factors controlling seedling growth are partly or entirely distinct from those controlling radicle emergence; this means that if impaired systems related to radicle emergence could be restored by the treatment, it does not automatically mean that other systems operative for seedling growth will be restored as well.

VI. PHYSIOLOGICAL EFFECTS OF SEED TREATMENT

Biochemical approaches very often are the only ways to give a closer explanation of early emergence or vigour differences amongst seeds since radicle emergence and seedling growth during germination are the end results of a series of biochemical changes. Since these approaches measure metabolic event linked to germination processes, therefore they might be used to evaluate vigour improvements gained from osmotic treatment. However, up until now little is known about the physiological mechanisms underlying vigour improvement from osmotic treatment. Although it has been suggested that part of the improvement in germination performance resulting from osmotic treatment may arise from repair of deterioration sustained previously by the seed during maturation or storage (Burgass and Powell, 1984; Dearman *et al.*, 1986), firm evidence concerning these suggestions has not been established completely. Respiration tests done by Sundstrom and Edward (1989) in osmotically-treated pepper seeds seemed doubtful since respiration usually rises as the water content of seed increases (Mayer and Poljakoff-Mayber, 1982). Sundstrom and Edward (1989) found that respiration reflected as CO₂ evolution was relatively high within 1 h of imbibition and rapidly dropped following 2-3 h, while it levelled off after 5 h imbibition. Thus high CO₂ evolution detected by Sundstrom and Edward (1989) during first hour of imbibition osmotically treated and untreated control seeds may be a purely physical process involving the liberation of gas colloiddally absorbed within the seed, not peculiar to metabolic status of seed (Harber and Brassington, 1959). This chapter attempts to investigate: (i) changes in respiration, and (ii) changes in dehydrogenase activity as a result of osmotic treatment.

6.1 Experiment 7. Respiration of Osmotically Treated *P. radiata* Seeds

6.1.1 Oxygen Uptake

Respiration measurements were done with seeds imbibed for different periods of time to find any possible contribution of respiration for germination enhancement gained from osmotic treatment. Seeds of grade V were used in this experiment, while osmotic treatment and drying after treatment were carried out as in Experiment 5, followed by imbibing either intact or decoated seeds before respiration measurements.

Gas exchange measurements showed only a small increase of oxygen uptake in intact untreated controls between 24 h to 120 h of imbibition, having around 1.5 $\mu\text{l}/\text{seed}/\text{h}$ of oxygen uptake during 24-48 h of imbibition and then around 2.2 $\mu\text{l}/\text{seed}/\text{h}$ at 120 h after imbibition. In osmotically treated seeds with intact coats, increases in oxygen uptake started to occur just after 48 h of imbibition, a period coinciding with the onset of radicle emergence (Figure 6.1.1A). Seedcoat removal allowed increased respiration rates of seeds but decoated treated seeds and decoated controls had similar oxygen uptakes during 24-48 h of imbibition at around 2.2 $\mu\text{l}/\text{seed}/\text{h}$; and further increases in oxygen uptake for decoated treated seeds started to occur after 48 h of imbibition whereas for decoated untreated controls the increase was after 72 h of imbibition (Figure 6.1.1B). Seedcoat removal advanced emergence of untreated controls by one day, however, in osmotically treated seeds seedcoat removal did not advance radicle emergence (Table 6.1.1).

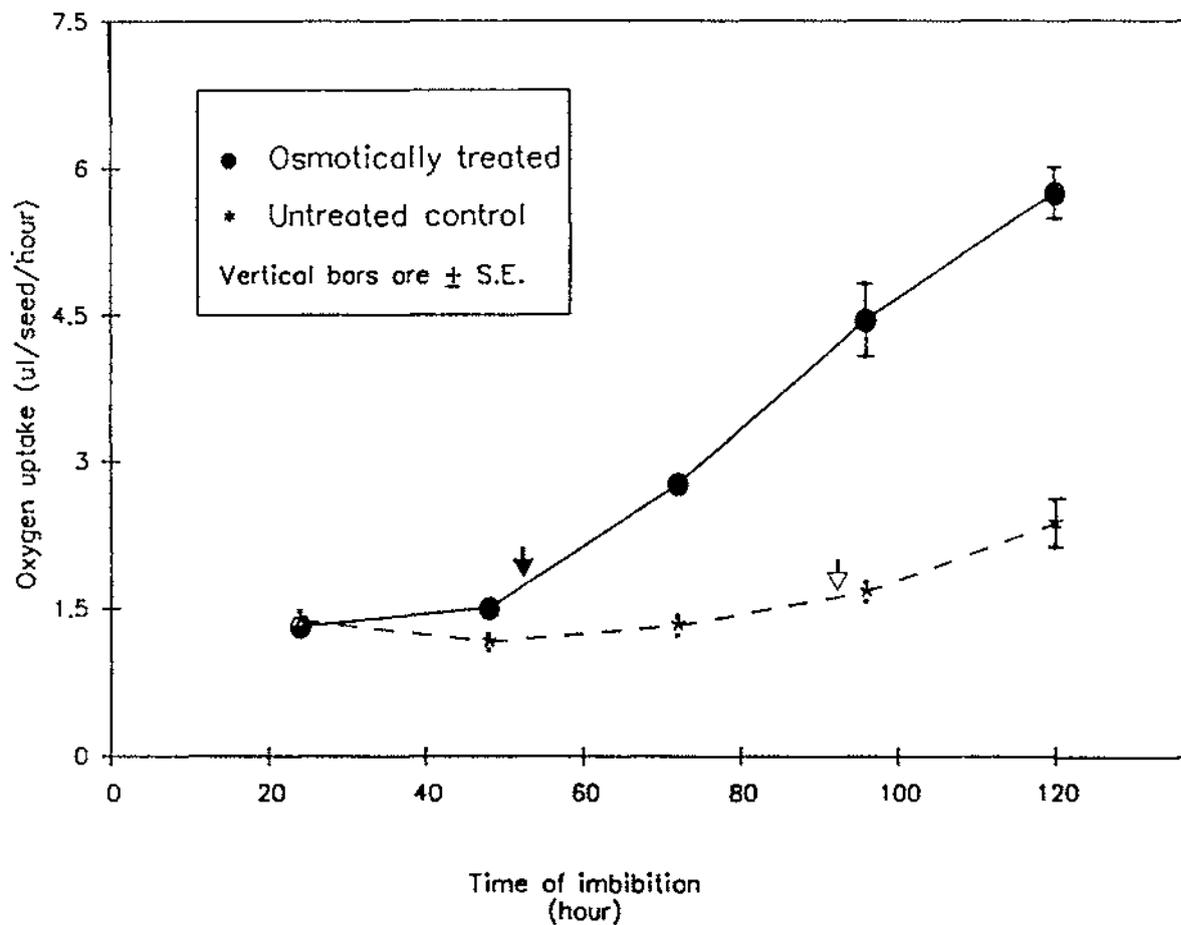


Figure 6.1.1A Osmotic treatment-induced changes in oxygen uptake during early germination of grade V intact *Pinus radiata* seeds. Osmotic treatment was carried out at -1.0 MPa of mixed $\text{KNO}_3 + \text{KH}_2\text{PO}_4$ solutions for 10d at 20°C . Seeds were then dried back to around 8% seed moisture content. Data presented are means of 6 replicates, vertical bars are \pm s.e. of individual means; \blacktriangledown and \triangledown indicate the time of the onset of radicle emergence.

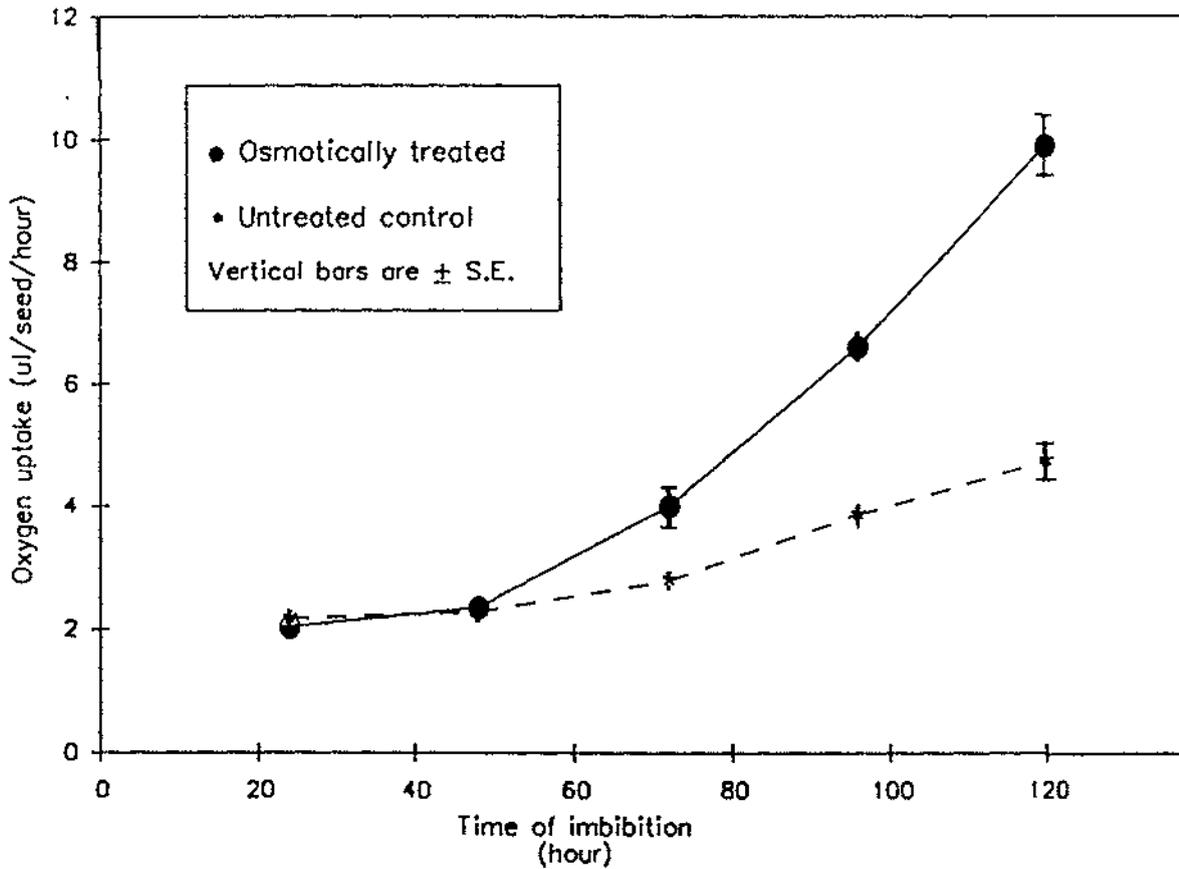


Figure 6.1.1B Osmotic treatment - induced changes in oxygen uptake during early germination of grade V decoated *Pinus radiata* seeds. Osmotic treatment was carried out at -1.0 MPa using mixed $\text{KNO}_3 + \text{KH}_2\text{PO}_4$ solutions for 10d at 20°C . Data presented are means of 6 replicates, vertical bars are \pm s.e. of individual means, \blacktriangledown and \triangledown indicate the time of the onset of radicle emergence of decoated seeds.

