SEED EXTRACTION METHODS AND QUALITY EFFECTS IN *PINUS RADIATA* D. DON

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ABSTRACT

The current study aims to investigate the effectiveness of various heated air and microwave oven treatments on seed extraction efficiency and subsequent seed quality in *Pinus radiata*. Radiata pine cones were collected from Foxton from a commercial plantation and used in preliminary studies considering a range of both heated air and microwave oven treatments. Cones of different genetic families were collected from an open pollinated seed orchard owned by Carter Holt Harvey Forests at Matakana Island.

In the air oven extraction method temperature and duration combinations of 50°C and 24 hours or 60°C and 12 hours were found to be most suitable for seed extraction while giving good seed quality in preliminary experiments. A temperature of 40°C was found to be too low for efficient seed extraction while 70°C was found to be lethal to seeds. Various temperature and duration combinations gave similar results since a decrease in extraction temperature could, in some cases, be compensated by an increase in the extraction period.

Exposure of cones in a microwave oven affected germination, particularly when only 1 or 2 cones were heated at each exposure time. However when 3-5 cone samples were used heating for 30 or 40 seconds was sufficient to break scale resin bonding. Ambient storage of treated cones for up to 7 days following microwave oven treatment allowed full scale reflexing and high seed extraction efficiency.

Cones from 10 different families showed variable germination responses to different seed extraction conditions. Two families showed consistently high germination across all treatments while the rest showed reduced germination. Whether this reflects genetic differences in cone serotiny, seed thermosensitivity differences, cone wood density, resin bond strength, or is related to seed size and/or moisture content is not known. Seedling dry weight was not affected by extraction temperature and/or duration of heating, being found to be more a function of seed size.

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CHAPTER 1

1. INTRODUCTION

In New Zealand the predominant commercial forest plantation timber species is *Pinus radiata*, a member of the *Pinaceae* family. Its common name is radiata pine, insignis pine or monterey pine and it was formerly known as *Pinus insignis* Dougl. Well over one million hectares are planted to *P. radiata* in New Zealand (Lee 1994) where it often performs better than in its natural habitat in coastal central California and northern Guadalupe Island, and in Mexico (Bannister, 1973; Krugman and Jenkinson, 1974; Rimbawanto *et al* 1988a).The var. *binata* occurs on Sun Guadalupe Island and is slower growing than the other 3 mainland populations; Monterey, Cambria and Año Nuevo Point. The Cambria population has the largest cones and seeds, and the Monterey population has the smallest. In provenance trials in Australia, Cambria trees did not do as well as those from Año Nuevo Point and Monterey populations (Krugman and Jenkinson, 1974).

The genus *Pinus* is one of the largest and most important coniferous genera comprising about 95 species and numerous varieties and hybrids. This genus occurs naturally throughout the world and contains the most valuable timber species in the world. Their value is due to their ability to grow outside their native range. Schopmeyer (1974) stated that naturally the height of mature *Pinus radiata* ranges from 5-150 feet (about 1.5-45m) and minimum seed bearing age is 5-10 years. The species is reported to have been first cultivated in 1833 (Krugman and Jenkinson, 1974). Its main uses are timber production, shelterbelts and environmental forestry. The interval between large seed crops is one year, and the pre-ripe colour of cones is green, while the cone colour at maturity is lustrous nut-brown to light-brown.

Pines are monoecious with male (microsporangiate) and female (megasporangiate) strobili borne separately on the same tree. Male strobili predominate on the basal part of new shoots, mostly on older lateral branches in the lower crown. Female strobili are found most often in the upper crown, primarily at the apical end of the main branches in the position of subterminal or lateral buds. Frequent exceptions are found to this general scheme. Usually the male develops one to several weeks before the female strobilus. After pollination, scales of the female strobili close, and the strobili begin a slow development. By the end of the first season they are about one-eight to one-fifth of the length of a mature cone. Development may continue in the winter under favourable conditions. Fertilisation in pines takes place in spring or early summer about 13 months after pollination, after which the cones begin to grow rapidly. In the North Island of New Zealand there is a 22-month interval between pollination in early spring (August/September) and attainment of maximum cone size in June, 2 years later for *Pinus radiata.*

One of the commercial issues in pine seed production is the effectiveness of seed extraction from the cone and the effect of seed extraction technique (particularly temperature) on subsequent seed quality and storability. In an attempt to identify the most appropriate temperature: time: quality relationships for *Pinus radiata* seed extraction from closed cones a study was conducted at the Seed Technology Centre at Massey University the objectives of which were:

1. To determine the optimum cone exposure temperature and duration for maximum seed extraction using a conventional heated air oven to obtain seed of high quality.
2. To investigate the application of microwave heating to cones to obtain maximum seed extraction and determine the optimum duration of exposure to also obtain maximum seed quality.
3. To determine post extraction seed quality in the laboratory using standard germination, seedling vigour and viability tests.
4. To determine the effect of the best oven heated air and microwave oven extraction treatments on the subsequent seed quality of cones from a range of different genetic backgrounds.

The study was divided into two major components. The first part was a preliminary study to find the best methods of seed extraction in terms of quality maintenance for each of the two heating methods. The second part involved a comparison of 10 different genetic cone families, using pine cones harvested from an open pollinated seed orchard with only maternal parents identified. The seed quality comparison was based on the results of germination tests and seedling dry weight.

CHAPTER 2

2. LITERATURE REVIEW

2.1 General

Rimbawanto *et al* 1988a reported that cone development takes about 28 months from female strobili initiation until maturation. They divided the development into two principal phases - firstly, initiation until just before fertilisation and secondly, from fertilisation to maturation. Sweet and Bollman (1970) however described cone development in three phases: a seasonal period of growth in which pollination occurs, a second period of seasonal growth in which fertilisation occurs, and finally a period of cone maturation. Rimbawanto *et al* 1988a also showed that the period of rapid growth does not seem to result directly from pollination or fertilisation and the seasonal growth period has some similarity with those of vegetative growth. They also reported that viable seed could be extracted some six months before cone maturity.

Each seed has one membranous wing and falls from the trees in a spiral fashion. The seeds exhibit epigeal germination, taking the seed coat above the ground; and when the seed coat falls, it exposes the needle-like leaves of the young seedling. The seed sometimes needs chilling (stratification) to overcome dormancy, but Rimbawanto *et al* 1988a reported that there were no significant differences in the germination of chilled or un-chilled seed in freshly harvested seed.

Seed is the major means of radiata pine propagation in New Zealand, although Coker *et al* (1991) have developed ways to use somatic embryogenesis as a plant propagation system. This method is very useful in clonal forestry although clonal forestry has associated problems due to its narrow genetic base, e.g. disease.

Seed size commonly ranges from 22 000 to 40 000 seeds per kilogram and this can affect germination and subsequent growth. Menzies *et al*, (1991) reported that seeds larger than 25 000 seeds per kilogram has significantly better germination and produces significantly taller seedlings that smaller seed (33 000 plus seeds per kilogram). Kusmintardjo *et al* (1991) reported that when commercial *Pinus radiata* seed is sorted into 4 size grades, the smaller grades often having poor emergence and vigour and thus have little commercial value.

During seed dewinging various types of seed damage can occur. These may be observed in several ways during or at the end of a germination test. While some damage is not visible or can not be detected by the X-ray technique it could be evident in the resultant seedling.

Heit (1961a) noted that damage by a dewinging machine typically involved swelling, blunt growing tip and a wide rupture of the seed coat along the whole length of suture in the injured lot. He also reported that this type of injury can be eliminated by proper mechanical adjustment and reduction of dewinging speed. Actual cracking of the seedcoat and crushing of the inner seed parts has resulted from poorly supervised or faulty cleaning machinery thus producing broken, deformed seedlings in subsequent laboratory tests. Too severe rubbing by hand to remove the wings also causes abnormal and injured seedlings. This result is the same as that obtained by a power driven machine with rotating brushes.