Table 6.1.1 Cumulative percentage radicle emergence of osmotically treated and untreated control grade V *Pinus radiata* seeds at different periods of imbibition.

Treatment		Period of imbibition (hours)							
		24	49	66	72	83	98	123	342
Decoated seeds	osmotically treated	0	2.66 (±1.33)	8.0 (±2.31)	18.16 (±4.81)	32.0 (±4.6)	45.33 (±3.53)	61.33 (±3.52)	89.33 (±1.33)
	untreated control	0	0	0	1.33 (±1.33)	2.66 (±1.33)	5.33 (±1.33)	21.33 (±3.53)	89.33 (±1.33)
Intact seeds	osmotically treated	0	1.33 (±1.33)	4.0 (±2.31)	9.33 (±2.66)	21.33 (±4.8)	36.0 (±1.33)	57.33 (±1.33)	90.66 (±3.52)
	untreated control	0	0	0	0	0	1.33 (±4.0)	6.66 (±2.66)	90.66 (±2.66)

Data presented are means of 3 replicates, numbers in brackets are individual standard errors (±s.e.)
Experiment carried out separately from the respiration tests.

6.1.2 Respiratory Quotients (RO)

As illustrated in Appendix Table G.1 and Table 6.1.2, the respiratory quotient was affected by the interaction between imbibition time, seedcoat removal and osmotic treatment. In untreated control, both intact and decoated seeds, early imbibition (24 h) had respiratory quotients around unity but increased imbibition time resulted in a decline in those values ranging from 0.62 to 0.80. In contrast, for osmotically treated seeds, respiratory quotients throughout the imbibition period were relatively stable around 0.65.

Table 6.1.2 Respiratory quotients of osmotically treated and untreated control grade V *Pinus radiata* seeds during early imbibition.

		Imbibition time (hours)				
		24	48	72	96	120
Decoated seeds	Treated	0.61	0.71	0.65	0.74	0.58
	Control	0.92	0.78	0.71	0.63	0.79
Intact seeds	Treated	0.72	0.68	0.55	0.62	0.68
	Control	0.95	0.80	0.70	0.62	0.66

Osmotic treatments were at -1.0 MPa solution of mixed $\text{KNO}_3 + \text{KH}_2\text{PO}_4$ for 10 d at 20°C. Data presented are means of 6 replicates, $\text{LSD}_{0.05} = 0.147$.

6.2 Experiment 8. Changes in Dehydrogenase Activity of Osmotically Treated Seeds

Formazan production in tetrazolium testing is essentially a measurement of total dehydrogenase enzyme activity and this type of testing has been used to estimate percentage germination, to detect embryo soundness, to diagnose the causes of embryo disturbance and to differentiate seedling vigour (e.g. Johnston *et al.*, 1986; Moore, 1973; Sung and Chen, 1988). Accordingly, a quantitative assay of formazan production was conducted in this experiment to look at changes in total dehydrogenase activity of fully imbibed seeds in an attempt to evaluate their contribution to the ensuing better performance of treated seeds. Compared to individual enzyme extractions and assays, the measurement of total dehydrogenase via TZ test is relatively quick and simple.

Results shown in Figure 6.2.1 clearly indicated that the rate of formazan production and the capacity of production were higher in osmotically treated seeds than that from untreated controls. The calculated rate of initial formazan production (Figure 6.2.2) differed considerably between treated and untreated seeds.

6.3 Discussion

6.3.1 Respiration

Results shown in Figure 6.1.1A and 6.1.1B indicated no difference in oxygen uptake during early imbibition (24-48 h) of osmotically treated and untreated controls. Early increases in oxygen uptake of osmotically treated seeds were associated with the event of radicle emergence. In his study on *Pinus radiata* seed development, Rimbawanto (1987)

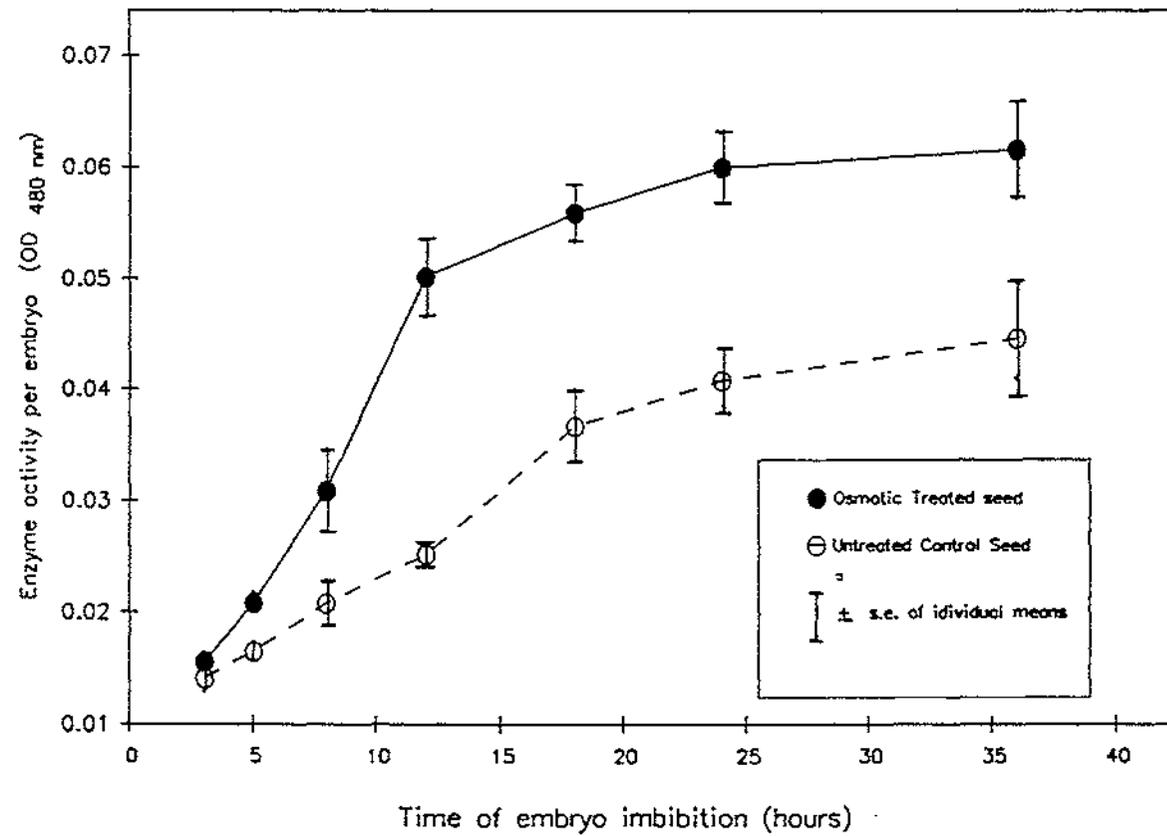


Figure 6.2.1

Dehydrogenase activity from imbedded embryos of osmotically treated and untreated control grade III *Pinus radiata* seeds which were imbibed in 2,3,5 Triphenyl Tetrazolium Chloride (TTC) for different periods of time. Osmotic treatment was carried out at -1.0 MPa of mixed $\text{KNO}_3 + \text{KH}_2\text{PO}_4$ solutions for 10d at 20°C . Data presented are means of 4 replicates, vertical bars are \pm s.e. of individual means.

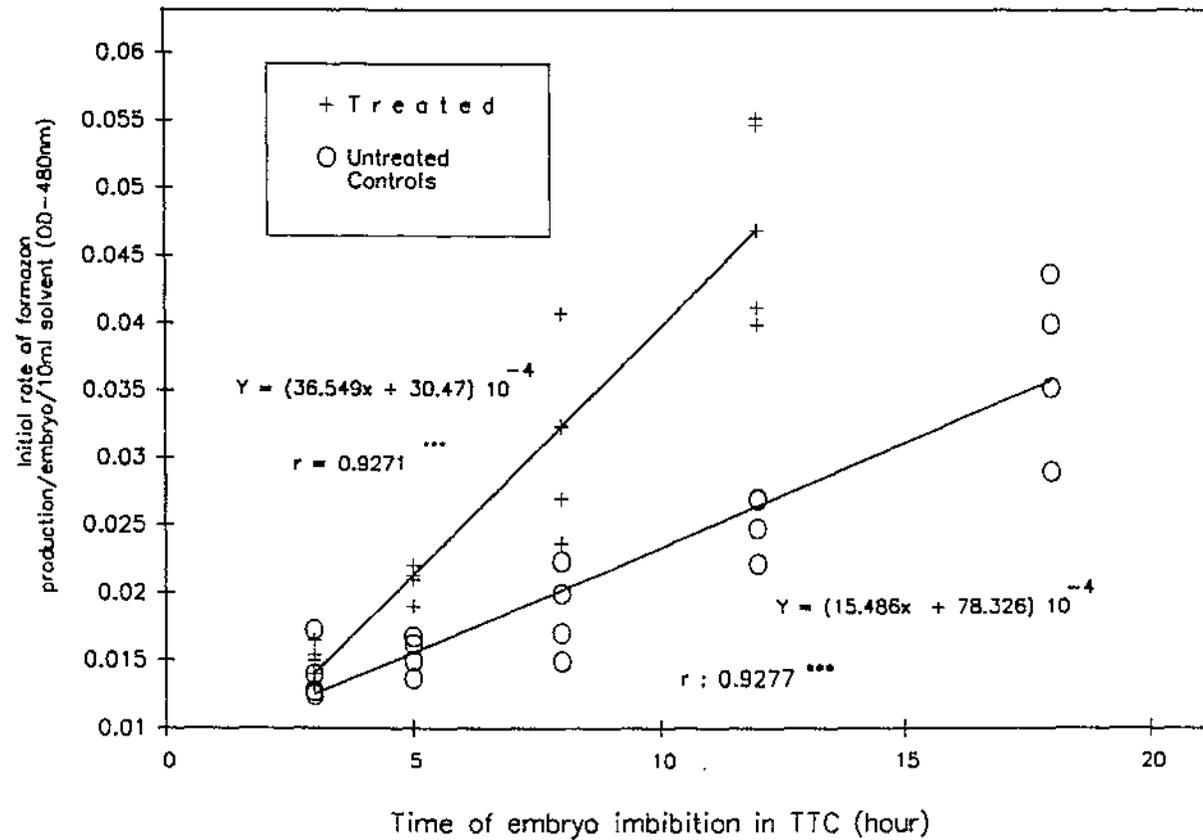


Figure 6.2.2 The initial rate of formazan production from embryos of osmotically treated and untreated control grade III *Pinus radiata* seeds which were imbibed in 2,3,5 Triphenyl Tetrazolium Chloride (TTC) for different periods of time. Osmotic treatment was carried out at -1.0 MPa using mixed $KNO_3 + KH_2PO_4$ solutions for 10d at 20°C.

argued that seed coats in more mature seeds seem to be less permeable to gaseous exchange than were less developed seeds. Accordingly, respiration in intact seeds may not reflect the metabolic potential of the seeds. Meanwhile, Miles *et al.* (1988) suggested that the availability of respiratory substrates during early imbibition of soybean seeds may be a limiting factor and depress early oxygen uptake.

In pepper (Malnassy, 1972) and tomato (Koehler, 1967), osmotic treatment increased seed respiration, a change which preceded radicle emergence. In contrast, this study found increases in respiration of osmotically treated seed did not precede early radicle emergence (Figure 6.1.1A). While seedcoat removal increased oxygen uptake, osmotically treated seeds without coats emerged as early as intact seeds, although in untreated controls, decoated seed emerged 1 d earlier than intact ones (Table 6.1.1). This suggested that the seedcoat was certainly a barrier to oxygen uptake and germination in untreated seeds.

Respiratory quotient is generally dependent on the state of oxidation of the substrate oxidised. Highly oxidised substrates such as organic acids result in RQ's between 1 and 1.5; fats or lipids give RQ around 0.7 to 0.8, while an RQ of the order of 1 is characteristic of carbohydrate respired (Mayer and Poljakoff-Mayber, 1982). Results presented in Table 6.1.2 indicated that generally fats or lipids were the substrates oxidised during respiration. However, there were some differences amongst the RQ values. In osmotically treated seeds both in intact and decoated state, RQ values were relatively stable around 0.6-0.74; untreated controls, however, had RQ's close to unity during early imbibition (24 h) but this value then dropped to around 0.62 to 0.79 with increased imbibition time. This suggests a shift of respiratory substrate during early germination of *P. radiata* seeds from sugars or carbohydrates at very early imbibition into fats or lipids at the later stage of germination. The work in soybean and other seeds demonstrated that substrates consumed in respiratory metabolism during early germination are sugars and oligosaccharides (Hsu *et al.*, 1973;

Mayer and Shain, 1974) while the breakdown of lipids is usually a fairly late event in the overall series of steps in germination (St Angelo and Altschult, 1968; Shewky *et al.*, 1972). Several studies also suggest that sugar breakdown in the first hour of germination differs from that in the later phase of seedling growth (Simon, 1984). If this is the case, probably *Pinus radiata* seeds have limited soluble sugar and oligosaccharide reserves. In osmotically treated seeds, a stable value of RQ throughout different times of imbibition indicates these soluble sugar reserves were oxidised during osmotic treatment (10 d) and the seeds are already using their main storage reserves. Reports indicate that the megagametophyte tissues, the main organ in pine seed for storing food reserves, contain very high levels of fats or lipids (45-48%) (Hilditch and William, 1964; Trelease and Doman, 1984).

6.3.2 Dehydrogenase Activity

Results shown in Figures 6.2.1 and 6.2.2 demonstrate considerably higher activities of dehydrogenase enzymes in osmotically treated seeds compared to untreated controls. Since enzyme activity was reflected in formazan production, classical questions have to be answered in this context:

- i. does higher formazan production from embryos of treated seeds result from higher amounts of reagent (2,3,5 triphenyltetrazolium chloride) entering embryos of treated seeds?
- ii. is higher formazan production from embryos of treated seeds due to easier formazan extraction from embryos of treated seeds than from untreated controls?

- iii. does higher formazan production from embryos of osmotically treated seeds indicate higher dehydrogenase activity in those embryos of treated seeds than that in untreated controls?

Results indicated that embryos from both osmotically treated and untreated control seeds produced formazan. However, with increasing periods of embryo imbibition in tetrazolium, the production of formazan was always higher in osmotically treated seeds (Figure 6.2.1). The rates of formazan production were also higher in embryos of osmotically treated seeds (Figure 6.2.2). This result, however, could not answer those three questions. However, a second extraction of some embryos which had already been assayed failed to show any sign of residual formazan. Embryos in this case were uncoloured. Indicating that all formazan produced had been completely extracted in the first assay. Therefore this later assay gave a clue that no differences in extractability of formazan between osmotically treated and untreated controls. Consequently, any difference in formazan production should be attributed to differences in the amount of reagent penetrating embryo tissue or due to exactly higher rate of enzyme activity in osmotically treated seeds.

Other studies have clearly indicated that osmotic treatment increased the activity of many different enzymes including malate dehydrogenase, 6-phosphogluconate dehydrogenase, isocitrate dehydrogenase, glucose-6-phosphate dehydrogenase and aldolase (Smith and Greg Cobb, 1988). Similarly, in lettuce, carrot and okra (Kundu, 1980) an increase in dehydrogenase activity as a result of hydration-dehydration treatment was observed. Many dehydrogenase enzymes are believed to be involved in respiratory pathways, particularly pyruvate dehydrogenase, isocitrate dehydrogenase, oxoglutarate dehydrogenase, succinate dehydrogenase and malate dehydrogenase are those key enzymes in the tricarboxylic acid (TCA) pathway (Wiskich and Dry, 1985) producing reduced NAD and FAD and ultimately yielding energy in the form of ATP (Ting, 1982). If higher formazan

production does reflect higher activity of total dehydrogenase enzymes, it is not surprising that osmotically treated seed had been claimed having higher ATP production than that of untreated controls (Mazor *et al.*, 1984a; Fu *et al.*, 1988). A correlation between dehydrogenase activity and seed vigour therefore appears to be positive, confirmed similar finding by many authors in different seeds, e.g. in rice (Sung and Chen, 1988), wheat (Johnston *et al.*, 1986), and horticultural seeds (Kundu, 1980; Dey and Mukherje, 1988; Rudrapal and Nakamura, 1988a, 1988b; Choudhuri and Basu, 1988).

In comparison with respiration measurements (Experiment 7), this enzyme assay seemed to give more sensitive index of vigour because early changes in oxygen uptake in osmotically treated seeds seemed to be the result of radicle protrusion rather than the cause of that event. If so, the quantitative tetrazolium testing would offer potential use for determining vigour level of seed or seed lot; the potential advantage may include greater speed, cheapness and its simplicity compared to manometric measurements in respiratory testing.

However, whether higher dehydrogenase activity in osmotically treated seeds is in fact closely correlated with the operation of TCA, the electron transport chain and oxidative phosphorylation needs to be confirmed by further experiment. A differential penetration of TTC reagent into embryo tissues perhaps also requires special elucidation where the use of macerated tissue could be explored, although my preliminary study (data not shown) found that grinding the seeds before treating them into TTC gave less TTC reduction; presumably grinding reduced dehydrogenase activity. Another limitation of this simple TTC testing is its inability to specify the types of dehydrogenase. The work of Oota *et al.* (1956) in *Vigna* sp concluded that oxidative mechanisms in cotyledons differed from those in other parts of seedling which were growing actively. TCA pathway might be absent from cotyledons as citric acid dehydrogenase was not functioning in them (Oota, 1956). However, that hypothesis needs to be tested since cotyledons are live tissue.

VII. GENERAL DISCUSSION

7.1 Best Conditions for Osmotic Treatment and Problems with Drying Back After Treatment

Rapid germination of *Pinus radiata* seeds can be obtained following osmotic treatment with either salt or polyethylene glycol solutions. Results indicated that water potential and treatment duration were the two major factors determining the level of treatment benefit. The correct choice of water potential seemed to be crucial for its success, since after treatment at -0.5 MPa, most seeds were lost due to pre-germination while at -1.5 MPa, the benefits of treatment were less than those gained from either treatment at -0.5 MPa or -1.0 MPa (Experiment 1). Presumably when water potentials are reduced, normal processes of germination take place more slowly, if at all, because of restrictions of seed moisture content. A 10 d duration of treatment rather than 7 d was required for maximum benefit of treatment at -1.0 MPa when median germination times were 40% lower than from untreated controls (Figure 4.2.3).