Somewhat surprisingly, however Wang (1973) reported that a comparison of laboratory test results with nursery emergence showed that seedlots with underdeveloped embryos or seeds injured by harsh seed processing often had better field performance than that observed in laboratory germination tests. However seed lots with low laboratory germination were often more variable in field performance than high germination seed lots. Seed injuries sustained during kiln extraction and during the dewinging and cleaning process have been known for many years (Heit, 1961b). Injury to *Pinus resinosa* seed from both high humidity in the kiln and from the dewinging machine in wing removal having been reported as long ago as 1940 by Eliason and Heit. High humidity in the kiln produces a cooking effect on the seed thereby lowering the capacity of the seed to germinate.

In nature, serotinous cones are opened by forest fires which allows regeneration of the forest. Teich (1970) suggested that lodgepole (*Pinus contorta*) and jack pine (*Pinus banksiana*) cones are subjected to temperatures in excess of 50°C during forest fires which melts the resin bonds and allows release of the seed in large quantities for dispersal and regeneration growth when environmental conditions are suitable.

Heat damage to seed can occur as a result of the cumulative effects of respiration and fungal heating, and by the accumulation and retention of radiant heat within the seed mass following harvest; both situations being referred to as 'field heating', (Hill and Johnstone, 1985). The same authors also state that heat damage can also occur in heated air drying systems especially when excessive heat is used, this subsequent damage is referred to as 'drying damage' and suggest that the heating process in seed with subsequent damage to germination is well known but the cause and mode of attack is not. The moisture content of seed has a strong bearing on its storage quality since it influences respiration rate and subsequent loss of vigour which in turn is accompanied by a loss of germination compounded by the development of storage fungi and increased insect activity. Hill and Johnstone (1985) suggested that there is no single temperature which can be quoted beyond which it is unsafe to heat seed during drying, because many factors are involved which include the type of seed, heat tolerance, seed moisture content, duration of exposure, rate of drying and the purpose for which the seed is intended. They do however recommend a drying temperature not exceeding 32°C for seed moisture contents above 20% and 43°C for seed moisture around 10%. Rapid drying can cause over-drying of the outer parts of the seed leaving the inner parts at a higher seed moisture. This phenomenon is called case-hardening and can be exhibited by some seeds, especially cereals. It must also be noted however that an increase in the air flow in a drier can substantially increase the drying rate at a given temperature and thus reduce the chances of heat damage to seed.

Jugenheimer (1958) suggested that seed with high moisture content should be dried at temperatures not higher than 106 °F (40 °C) to 110 °F (43 °C) in order to preserve germination standards. He also suggested that air temperatures as high as 115°F (46°C) may be used with mature seed of 25% moisture content or less and when the seed type is not susceptible to heat damage.

2.2 Cone structure and serotiny

The cones of *Pinus radiata* are serotinous. This means they may remain closed on the tree for years after maturity. The term serotinous is derived from the Latin word *seur* meaning late. Foresters therefore refer to cones which do not open spontaneously after maturation as serotinous. The radiata cone has a specific gravity of less than 1 at maturity, and therefore water can be used to distinguish between ripe and unripe cones in a flotation test (Krugman and Jenkinson, 1974).

The mature cone consists of overlapping woody scales, each of which bears two seeds at the base on the upper surface. Drying causes the cone scales to separate owing to differential contraction of the two tissue systems: woody strands of short, thick-walled, tracheid-like cells extending from the cone axis to the tip of the cone scales, and thick-walled sclerenchyma cells in the abaxial zone of the scale (Aase, 1915). The closed cone habit (serotinous cones) is the result of 3 factors:

1. extremely strong adhesion between adjacent, overlapping cone scales beyond the ends of the winged seeds,
2. cone structure; and
3. the nature of the 2 tissue systems in the scales described above.

The scales are held together by a resinous substance which melts when heat is applied, allowing the scales to separate. Because of their serotinous nature; in the North Island of New Zealand there is no risk of losing seed by cone opening. However, there is such a risk in the hotter summer climate in Canterbury, South Island if cones are collected later than the end of December (Anonymous, 1946a).

Historically, fire has been the most important causative factor in the establishment and regeneration of natural coniferous forests with serotinous cones, (Beaufait, 1960). Cones which remain closed do so because of a resinous bonding material which forms a vapour resistant protective coating over the entire cone. Cameron (1953) found that temperatures (about 50°C) exceeding those usually found in tree crowns are required to melt this resin and free the scales. The lower scales of the radiata pine cone do not seem to contain any seed as reported by Beaufait (1960) for *Pinus contorta*, but they often restrict full opening of those above. Even when mechanically opened, the lower scales are found to contain no seed or only wings with undeveloped seed at the tip.