Since seeds are kept in the imbibed state during treatment, protection against microbial attack is of prime importance. The use of Thiram at a rate of 1% seed weight gave good protection and should be employed before treatment, resulting in a low incidence of infection (Figure 4.2.1). However, the spread of germination time may be increased if Thiram is applied twice (before and after, Table 4.2.4). This seemed to be a retardation effect of Thiram when applied beyond its optimum rate and has also been noted by other workers in a range of species (e.g. Joshua, 1973; Grundler, 1974; Heydecker and Coolbear, 1977).

A comparison between salt and polyethylene glycol as osmotica was made in experiments using grade IV *Pinus radiata* seeds. Faster germination of salt treated seeds coincided with a small level (of around 1%) pre-germination occurring during treatment. This indicated that the completion of germination processes was faster in salt treatments rather than in polyethylene glycol. It might be argued that the presence of pre-germination is undesirable as these seeds must be discarded before sowing. However, small proportion, one or two percent perhaps maybe necessary as indication that most of the seeds are near the emerging state and could germinate quickly upon transfer to water.

Osmotically treated seeds are, however, sensitive to rapid drying. A comparable absolute moisture content (7-8%) was obtained from either rapid drying where seeds were dried back for 2 d at ambient temperature + 12 h drying over silica gel within desiccators (Experiment 3) or from slow drying where seeds were dried back for 9-10 d at 20°C, 80-85% RH + 12 h drying at ambient temperatures (Experiment 4). Rapid drying in this case was a phenomenon where a complete loss of treatment benefit can occur while slow drying preserves treatment benefits in terms of rapid germination. As discussed in section 5.4.4, rapid drying has been shown to be associated with disruption of many ultrastructural components of the cell (Bewley and Pacey, 1978; Krockho *et al.*, 1978). An example of this is found in the moss, *Crotonuron filicinum* (Bewley, 1979). Speed of water loss had a determining effect upon ultrastructural changes. Forty minutes of the rehydration of gametophyte *C. filicinum* following rapid desiccation, the cytoplasm of all cells of the phyllia were disorganised, mitochondria were swollen and had an ill-defined internal structure, the outer chloroplast membrane was lost and lamellae were dispersed. At the same stage of rehydration after very slow desiccation, however, only about 20% of the cells were similarly disrupted. The rest, 80% of the cells, were fairly normal in appearance. Twenty-four hours after rehydration, all cells of the rapidly desiccated moss were extensively disrupted, but some 50% of the very slowly dried moss retained their integrity (Bewley,

1979). In celery (Biddington *et al.*, 1982a), rapid drying following treatment resulted in more electrolyte leakage from seeds compared to slow drying; indicating that rapid drying increased membrane damage. In the present experiment, it was not known whether rapid drying impaired the membrane system since no measurement of electrical conductivity of seed leakage was done. However, results in pine and larch (Huang and Zou, 1989) provided evidence that drying osmotically treated seeds even part moderate drying for only 8 h at ambient temperatures with 60% RH resulted in an increase in electrical conductivity of seed leakage. Prolonged drying under those conditions tended to increase seed leakage while vigour index declined (Huang and Zou, 1989). Therefore, perhaps we could speculate that rapid drying in this experiment probably resulted in membrane damage while slow drying in some way prevents this damage thus retaining the benefits of osmotic treatment.

7.2 Application of Osmotic Treatment in Different Seed Grades and Aged Seeds

7.2.1 Effect on Different Seed Grades

Previous results (Experiment 4) showed that osmotic treatment at -1.0 MPa for 10 d resulted in an increase in germination rate of grade IV *Pinus radiata* seeds of the order of 30%. As this grade is considered to be low vigour as it derived from small seed, a subsequent experiment was established with different grades to test the hypothesis that the benefits of osmotic treatment might be much more evident in low vigour seeds than in high vigour seeds. Results presented in Experiment 5 clearly indicated that osmotic treatment consistently improved germination rate as well as emergence rate of all different seed grades without any ameliorative effect on percentage germination, with an exception in grade II seeds in laboratory germination (Figures 5.1.1, 5.1.3, 5.1.4). Advancement of germination in the laboratory due to osmotic treatment was around 2.4-3 d in all different grades while in the

glasshouse it was around 5-6 d, relative to untreated controls. This result would dispute the argument that low vigour seeds (small seeds) would have more benefits from osmotic treatment compared to high vigour seeds. Similarly, in terms of seedling dry weight, although data were uncorrected for differing emergence rates between treated and untreated controls, two independent regression lines seemed to show that osmotic treatment improved seedling growth having higher benefits in larger or heavier seeds than in smaller or lighter seeds (Figure 5.1.5). Although there have been suggestions that pre-sowing seed treatment improved subsequent growth of plants (e.g. Heydecker and Coolbear, 1977; Brocklehurst and Dearman, 1983b), there are also some sceptical views of improved growth *per se*. In tomato, osmotic treatments (Coolbear, 1978; Odell and Cantliffe, 1986; Argerich and Bradford, 1989) or low temperature pre-sowing treatments (Francis, 1985; Coolbear *et al.*, 1987; Ranganarasimhiah, 1989) reduced median germination times but failed to have effects on subsequent seedling growth rates, a finding being noted similarly by Heydecker (1974) in onions. Thus any advantages retained by a crop from treated seeds would be largely due to early emergence and perhaps occur in a limited period during early growth (Coolbear, 1978; Passom *et al.*, 1989). In the present study, it was not possible to ascertain whether or not the benefits obtained from osmotic treatment would be retained until seedlings mature (1 or 2 years). As seedlings were harvested 45 d after sowing, this period is too short for predicting the performance of the mature seedling which usually takes one to two years. In *Pinus taeda* and *Pinus ponderosa* (Dunlap and Barnett, 1984; Mexal and Fisher, 1987), rapid and complete emergence are highly desirable characters of seedlings in nursery operation. Seedlings emerging 10 d after first emergence began were smaller (35%) at harvest than those emerging first (Mexal and Fisher, 1987). In this experiment, although treatment advanced seedling emergence around 5-6 d in all seed grades, growth improvement in small seeds seemed to have little value since seedling from small seeds were smaller than from untreated control large seeds. This suggests that seedling growth does not entirely depend on the time of seedling emergence as suggested by Scott and Jones (1985).

7.2.2 Effects on Aged Seeds

Low resistance of osmotically treated seeds to adverse storage conditions has been observed in many different seeds, e.g. tomato (Alvarado and Bradford, 1988; Argerich *et al.*, 1989), lettuce, leeks and carrot (Dearman *et al.*, 1987). In the present study, although osmotic treatment improved seed vigour according to the criteria of reduced median germination times and improved seedling dry weight, osmotically treated seeds could not be stored at elevated temperature and moisture content (45°C, 30% SMC) since loss of viability and increased median germination times of osmotically treated seeds were faster than in untreated controls (Figures 5.2.1, 5.2.2 and 5.2.4). This situation is contradictory to generally accepted view of seed vigour where vigorous seeds should be associated with longer and better storability and greater tolerance to any adverse storage conditions (AOSA, 1983; Roberts, 1986). Vigorous osmotically treated seeds in this case simultaneously have high susceptibility to deterioration, accordingly, treated seeds could be considered to be vigorous but with a reduced storability. These results are in contrast to the extended longevity obtained with seed hydration treatments (e.g. Mitra and Basu, 1979; Peri, 1979; Burgass and Powell, 1984), such hydration treatments seemed to be carried out for shorter periods of time compared to the duration of osmotic treatment which could need several days, even a couple of weeks. Short hydration treatments may only influence early stages of imbibition where repair processes could be the major events at this stage. In either long hydration or osmotic treatment, the repair process is perhaps only part of the overall components required during the early processes of germination, hence the benefits of osmotic treatment are usually much more evident with longer duration treatments (Haigh and Barlow, 1987). However, this advancement of germination processes simultaneously induces susceptibility of seeds to deterioration, presumably this is a result of a continuation of hydrolytic enzyme activity which is considered to be involved in deterioration (Perl *et al.*, 1978; Francis and Coolbear, 1988). Although these enzymes are required for reserve mobilisation and/or biosynthetic

processes, which may be essential for rapid germination of treated seeds, they could pose a detrimental effect if seeds are to be stored. Meanwhile, improved percent germination and increased germination rates of aged seeds following osmotic treatment might reflect that some sorts of damage were repairable or replaceable. An appreciable number of 3 d aged seeds which failed to germinate, but could be induced to germinate following treatment (Figures 5.1.1, 5.1.2) should not be interpreted as dead seeds but rather due to small defects which have not progressed too far. Therefore allowing repair and replacement to proceed during treatment may be the reason of improved percentage germination and increased germination rate of aged seeds.