There is a remarkable change in length of a cone scale from the wet to the dry state (Harlow *et al*, 1964). This change is the cause of cone scale opening when heat is applied in serotinous cones. Harlow (1964) showed that there are two principal tissues separated by a sort of neutral zone. Histological study shows that in longitudinal radial section, a pine cone scale consists of two zones. Dorsally, the cells are principally woody fibres extending from the cone axis; ventrally there is a band of tissue consisting of short rectangular thick-walled cells. The melting of resin allows the cone scales to open in a manner analogous to the action of the bi-metallic element in a household thermostat, with the strongest curvature occurring nearest the point of attachment to the cone axis, decreasing with proximity to the scale tip. Depending on the species, lengthwise shrinkage of this tissue varies from 10 to 36%. Shrinkage of the fibrous portion is negligible. The fibril orientation in the short rectangular cells would be at right angles to the long axis of the cell. Electron microscope studies support this assumption and show that the micro-fibrils are indeed oriented at 90° to the long axis of the cell. The shrinkage of wood is normally associated with the removal of water from the inter-microfibril zones of the cell walls and from the amorphous (noncrystaline) regions within the microfibril. The loss of water from the cell lumens does not affect cell dimensions. However the removal of bound water (below the fibre saturation point) from the normally three layered secondary wall initiates shrinkage. The differential shrinkage of the ventral and dorsal regions of the cone scale results in cone scale flexing which provides sufficient force to overcome the bonding of scales by the resin and thereby opening them to release seed. Further scale curvature results in more scale opening.

2.3 Cone ripeness and seed maturity

Rimbawanto *et al* (1988a) showed that *Pinus radiata* seeds ripening on the trees were fully germinable and of high vigour by the end of July, much earlier than previously thought, although at this stage they were difficult to extract from the cones without several weeks of air drying. They also showed that cone colour and moisture content can be eliminated as indices of seed development, but it is suggested that specific gravity may prove a useful guide. Rimbawanto *et al* (1988b) stated that cones harvested 3 months earlier than previously recorded, produced seeds of high germinability and vigour. They also stated that prolonged artificial ripening beyond 12 weeks caused some loss of seed vigour, but no index on cone maturation during storage formed a sound basis for assessment of viability and/or extractibility (degree of scale opening) of the seeds. They also indicated that artificial ripening triggered a change from the developmental to the germinative mode and there was a concurrent loss of seed moisture, but this was not the only change involved in this process. Seeds from artificially ripened cones showed a limited response to stratification, suggesting a small degree of residual dormancy, (Rimbawanto *et al*, 1988a). The reason why there is such limited information on seed development in *P. radiata* is the difficulty in extracting the seed from immature cones, especially using heat. (Rimbawanto *et* *al*, 1988a) also showed that the results of seed extraction of open pollinated (OP) cones can be extrapolated to controlled pollinated (CP) cones, since no differences were observed in their studies.

Edwards (1980) suggested that in a number of pines species cone maturity is attained when its specific gravity falls below 0.9 and the moisture content of the cones is below 50%. Rimbawanto *et al*, 1989 suggested that at this stage the respiratory capacity of developing seeds of *Pinus radiata* decreased as seed growth is completed and indicated that the developed seed coat contributed to the reduced metabolic activity of mature seeds. They concluded that the timing of seed development in *Pinus radiata* and changes in seed coat colour are the only reliable index of seed maturity. Wilcox and Forth (1980) suggested that artificial ripening of green *Pinus radiata* cones in paper bags reduced the ripening time of the cones from 6 months to 10 weeks, but did not significantly affect germination success or seedling height. This shows that physiological maturity is attained well before the seed is ready to be triggered into the germinative mode.

Arisman and Powell (1986a) noted that mature brown and green/brown cones opened better and yielded more seeds than green cones of *Pinus merkusii*, and seeds from the former cones also had higher percentages of sound seed, germination value and germination vigour. They noted that direct sunlight was satisfactory for seed extraction, especially from brown cones, (green/brown and green cones seed extraction improved after 2 or 4 weeks storage and then sun drying), and that germination values and germination vigour were also improved by pre-extraction cone storage.

Barnet (1988) reported that cone and seed maturity could not be accurately determined by specific gravity, but cone moisture content appeared to be directly related to both. A moisture content of 20% (dry weight basis) indicated maturity of both cones and seeds. The report also stated that cone storage for 4 weeks after harvest improved both seed yield and quality, in *Pinus strobus.*

Edwards (1980) noted that developing seeds of both angiosperms and gymnosperms are a rich source of growth regulating substances and changes in the levels of these compounds have also been associated with ripening. The major problem with tree seeds is that they never mature at the same time making seed collection difficult, since variation occurs from tree to tree within the same stand and also within the same tree. An increase in germination rate until peak maturity is considered as a maturity index for some species. This is however complicated by the onset of dormancy, making it difficult to draw the line between mature and immature seed. However from most literature cone specific gravity is suggested as the best method for ensuring seed maturity in *Pinus radiata* whose cone specific gravity has to drop below 1. It is also observed that for many pine species the more mature the seed the higher the seed vigour, immature seed being associated with low seedling dry weight, poor and slow germination, increased seed susceptibility to disease during germination and a higher proportion of abnormal germinants.

Wilcox and Firth (1980) recorded a 77% and 80% germination level in seed from naturally ripened and artificially ripened *Pinus radiata* cones respectively. This illustrates that artificial ripening does not reduce seed germinability (Rimbawanto, 1987). They also suggest that artificial ripening is important in avoiding a time delay of one year between the harvesting of seed cones and the establishment of a progeny test. The same authors also found that differences between mother trees was highly significant, with individual tree values ranging form 54 to 95%. This showed clearly that mother tree effects which presumably are partly genetic, have a much greater influence on germination than does the ripening process. There were some interaction effects of mother trees and ripening process on germination but less so for seedling height. Some mother trees showed very adverse effects of artificial ripening while others showed improvement in germination by this process. This clearly demonstrates the genetic influence of mother trees on the seed they produce. Despite this, the effects of the paternal parent would also be likely to influence the seed quality and subsequent progeny. This is most likely be influenced by the general and specific combining ability (g.c.a. and s.c.a. respectively) of the parents, since combining abilities are known to be an indication of the potential of parents to produce good progeny with hybrid vigour when crossed.

Arisman and Powell (1986b) showed that brown and green/brown cones of *Pinus merkusii* opened better and yielded greater weights and numbers of seeds than green cones. They also found that seeds from brown and green/brown cones had superior full seed percentages, germination value and germination vigour, and that splitting the cones yielded more seeds from green cones than any other extraction method. They also quote that split cone extraction was as effective as sun-drying after storage of green/brown cones for 4 weeks, and reported that the storage of cones of all maturity classes (green, green/brown and brown) increased cone opening and seed vigour and produced more physiologically mature seeds. In their experiments 20% of green cones opened fully and more than 40% remained closed without pre-extraction storage; while pre-extraction storage for 2 weeks raised the level of fully opened green cones to 50%, but this effect did not increase with further storage.

The degree of opening affected the seed yields from cones (Arisman and Powell 1986a). The split cone method yielded the same number of seeds from all classes of cone colours although fewer were extracted from green cones. This is a sign that some of the seeds were not mature and could not be extracted or separated from the cone scales. The courses of germination for the seed all followed a typical sigmoid pattern; with seed from brown cones germinating earlier than that from green/brown and green cones and germination from brown cones being always higher than from any other cone colour class. They also stressed that brown or brownish cones could be immature and turn brown due to nutritional deficiencies or insect infestation. Arisman and Powell (1986) found that sun-extraction of seed was suitable for *Pinus* *merkusii*, although they noted that sudden exposure of green/brown and green cones to heat resulted in the case hardening of cone scales; the causes of which are not fully understood. However it seems likely that high moisture content is probably the main cause.