7.3 Physiological Changes Associated with Osmotic Treatment

The present study demonstrated that osmotically treated seeds had higher respiration during early germination compared to untreated controls as increases in oxygen uptake occurred earlier in osmotically treated seeds. However, increased respiration should not necessarily be considered the cause of early germination of osmotically treated seeds as it may be a consequence of that event since changes in oxygen uptake occurred at the time of radicle protrusion (Figure 6.1.1A and Table 6.1.1). Since there were no differences in oxygen uptake between osmotically treated and untreated controls (around $1.5 \mu\text{l/h/seed}$ with intact coat and $2.2 \mu\text{l/h/seed}$ in decoated) prior to radicle emergence, some possible explanation could be established in conjunction with germination advancement gained from osmotic treatment. One could be factors other than energy supply perhaps to pose a limit upon early metabolic events prior to radicle emergence. This has been demonstrated in wheat (Brooker *et al.*, 1977) that energy producing systems were operative very early (20 min after hydration produced very high ATP), but energy requiring systems, e.g. protein synthesis did not rise in concert with ATP. This supports findings in many different seeds (e.g. Slyer *et al.*, 1980)

that no consistent correlations were found between ATP and seed vigour. Aside of protein synthesis, other energy requiring systems operative very early prior to radicle emergence, e.g. RNA synthesis, enzyme synthesis and DNA repair (Osborne, 1983) could also play an important role in ensuing rapid emergence. Therefore, increasing these control mechanisms prior to radicle emergence presumably did not necessarily require an increase in respiration as available ATP could provide enough energy. The other alternative, probably energy required at very early germination, was not entirely derived from aerobic respiration but could be via phosphorylation at substrate level during glycolysis. This has been shown in pea (Givan, 1972), *Cucurbita pepo* (Thomas and Ap. Rees, 1972) and *Phaseolus mungo* (Morohashi and Shimokoriyama, 1975) that glycolysis was operative during early germination but subsequently mitochondria activity took over and increased considerably while glycolytic enzymes showed much smaller changes (Morohashi and Shimokoriyama, 1975). If these are the case, increased respiration at the same time of radicle emergence should be considered that energy status available within seed probably could not support any longer a dramatic increase in major metabolic events including: cell expansion, DNA replication, cell division, active transport of food reserves etc; and therefore active aerobic respiration is needed to support energy-dependent activities. This high energy requirement could only be supplied sufficiently from tricarboxylic acid operation since ATP produced are several times that produced by glycolytic pathway (Ting, 1982).

In the case of enzyme activity, higher rates and overall total production of formazan in osmotically treated seeds perhaps indicated that dehydrogenase enzyme activity in treated seed was higher than that of untreated controls. However, it was not known whether those dehydrogenase are mitochondrial enzymes or not mitochondrial enzymes. As discussed in Chapter 6.3 many dehydrogenase enzymes have been suggested as having a major role in energy transducing systems which are involved in respiration (Kolloffel, 1967; Wiskish and Dry, 1985). As an example, NADP-isocitrate dehydrogenase has been shown to be a key

enzyme which may limit mitochondrial respiration at low temperature of soy bean (Duke *et al.*, 1977). Thus it was likely that higher dehydrogenase activity in treated seeds seemed to be closely associated with the operation of respiratory chain. However, whether this particular enzyme has a major role for rapid germination of treated seeds is still obscure since other studies also demonstrated increases in RNA synthesis, protein and enzyme synthesis and DNA repair during osmotic treatment in different seeds (e.g. Coolbear *et al.*, 1980; Fu *et al.*, 1988; Bray *et al.*, 1989; Davidson and Bray, 1991; Davidson *et al.*, 1991). In this connection rapid germination characteristics of treated seeds presumably could not be attributed to one particular mechanism but rather a combination of many factors where metabolic changes occurring during priming allow early processes of germination to be completed. Thus, upon transfer to water treated seeds will germinate faster.

7.4 Limitations of Study and Scope of Further Work

In the present study, improved seedling growth owing to osmotic treatment was evaluated in the artificial environment of the glasshouse where soil media and watering seemed to be optimal. Such conditions like that might not occur in the field and thus a field evaluation for further work would certainly be worthwhile in an attempt to show the potential economic value which might be derived from osmotic treatment.

Osmotically treated seeds are sensitive to drying, the method of drying has a significant effect in preserving treatment benefits. Drying should not be carried out at ambient temperature continuously (50-60% RH) but slow drying at 80-85% RH is effective as little treatment benefit was lost. However, no work has been done in this study to identify the rate of water loss during drying at ambient temperature after treatment as well as the type of damage, if any, generated from that drying. If drying is related to membrane damage,

an analysis of membrane components before and after drying perhaps should be carried out to determine the extent of damage from both rapid and slow drying. Apart from membrane components, studies to define other cellular sites of damage caused by rapid drying may be included in conjunction with the ability of cells to synthesise nucleic acids and proteins as well as the stabilisation of enzymes resulted from drying.

The revelation of better performance of salt treatment over polyethylene glycol in terms of rapid germination offers scope for further work to look into the causes of reduced effectiveness of PEG treatment. This study certainly does not support any longer the theory of ion penetration of salt entering seed tissues which would cause a deleterious effect.

From a physiological and biochemical standpoint, the germination advancement gained from osmotic treatment perhaps could not be reflected only a single control mechanism such as respiration or energy producing system. Other control mechanisms operating during early germination including: synthesis of RNA, proteins, enzymes, DNA repair, cell elongation, DNA replication, cell division and reserve mobilisation, all could play an important role in germination processes (Osborne, 1983). Accordingly, an integrated study on respiration with other aspects of control mechanisms operating during germination could lead to conclusions on the relative importance of one aspect to others in ensuing better performance of osmotically treated seeds compared to untreated controls. As an example, in tomato (Coolbear, 1978), increased RNA occurring during osmotic treatment perhaps was responsible for rapid germination of treated seeds.

Possibly more important than physiological and biochemical studies, technical aspects for large scale applications need to be further explored. Priming for large amounts of seeds certainly requires special equipment such as stirred bioreactor where the supply of oxygen during treatment is of prime importance. A priming system which can be employed for large

tree seeds has not been studied far enough. In addition, if treated seeds need to be sent to other areas, studies on maintaining treatment benefits by using special packaging and appropriate shipping techniques would be worthwhile.

VIII. CONCLUSION

8.1 Conclusion

From the preceding results and discussion, conclusions which can be drawn from this study include:

1. Osmotic treatment improved germination performance of *Pinus radiata* seeds by reducing the median germination and/or emergence times without altering final percentage germination and/or emergence. The optimum conditions of osmotic treatment were -1.0 MPa of mixed $\text{KNO}_3 + \text{KH}_2\text{PO}_4$ solutions (1:1 w/w) for 10 d at 20°C which resulted in 40% reduction in median radicle emergence times if seeds were not dried back (Experiments 2 and 4).
2. Osmotically treated seeds are vulnerable to drying back. Drying back to original moisture content in ambient conditions or over silica gel resulted in complete losses of treatment benefit (Experiments 1 and 3). If, however, drying back osmotically treated seeds is carried out slowly under controlled conditions such as 80-85% RH, 20°C for 9-10 d followed by overnight drying at ambient conditions ($22-27^\circ\text{C}$, 50-60% RH), the benefits of osmotic treatment were largely retained.
3. During the course of osmotic treatment, prevention of seeds from microbial attacks are important. The use of Thiram at a correct rate and correct time is necessary to have maximum effect without any delaying effect on germination.
4. Both polyethylene glycol and mixed $\text{KNO}_3 + \text{KH}_2\text{PO}_4$ (1:1 w/w) can be used as osmotica to improve germination rates in comparison to untreated controls; with no

effect on uniformity and total percentage germination. However, the level of improvement was better in salt treated seeds than in polyethylene glycol treated seeds.

5. Consistently osmotic treatment improved germination rates of all different seed grades in a similar way. Improved seedling dry weight was mainly a consequent of early emergence of osmotically treated seeds, having more benefits in larger or heavier seeds than in smaller or lighter seeds.
6. Osmotically treated seeds might be considered vigorous according to criteria low median times of germination and improved seedling dry weight (Experiment 5). However, osmotically treated seeds could not be suggested as vigorous in terms of storage potential since loss of viability and increased median germination times of osmotically treated seeds occurred faster than untreated control if seeds were stored at elevated temperature and moisture content (45°C, 20% SMC) (Experiment 6).
7. Osmotically treated seed had higher respiration rates than untreated controls during early germination. However, increased respiration was more likely to be the result of germination rather than the cause. Similarly, total dehydrogenase activity in osmotically treated seeds were higher than untreated controls, but their contribution towards rapid germination is unclear.

8.2 Commercial Applications

From the standpoint of potential applications, the results presented in this study show highly promising advantages in differing respects. It is obvious in nursery practice that such early emergence would allow less vigorous seedlings to have time to grow sufficiently before

transplanting. Since one of many factors affecting the success of *Pinus radiata* establishment is survival of outplanted seedlings, better survival could only occur when seedlings have specific characteristics (FRI, 1988; Table 2.2), therefore early emergence should result in better root systems, bigger seedling stem diameter and other better physical seedling characteristics. If seedlings with those characteristics are planted in the field, it should result in better survival.

By emerging early, seeds should not remain longer in nursery beds, particularly if seeds are sown early in the season in temperate climates. Longer seeds in soil would mean seeds prone to disease attacks and to deteriorating physical soil conditions, but also to birds since seed losses after sowing have been reported sometimes from nurseries in New Zealand (Heederwick, 1981). The only methods to deter birds (Greenfinches) in forest nurseries so far that show promise are to combine sowing stratified seeds at shallow depths into warm soil to minimise the germination and emergence period, coupled with using "Mesuroi" as a poisoning agent in seed coating, and continuous shot gun patrols over susceptible period (Heederwick, 1981). This clearly indicates the importance of rapid germination and/or emergence derived from osmotic treatment for that purpose.

This study found that some seeds rendered non-germinable seeds as a result of ageing can be induced to germinate by the method of osmotic treatment. This suggests that physiological repair mechanisms can operate effectively during osmotic treatment which enables aged seeds to retain or restore their germinability. Perhaps this treatment could become routine in some activities like gene banks, not only for germination tests used to monitor viability, but also for sowing seeds to produce plants for regeneration or utilisation in breeding programmes.

The revelation of better performance of salt treated seeds relative to polyethylene glycol treated seeds also provides new possibilities to be established in other forest tree seeds, since up until now osmotic treatment in tree seeds has only used polyethylene glycol (Table 2.2). Compared to polyethylene glycol, salts seem to be more readily available, cheaper, more economic as the amounts required is relatively small, and easily found in markets.