Rimbawanto *et al* (1988b) suggested that the factor limiting seed extraction efficiency was not the development of the seed itself but the point at which the cones became amenable to seed extraction by conventional kilning techniques. They also reported that artificial ripening triggered the change from developmental to germinative mode and there was a concurrent loss in seed moisture. But this was not the only change. They reported that seed from artificially ripened cones showed limited response to stratification treatment, suggesting a degree of residual dormancy. Rimbawanto *et al* (1988a) also suggested that seed maturation occurred before cone maturation or drying which would be of considerable benefit to the seed production programme from controlled crosses in seed orchards.

2.4 Seed extraction methods

Serotinous cones can be opened by dipping them in boiling water for 10 to 120 seconds, and sometimes up to 10 minutes. This procedure produces maximum cone reflexing by melting the resins between the scales and by wetting the woody cone (Krugman and Jenkinson, 1974). Kiln extraction can be done at 120°F (about 50°C) for 48-72 hours. Seeds are then dewinged by machines of various types, by flailing in a sack or rubbing. Dewinging of some species can be simplified by first wetting the seeds, then letting them dry; wings are then loosened by this method and can be fanned out after drying. Care must be taken for mechanical dewingers, since they may injure the seeds. After dewinging and cleaning empty seeds can be removed by flotation in a suitable liquid for some species e.g. *Pinus taeda* in water. A liquid of suitable known specific gravity is used for this. Viability may be reduced by this method and so complete drying after immersion should be ensured.

Sometimes the cones open when they fall to the ground where they could be exposed to higher forest floor temperatures than are experienced in the forest canopy. Lotan (1967) reported that temperatures of between 45 and 69°C are adequate to open cones, with 45°C being the approximate minimum temperature needed to melt the resin itself. Krugman and Jenkinson (1974) however suggested that heat alone does not lead to complete seed release in the same time that heating moistened cones does.

Hellum and Pelchat (1979) used temperatures of between 60 and 82.2°C and durations of 4, 6 and 8 hours for the extraction of seed from cones of logdepole pine (*Pinus contorta* Dougl. var. *latifolia* Englem.) after soaking them in 50°C water for 5 minutes. As the temperatures were being raised from 60 to 80.2°C, seed release from cones increased from 20% to full seed release. Full seed release was taken as the maximum seed released under the given test conditions, realising that a small amount of seed was retained in the cone. They found that average germination declined from about 85% to less than 40% over the same temperature range. The best average germination, called the 'seed value', approached 80% when the cones were exposed to 60°C for 8 hours in the kiln. They concluded that temperatures slightly lower than 60°C should be used and that kiln time should be increased to about 12 hours; and that these suggested conditions might yield seed values approaching 90%.

Beaufait (1960) showed that the duration of exposure to heat was critical in affecting loss of viability when cones were subjected to high fire temperatures. For example the average total germination on 5 tests of cones exposed to 900°F (480°C) for 30 seconds was 88% while those for 60 seconds had no viable seeds remaining. Exposure of extracted seed to 700°F (370°C) killed all the seed in 10 to 15 seconds while a temperature of 1000°F (530°C) killed the seed in 1 to 5 seconds. It is concluded that high temperatures during prescribed burning will aid seed dispersal with little seed being lost. It is also reported by Hellum and Pelchat (1979) and Wang (1978) that seed obtained from cones in a second extraction germinated more poorly and sluggishly than seed from the same cones during initial extraction.

It seems apparent therefore that both temperature and duration are important in explaining why seed is damaged during the extraction process. It must also be borne in mind that seed release is more a function of cone scale flexing than of resin bond breaking.

Beaufait (1960) reported that re-wetting and desiccation of an already open scale will result in less than full seed recovery because permanent deformation causes the scale to remain in a fully opened position regardless of its moisture content. In such cases the scale is stressed beyond its elastic limit and the cellular material is likely to have set. He also reported that a full size cone has a moisture content of between 250 and 350 percent of its dry weight. This reduces to 15-25 percent when the cone has turned uniformly brown. Oldest cones are consistently within 2 percent of that found for cones one year older. All the cones are therefore in equilibrium with the atmosphere. This shows the importance of the equilibrium moisture content concept in drying biological materials.