IX. LITERATURE CITED

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APPENDIX I

**Worked Example of Calculating the Desired Water Potential of a
Mixed KNO₃ + KH₂PO₄ Solution**

The CRC Handbook of Chemistry and Physics 69th Edition (Wolf *et al.*, 1988-1989) provided the values of concentrative properties of both KNO₃ and KH₂PO₄ (Tables 52 and 55). As an example, a mixture of 1.320% by weight (13.20 gram per 1000 gram solution) of KNO₃ + KH₂PO₄, 1:1 (w/w) at 20°C can generate a water potential as follows:

Concentrative properties of KNO₃ and KH₂PO₄ monobasic at 6.6 grams each per 1 litre solution (1.320% by weight).

	KNO ₃	KH ₂ PO ₄
1. Freezing point depression of each solution (Δ)	0.432°C	0.328°C
2. Total water concentration of each solution (C_w)	993.25 g l ⁻¹	994.34 g l ⁻¹
3. R, a constant	0.08206 l.atm.mol ⁻¹ .deg ⁻¹	
4. Absolute temperature	293°K	

Source: Wolf *et al.* (1988-1989).

The calculated water potential (Y) of mixed KNO₃ + KH₂PO₄ solutions is:

$$\psi = \frac{\frac{\text{KNO}_3 + \text{KH}_2\text{PO}_4}{1.86} \times R \times T}{[C_w\text{KNO}_3 - C_w\text{KH}_2\text{PO}_4 - 1000]/1000}$$

$$\psi = \frac{0.432 + 0.328}{1.86} \times 0.08206 \times 293$$

$$\psi = \frac{[993.23 + 994.32 - 1000]/1000}{1.86} \times 0.08206 \times 293$$

$$\psi = 9.9474 \text{ Atmospheres}$$

$$1 \text{ Atmosphere} \sim -101,325 \text{ Pascals}$$

$$9.9474 \text{ Atms} \sim -1007920 \text{ Pascals} \sim -1.0 \text{ MPa}$$

In a similar way any value of water potential generated from different concentration of mixed $\text{KNO}_3 + \text{KH}_2\text{PO}_4$ solution can be calculated on the basis of data from CRC Handbook of Chemistry and Physics (1988-1989) by West, Astle and Beyer (eds.), Tables No. 52 and 55 (D-246 and D-247). Plotting the data of concentration of solution of $\text{KNO}_3 + \text{KH}_2\text{PO}_4$ against the values of water potential established the standard curve shown in Appendix Figure 1. From this figure a water potential valued -1.0 MPa generated from mixed $\text{KNO}_3 + \text{KH}_2\text{PO}_4$ (1:1 w/w) at 20°C is equal to mixed concentration of 6.6 g.l^{-1} of each salt.

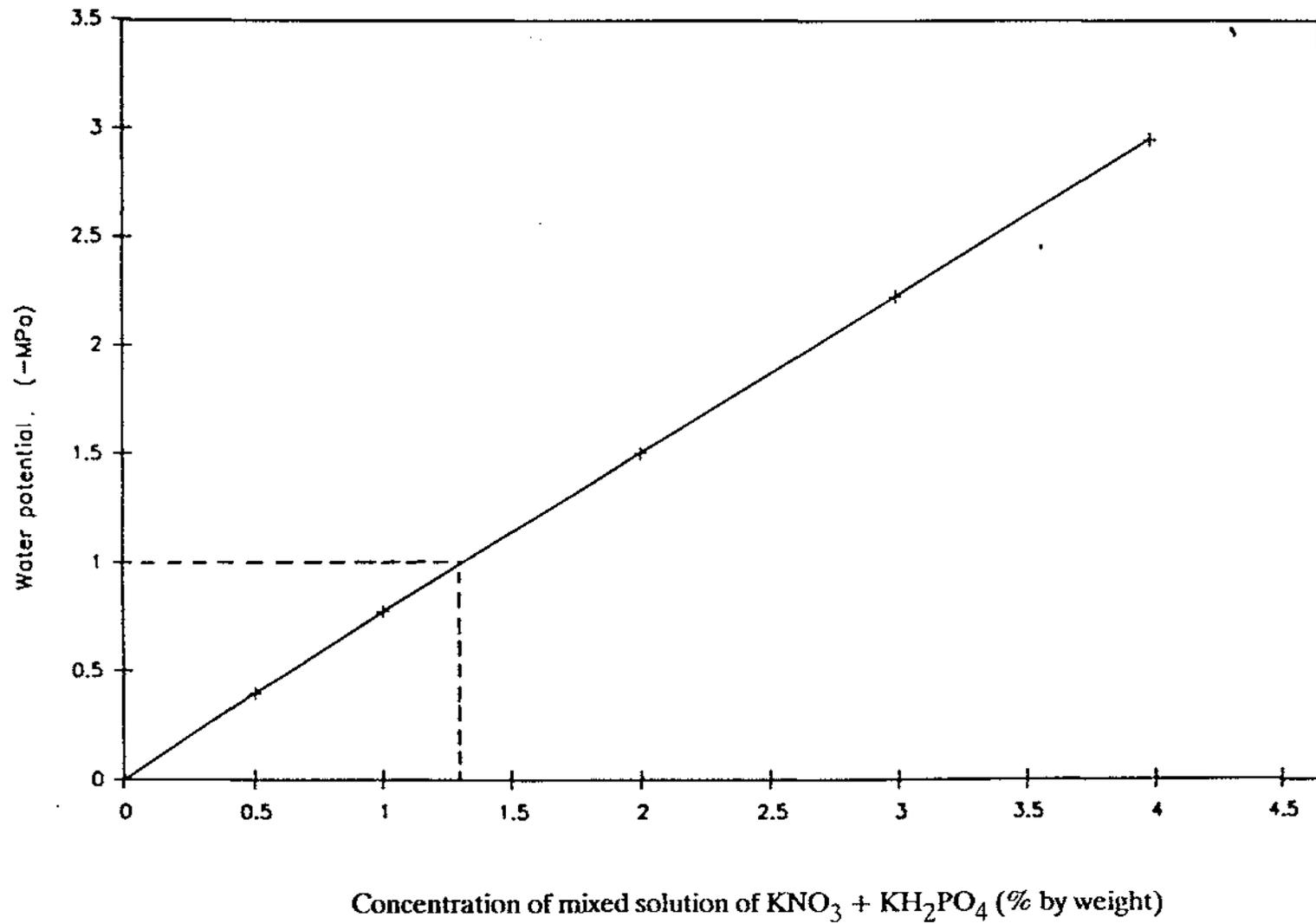


Figure 1. The water potentials generated from different concentration of mixed $\text{KNO}_3 + \text{KH}_2\text{PO}_4$ salts (1:1 w/w) at 20°C .

APPENDIX II

**Worked Example of Calculating the Desired Water Potential
Using Polyethylene Glycol Solution (PEG 6000)**

The desired water potential of PEG 6000 at 20°C was -1.0 MPa which is equal to -10 Bars; while the formula to calculate water potential of PEG 6000 solution was adopted from Michel and Kaufman (1973) as follows:

$$\varphi = -(1.18 \times 10^{-2})C - (1.18 \times 10^{-4}) C^2 + (2.67 \times 10^{-4}) CT + (8.39 \times 10^{-7}) CT^2$$

where: φ = water potential of PEG 6000, in bars
 C = gram solute required for 1 kg of water
 T = temperature, in °C

Thus, φ and T should be substituted with values of -10 bars and 20°C respectively, resulted in an equation of:

$$-10 = -(1.18 \times 10^{-2}) C - (1.18 \times 10^{-4}) C^2 + (2.67 \times 10^{-4}) C \times 20 + (8.39 \times 10^{-7}) C \times 400$$

or $1012.2 C^2 + 64600 C - 100,000,000 = 0$

Solving for C:- C = 284.02 g/kg water.

APPENDIX III**Table A.1** Analysis of variance (Anova) for percentage radicle emergence of salt-treated *P. radiata* seeds at different water potentials, treatment durations and drying back after treatment.

SOV	DF	SS	MS	F _{calc.}
Block	2	270.8	135.4	1.88
Water potentials (WP)	2	128.6	64.3	0.89
Duration (D)	1	2294.5	2294.5	31.85***
Drying back (DB)	2	0.59	0.30	<0.01
WP vs D	2	103.7	51.8	0.72
WP vs DB	4	202.1	50.5	0.70
D vs DB	2	537.5	268.7	3.73*
WP vs D vs DB	4	45.6	11.40	0.16
Error	34	2449.2	72.03	
Total	53	6032.6		

* Significant at P < 0.05

*** Significant at P < 0.001

Table A.2 Anova for median radicle emergence times at 20°C of salt-treated *P. radiata* seed at different water potentials, treatment durations and drying back after treatment.

SOV	DF	SS	MS	F _{calc.}
Block	2	0.64	0.32	0.81
Water potentials (WP)	2	2.20	1.10	2.77
Duration (D)	1	3.22	3.22	8.15**
Drying back (DB)	2	14.82	7.41	18.72***
WP vs D	2	0.51	0.26	0.65
WP vs DB	4	2.59	0.65	1.64
D vs DB	2	0.33	0.17	0.42
WP vs D vs DB	4	0.39	0.99	0.25
Error	34	13.49	0.39	
Total	53	38.19		

** Significant at P < 0.01

*** Significant at P < 0.001

Table A.3 Anova for mean spread radicle emergence times of salt-treated *P. radiata* seeds at different water potentials, treatment duration and drying back after treatment.

SOV	DF	SS	MS	F _{calc.}
Treatment	19	51.34	2.70	0.44 ns
Error	34	206.67	6.08	
Total	53	258.01		

Overall mean = 7.2 d

Table A.4 Anova for percentage normal seedling of salt-treated *P. radiata* seeds at different water potentials, treatment durations and drying back after treatment.

SOV	DF	SS	MS	F _{calc.}
Block	2	128.59	64.3	0.92
Water potentials (WP)	2	36.15	18.07	0.26
Duration (D)	1	4410.0	4410.0	63.05***
Drying back (DB)	2	128.59	64.3	0.92
WP vs D	2	25.48	12.74	0.18
WP vs DB	4	573.5	143.38	2.05
D vs DB	2	277.9	138.96	1.99
WP vs D vs DB	4	193.19	48.3	0.69
Error	34	2278.0	69.94	
Total	53	8051.4		

*** Significant at $P < 0.001$

Table A.5 Anova for median normal seedling times of salt-treated *P. radiata* seeds at different water potentials, treatment durations and drying back after treatment.

SOV	DF	SS	MS	F _{calc.}
Block	2	5.57	2.78	2.28
Water potentials (WP)	2	15.75	7.86	6.46**
Duration (D)	1	5.70	5.70	4.67*
Drying back (DB)	2	74.39	37.19	30.49***
WP vs D	2	2.73	1.37	1.12
WP vs DB	4	2.23	0.56	0.46
D vs DB	2	1.92	0.96	0.79
WP vs D vs DB	4	3.88	0.97	0.80
Error	34	41.47	1.22	
Total	53	158.55		

* Significant at $P < 0.05$ ** Significant at $P < 0.01$ *** Significant at $P < 0.001$ **Table A.6** Anova for mean spread of normal seedling times of salt-treated *P. radiata* seeds at different water potentials, treatment durations and drying back after treatment.

SOV	DF	SS	MS	F _{calc.}
Treatment	19	17.04	0.90	0.31 ns
Error	34	98.6	2.90	
Total	53	115.64		

Overall mean = 7.6 d

Table B.1 Anova for final percentage radicle emergence of grade IV *Pinus radiata* seeds after salt treatment at -1.0 MPa with different durations, fungicide applications and drying after treatment.

SOV	DF	SS	MS	F _{calc.}
Treatment	25	531.7	21.27	0.74 ns
Error	46	1318.3	28.66	
Total	71			

ns = non significant

Overall mean = 92.7%

Table B.2 Anova for median times of radicle emergence of grade IV *P. radiata* seeds after salt treatment at -1.0 MPa with different treatment durations, fungicide applications and drying after treatment.

SOV	DF	SS	MS	F _{calc.}
Block (A)	2	0.13	0.06	0.31
Osmotic duration (B)	2	43.17	21.59	103.6***
Fungicide (C)	3	0.72	0.24	1.15
Drying back (D)	1	2.07	2.07	9.92**
B vs C	6	0.62	0.10	0.50
B vs D	2	2.05	1.03	4.93*
C vs D	3	0.43	0.14	0.68
B vs C vs D	6	0.91	0.15	0.73
Error	46	9.58	0.21	
Total	71	59.68		

* Significant at P < 0.05

** Significant at P < 0.01

*** Significant at P < 0.001

Table B.3 Anova for mean spread times of radicle emergence of grade IV *P. radiata* seeds after salt treatment at -1.0 MPa with different treatment durations, fungicide applications and drying after treatment.

SOV	DF	SS	MS	F _{calc.}
Block (A)	2	1.24	0.62	0.27
Osmotic duration (B)	2	16.13	8.06	3.52*
Fungicide (C)	3	26.23	8.91	3.89*
Drying back (D)	1	14.40	14.40	6.28*
B vs C	6	13.73	2.29	1.00
B vs D	2	8.57	4.29	1.87
C vs D	3	4.19	1.40	0.61
B vs C vs D	6	5.97	1.0	0.43
Error	46	105.42	2.29	
Total	71	196.38		

* Significant at $P < 0.05$

Table B.4 Anova for percentage normal seedling of grade IV *P. radiata* seeds after salt treatment at -1.0 MPa with different treatment durations, fungicide applications and drying after treatment.

SOV	DF	SS	MS	F _{calc.}
Treatment	25	713.3	28.53	1.02 ns
Error	46	1284.7	27.93	
Total	71	1998.0		

ns = non significant

Overall mean = 91.7%

Table B.5 Anova for median times of normal seedling production of grade IV *P. radiata* seeds after salt treatment at -1.0 MPa with different treatment durations, fungicide applications and drying after treatment.

SOV	DF	SS	MS	F _{calc.}
Block (A)	2	0.57	0.28	0.36
Osmotic duration (B)	2	18.29	9.15	11.37***
Fungicide (C)	3	6.31	2.10	2.63
Drying back (D)	1	6.85	6.85	8.51**
B vs C	6	3.53	0.59	0.73
B vs D	2	5.54	2.77	3.46*
C vs D	3	0.60	0.20	0.25
B vs C vs D	6	3.55	0.59	0.75
Error	46	37.06	0.80	
Total	71	82.30		

* Significant at P < 0.05

** Significant at P < 0.01

*** Significant at P < 0.001

Table B.6 Anova for mean spread times of normal seedling of grade IV *P. radiata* seeds after salt treatment at -1.0 MPa with different treatment durations, fungicide applications and drying after treatment.

SOV	DF	SS	MS	F _{calc.}
Block (A)	2	1.03	0.51	0.35
Osmotic duration (B)	2	1.65	0.82	0.56
Fungicide (C)	3	21.05	7.02	4.77**
Drying back (D)	1	6.24	6.24	4.25*
B vs C	6	8.54	1.42	0.97
B vs D	2	2.77	1.39	0.94
C vs D	3	8.50	2.83	1.92
B vs C vs D	6	16.77	2.79	1.90
Error	46	67.62	1.47	
Total	71	140.18		

* Significant at P < 0.05

** Significant at P < 0.01

Table C.1 Anova for the percentage radicle emergence from grade IV *P. radiata* seeds after treatment with different osmotica and drying back after treatment.

SOV	DF	SS	MS	F _{calc.}
Treatment	7	208.0	29.71	1.09 ns
Error	10	272.0	27.2	
Total	17	480.0		

ns = non significant
Overall means = 88%

Table C.2 Anova for median times (T_{50}) of radicle emergence from grade IV *P. radiata* seeds after treatment with different osmotica and drying back after treatment.

SOV	DF	SS	MS	F _{calc.}
Block	2	0.13	0.065	0.54
Drying (A)	1	4.11	4.11	33.9***
Osmotic treatment (B)	2	5.69	2.85	23.55**
A vs B	2	1.62	0.81	6.69**
Error (b)	10	1.21	0.121	
Total	17	12.76		

** Significant at $P < 0.01$
*** Significant at $P < 0.001$

Table C.3 Anova for mean spread times (T_{90} - T_{10}) of radicle emergence from grade IV *P. radiata* seeds after treatment with different osmotica and drying back after treatment.

SOV	DF	SS	MS	F _{calc.}
Treatment	7	5.54	0.79	0.99 ns
Error	10	7.97	0.797	
Total	17	13.51		

ns = non significant
Overall means = 6.2 d

Table C.4 Anova for the percentage normal seedling of grade IV *P. radiata* seeds after treatment with different osmotica and drying after treatment.

SOV	DF	SS	MS	F _{calc.}
Treatment	7	296.7	42.41	0.55 ns
Error	10	776.9	77.69	
Total	17	1073.8		

ns = non significant
Overall means = 84.9%

Table C.5 Anova for median times (T_{50}) of normal seedling production from grade IV *P. radiata* seeds after treatment with different osmotica and drying after treatment.

SOV	DF	SS	MS	F _{calc.}
Block	2	0.40	0.20	0.69
Drying (A)	1	9.24	9.24	31.9***
Osmotic treatment (B)	2	12.83	6.42	22.1***
A vs B	2	2.19	1.08	3.72
Error (b)	10	2.91	0.29	
Total	17	27.55		

*** Significant at $P < 0.001$

Table C.6 Anova for mean spread times ($T_{90}-T_{10}$) of normal seedling production from grade IV *P. radiata* seeds after treatment with different osmotica and drying after treatment.

SOV	DF	SS	MS	F _{calc.}
Treatment	7	6.23	0.89	1.19 ns
Error	10	7.45	0.745	
Total	17	13.68		

ns = non significant
Overall means = 7.0 d

Table C.7 Anova for mean seedling dry weight from grade IV *P. radiata* seeds after salt treatment with different osmotica and drying back after treatment.

SOV	DF	SS	MS	F _{calc.}
Treatment	7	0.98	0.14	0.24 ns
Error	10	5.79	0.579	
Total	17	6.77		

ns = non significant

Overall means = 13.07 mg

Table E.1 Anova for percentage radicle emergence of treated and untreated *P. radiata* seeds from different grades.

SOV	DF	SS	MS	F _{calc.}
Block	2	29.9	14.95	0.81
Seed grades (A)	4	274.1	68.53	3.71*
Osmotic treatment (B)	1	26.1	26.13	1.41
A vs B	4	248.5	62.13	3.36*
Error	18	332.8	18.49	
Total	29	911.47		

* Significant at $P < 0.05$ **Table E.2** Anova for median times (T_{50}) of radicle emergence of treated and untreated *P. radiata* seeds from different grades.

SOV	DF	SS	MS	F _{calc.}
Block	2	1.92	0.96	1.39
Seed grades (A)	4	0.67	0.17	0.74
Osmotic treatment (B)	1	81.67	81.67	360.1***
A vs B	4	0.28	0.07	0.30
Error	18	4.08	0.23	
Total	29	90.62		

*** Significant at $P < 0.001$

Table E.3 Anova for mean spread times ($T_{90}-T_{10}$) of radicle emergence of treated and untreated *P. radiata* seeds from different grades.

SOV	DF	SS	MS	F _{calc.}
Treatment	11	4.89	0.444	0.94NS
Error	18	8.50	0.472	
Total	29	13.39		

Overall mean = 6.4 d

Table E.4 Anova for percentage normal seedling production by salt treated and untreated *P. radiata* seeds from different grades.

SOV	DF	SS	MS	F _{calc.}
Block	2	20.3	10.13	0.49
Seed grades (A)	4	389.3	97.33	4.68*
Osmotic treatment (B)	1	53.3	53.33	2.56
A vs B	4	229.3	57.33	2.76
Error	18	374.4	20.80	
Total	29	1066.7		

* Significant at $P < 0.05$

Table E.5 Anova for median times (T_{50}) of normal seedling production of salt treated and untreated *P. radiata* seeds from different grades.