Beaufait (1960) also reported that *Pinus contorta* cones were found to open in nature after extended winter cold periods with temperatures well below zero. Very low temperatures can cause moisture loss from biological materials through crystallisation and subsequent sublimation of free water. To test the effects of this phenomenon the author used freeze-drying to simulate winter conditions and managed to reduce the moisture content of the cones to 0.2% and maintained it for 3 months. The resin bonds remained stable and no cones opened. This suggests that winter opening of cones must therefore involve a phenomenon other than desiccation alone. It is also reported that a temperature of about 200°C opens cones within seconds, and that this would be within the temperature range experienced in the crown of a tree during a wild forest fire.

Anonymous (1946a) stated that prolonged drought and low humidity rather than temperature are critical in assisting cones of *Pinus radiata* to open and shed seed on trees before collection. Normally, cones of radiata pine remained closed for as long as 10 years, enabling cone collection for seed production to be carried out during any season of the year. This coupled with the fact that *Pinus radiata* seed shows no apparent difference in germination capacity related to storage temperature; (33-78°F (0-25°C) versus 35-40°F (1-5°C)). Anonymous, (1946b) suggests that seed can be collected from closed cones which may be several years old.

Bonner (1986a) reported that maximum seed extraction and germination occurred after cones had been stored for 8 days after harvest, when cone moisture content was less than 50% and the best extraction temperature was 35°C for *Pinus strobus*. Bonner (1987) also reports that a cone moisture of less than 50% is recommended before drying of *Pinus palustris* cones can be carried out without loss of seed quality.

Hellum (1978) reports up to 30% of seed may remain in the cone even after rigorous kiln drying. This often results in higher temperature extraction conditions being used in an effort to maximise seed release from cones (Hellum and Pelchat 1979; Writ 1931). Loss of seed viability often results in such circumstances.

Seed extractibility from pine cones has been a key concern with many foresters. Cone scales on totally green or only partially mature cones fail to expand when kilned, and remain in a closed, or semi-closed, position which prevents the seeds from falling out (Edwards, 1980). Only 78% of the seed could be extracted from *Pinus resinosa* cones which were immature. Also extraction costs were higher and seed quality was lower. Immature cones are high in seed moisture content and require longer kilning which may be lethal to seed. Cones collected closer to maturity open more readily when kilned, thereby increasing seed yields. A greater proportion of seed is filled at maturity although artificial ripening has been found to increase seed extractibility for some conifers. In general seed from immature cones has been found not to retain viability as well as that from mature cones. It has also been suggested that seeds extracted immature continue to mature in storage while those extracted when mature deteriorate slightly (Edwards, 1980). Despite this, immature seeds can not be expected to ripen in storage unless they have reached a critical point in their development while still on the parent tree. The specific gravity principle is more useful for pine cones as a maturity index than any other parameter. The use of multiple fruit characters has however been suggested for some fruits as the basis for assessing whether or not they are ripe. Although maturity estimates of fruits based on weather variables, such as radiation and precipitation have yet to be developed; such indices could be more universally applicable than fruit and seed parameters by producing more consistent and reproducible results.

2.5 Variation in cone serotiny

Cones of *Pinus radiata* are generally known to remain closed on the tree for several years. Naturally, agents for cone opening are known to be forest fires. However there is a general understanding that some cones open earlier than others under the same conditions. This could be attributed to genetic differences in parent trees. Cram and Worden (1957) found that differences with respect to maturity existed between cones on the same tree, between years for the same tree, and between trees in the same year (Cram and Lindquist, 1979). Also, Teich (1970) found cone serotiny to be genetically variable. While Shaw (1914) suggested cone serotiny to be an indicator of advanced evolutionary position. More recently cone serotiny has been said to vary with tree age, tree size, tree vigour, and crown physiology (Hellum and Pelchat, 1979).

Seed extraction from cones of lodgepole pine (*Pinus contorta* Dougl var. *latifolia* Engelm.) has kept scientists puzzled for nearly 90 years since 1910. Hellum (1980) identified temperature as the factor needed to open the cones since some cones with as low a moisture content as 6-7% remained closed. It has been assumed ever since that seeds are released automatically once the resin has melted or vaporized from the cone scales. This assumption has lead to a series of confusing reports by several authors on the subject of cone serotiny in lodge pole pine and jack pine. Hellum (1980) also quoted that cone opening required high or scorching temperatures, while seed release takes place under the influence of moderate but warm temperatures and some additional moisture. This is in agreement with Krugman & Jenkinson 1974. The report also noted that the two processes of resin bond breaking and cone scale flexing should be considered separately.