SOV	DF	SS	MS	F _{calc.}
Block	2	0.096	0.48	0.05
Seed grades (A)	4	13.74	3.44	3.77*
Osmotic treatment (B)	1	162.8	162.8	178.2***
A vs B	4	6.48	1.62	1.77
Error	18	16.45	0.91	
Total	29	199.60		

* Significant at $P < 0.05$ *** Significant at $P < 0.001$ **Table E.6** Anova for mean spread time ($T_{90}-T_{10}$) of normal seedling of salt treated and untreated *P. radiata* seeds from different grades.

SOV	DF	SS	MS	F _{calc.}
Block	2	0.51	0.25	0.85
Seed grades (A)	4	1.92	0.48	1.60
Osmotic treatment (B)	1	1.54	1.54	5.15*
A vs B	4	2.53	0.63	2.11
Error	18	5.39	0.30	
Total	29	11.89		

* Significant at $P < 0.05$

Table E.8 Anova for percentage seedling emergence in the glasshouse from treated and untreated *P. radiata* seeds of different grades.

SOV	DF	SS	MS	F _{calc.}
Treatment	9	629.9	69.99	1.53NS
Error	20	917.3	45.9	
Total	29	1547.2		

Overall mean = 83.6%

Table E.9 Anova for median times (T₅₀) of seedling emergence of treated and untreated *P. radiata* seeds from different grades.

SOV	DF	SS	MS	F _{calc.}
Seed grades (A)	4	21.92	5.48	2.27
Osmotic treatment (B)	1	236.9	236.9	97.9***
A vs B	4	6.47	0.67	0.67
Error	20	48.37	2.42	
Total	29	313.64		

*** Significant at P < 0.001

Table E.10 Anova for mean spread times of seedling emergence in the glasshouse of treated and untreated *P. radiata* seeds from different grades.

SOV	DF	SS	MS	F _{calc.}
Treatment	9	17.35	1.93	0.88NS
Error	20	44.04	2.20	
Total	29			

Overall mean = 12.3 d

Table E.11 Anova for mean dry weight of seedlings grown for 40 d in the glasshouse of treated and untreated *P. radiata* seeds from different grades.

SOV	DF	SS	MS	F _{calc.}
Seed grades (A)	4	1762.1	440.5	430.5***
Osmotic treatment (B)	1	435.3	435.3	425.5***
A vs B	4	68.5	17.13	16.74***
Error	20	20.5	1.02	
Total	29	2286.4		

*** Significant at P < 0.001

Table F.1 Actual seed moisture contents after different durations of controlled deterioration (at a nominal 20% SMC) of grade V *P. radiata* seeds which were osmotically treated or untreated with mixed $\text{KNO}_3 + \text{KH}_2\text{PO}_4$ solutions.

	Duration of ageing (days)				
	0	1	2	3	4
AO	19.37 (± 0.22)	19.13 (± 0.22)	18.36 (± 0.12)	19.30 (± 0.47)	18.30 (± 0.32)
TBA	19.10 (± 0.36)	20.40 (± 0.53)	18.30 (± 0.26)	17.93 (± 0.24)	19.46 (± 0.75)
TAA	19.30 (± 0.26)	20.10 (± 0.55)	18.30 (± 0.29)	18.86 (± 0.52)	20.20 (± 0.31)

Data are means of three replicates, numbers in brackets are s.e.s of individual means.

AO: ageing only with no osmotic treatment.

TBA: Osmotic treatment at -1.0 MPa for 10 d before ageing

TAA: Osmotic treatment at -1.0 MPa for 10 d after ageing

Table F.2 Anova for percentage radicle emergence of grade V *P. radiata* seeds which were subjected to salt treatment (-1.0 MPa for 10 d at 20°C) before or after different durations of controlled deterioration at 45°C and 20% SMC.

SOV	DF	SS	MS	F _{calc.}
Block	2	38.2	19.1	0.37
Osmotic treatment (A)	2	3822.2	1911.1	37.4***
Ageing (B)	3	6111.1	2203.7	43.1***
A vs B	6	2627.1	437.9	8.6***
Error	22	1124.1	51.1	
Total	35	14223.5		

*** Significant at $P < 0.001$

Table F.3 Anova for median times (T_{50}) of radicle emergence of grade V *P. radiata* seeds which were subjected to salt treatment (-1.0 MPa for 10 d at 20 °C) before or after different durations of controlled deterioration at 45 °C and 20% SMC.

SOV	DF	SS	MS	F _{calc.}
Block	2	1.26	0.63	0.65
Osmotic treatment (A)	2	257.4	128.7	132.6***
Ageing (B)	3	261.2	87.0	89.7***
A vs B	6	144.0	24.0	24.7***
Error	22	21.4	0.97	
Total	35	685.2		

*** Significant at $P < 0.001$

Table F.4 Anova for mean spread times ($T_{90}-T_{10}$) of radicle emergence of grade V *P. radiata* seeds which were subjected to salt treatment (-1.0 MPa for 10 d at 20 °C) before or after different durations of controlled deterioration at 45 °C and 20% SMC.

SOV	DF	SS	MS	F _{calc.}
Block	2	0.11	0.05	0.16
Osmotic treatment (A)	2	5.44	2.72	7.89*
Ageing (B)	3	10.99	3.66	10.62*
A vs B	6	1.99	0.33	0.97
Error	22	7.59	0.34	
Total	35	26.13		

* Significant at $P < 0.05$

Table F.5 Anova for percentage normal seedling production of grade V *P. radiata* seeds which were subjected to salt treatment (-1.0 MPa for 10 d at 20 °C) before or after different durations of controlled deterioration at 45 °C and 20% SMC.

SOV	DF	SS	MS	F _{calc.}
Block	2	67.6	33.8	0.76
Osmotic treatment (A)	2	6955.5	3477.8	78.25***
Ageing (B)	3	8544.0	2848.0	64.08***
A vs B	6	4258.6	709.8	15.97***
Error	22	977.8	44.44	
Total	35	20803.6		

*** Significant at $P < 0.001$

Table F.6 Anova for median times (T_{50}) of normal seedling production of grade V *P. radiata* seeds which were subjected to salt treatment (-1.0 MPa for 10 d at 20 °C) before or after different durations of controlled deterioration at 45 °C and 20% SMC.

SOV	DF	SS	MS	F _{calc.}
Block	2	2.81	1.40	1.27
Osmotic treatment (A)	2	258.9	129.4	117.27***
Ageing (B)	3	239.6	79.9	73.37***
A vs B	6	111.4	18.6	16.82***
Error	22	24.28	1.10	
Total	35	637.0		

*** Significant at $P < 0.001$

Table F.7 Anova for mean spread times (T_{90} - T_{10}) of normal seedling production of grade V *P. radiata* seeds which were subjected to salt treatment (-1.0 MPa for 10 d at 20 °C) before or after different durations of controlled deterioration at 45 °C and 20% SMC.

SOV	DF	SS	MS	F _{calc.}
Block	2	0.88	0.44	1.57
Osmotic treatment (A)	2	2.68	1.34	4.78*
Ageing (B)	3	2.78	0.93	3.86*
A vs B	6	3.56	0.59	2.11
Error	22	6.20	0.28	
Total	35	15.10		

* Significant at $P < 0.05$

Table F.8 Anova for mean seedling dry weight from grade V *P. radiata* seeds which were subjected to salt treatment (-1.0 MPa for 10 d at 20 °C) before or after different durations of controlled deterioration at 45 °C and 20% SMC.

SOV	DF	SS	MS	F _{calc.}
Block	2	0.198	0.099	2.52
Osmotic treatment (A)	2	0.038	0.019	0.49
Ageing (B)	3	0.447	0.149	3.80*
A vs B	6	0.309	0.051	1.31
Error	22	0.864	0.039	
Total	35	1.857		

* Significant at $P < 0.05$

Table G.1 Anova for the values of respiratory quotient during imbibition of decoated or intact coats of grade V *Pinus radiata* seeds which were treated or untreated at -1.0 MPa or mixed $\text{KNO}_3 + \text{KH}_2\text{PO}_4$ for 10 d at 20°C.

SV	DF	SS	MS	F _{calc.}
Imbibition time (A)	4	0.00875	0.10029	6.14**
Seedcoat removal (B)	1	0.00675	0.00675	0.41
Osmotic treatment (C)	1	0.31008	0.31008	19.0***
A vs B	4	0.6189	0.01547	0.95
A vs C	4	0.32014	0.08003	4.91**
B vs C	1	0.00085	0.00085	0.52
A vs B vs C	4	0.51293	0.12823	7.86***
Error	100	1.63146	0.01634	
Total	119	3.2450		

*** Significant at $P < 0.001$

** Significant at $P < 0.01$

APPENDIX IV

The calculated regression line for the relationship between seed weight and seedling dry weight for osmotically treated seeds was $Y = 0.8223 X + 5.192$ with standard errors of constants of ± 0.0353 and ± 1.2552 respectively. Significant test for constants (b_1 and b_0) would be:

$$T_{b_1} = \frac{b_1}{\text{S.E. of } b_1} = \frac{0.8223}{0.0353} = 23.3 \quad (P < 0.01)$$

$$T_{b_0} = \frac{b_0}{\text{S.E. of } b_0} = \frac{5.192}{1.2552} = 4.137 \quad (P < 0.01)$$

In a similar way, the calculated regression line for the relationship between seed weight and seedling dry weight for untreated controls was $Y = 0.552 X + 6.7625$ with standard errors on constants of ± 0.0353 and ± 1.2551 respectively. Significant test for constants (b_1 and b_0) would be:

$$T_{b_1} = \frac{b_1}{\text{S.E. of } b_1} = \frac{0.5522}{0.0353} = 15.61 \quad (P < 0.01)$$

$$T_{b_0} = \frac{b_0}{\text{S.E. of } b_0} = \frac{6.7625}{1.2551} = 5.37 \quad (P < 0.01)$$