Hellum and Barker (1980) reported that seed release is closely associated with cone moisture content in lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Englm). While moisture content varied between 12 and 16% in cones stored in unheated shelters in Alberta, moisture contents between 15.5 and 18.5% were required to obtain full seed release in bulk lots of cones, depending on the lot. Past research reports the variable nature of cone serotiny in lodgepole pine, and this study suggests that a large part of this variability is due to variable cone moisture.

Hellum and Barker (1980) also reported that for every 1% seed retained in 14 000 hecto litres (HL) of cones, or killed through severe extraction, over CAD$5 000 was lost at 1980 current seed prices. The authors reported that extraction time varied considerably from the slowest to the most rapid lot (4.5 hours versus 11 hours). This implies that extraction costs can be nearly 2.5 times more expensive for slow compared with rapid cone opening lots. Their study also showed it did not matter whether or not the seeds were released quickly, viability remained generally high when a constant 60°C was maintained. Furthermore they discovered that the drier the cone is when it enters the oven the fewer of its seed it can release. They report that cones at about 7% moisture content retained about 25% of their seed while if they were at 12-16% moisture content they only retained about 2-10% of the seed depending on the cone lot. This variation between cone lots would indicate more of a genetic variation than a cone moisture variation.

Hellum and Barker (1980) reported that variable cone wood density, as influenced by site or genetics, may be another cause for variability in cone serotiny. While they reported that serotiny was due to the variable cone moisture contents of different cone lots. This could be a consequence of differences in cone density which in turn would be genetically controlled just as wood density is. They therefore urged an increase in cone moisture level up to 19% before extraction started. Such a situation leads to 100% seed release from all the cone lots they tested. The use of excessive heat therefore becomes unnecessary to obtain higher seed yields from a given lots; and re-soaking as reported by Van Haverbeke (1976) for *Pinus sylvestris* would also be unnecessary to obtain full seed extraction and therefore yield from valuable cone lots.

Hellum (1980) noted that the genetic aspects of cone serotiny are largely unknown and the importance of rates of cone moisture loss are still under study. Cones with less than 20% moisture content are reported to retain some seed during extraction. However when cones were put into a scorcher at ±180 °C for 2 minutes or less and then held at room temperature the resin bonds of all cones were broken and this reduced heat damage to the seeds due to over-heating. The report noted that silvery old cones should be treated the same as younger brown cones and that there was a great variation from tree to tree even within one stand. It was also noted that the higher the cone moisture content the longer it takes to break the resin bonds of the cone scales. The report also mentions that if cones are soaked in water for about 30 seconds they absorb as much as 14% moisture. Cones containing about 20% moisture content are reported to open easier than at any other moisture content provided the moisture is vented out of the kiln as soon as its is released. Too low and too high a cone moisture content results in a delayed time of seed release from the cones. Hellum (1980) also noted that seed could be released in less than 12 hours but maintained that a temperature of 60°C was not detrimental to seed germination even after 14 hours suggesting that this is a perfectly safe temperature: time combination for seed extraction. Empty seed content increased gradually from the second to the tenth hour of extraction and could account for 40% of the sample with time. So if extraction costs are high then there is need to use an optimum extraction time since a safe temperature does not result in low germination seed even after long exposure hours.

Teich (1970) studied pine cone serotiny in jack pine (*Pinus banksiana*) and lodgepole pine (*Pinus contorta*) using the Hardy-Weinberg law on data from several populations and by examining data on progeny of individual trees. The report showed that cone serotiny appeared to be controlled by a single gene with two alleles. It is also reported that the phenotype of the heterozygote has intermediate properties of both homozygotes and is subject to environmental influence; which may cause few or many of its cones to open. Because of this confounding effect, it was difficult to separate the heretozygotes from the homozygotes.

Teich (1970) also reported that the frequency of closed cones in natural populations of jack pine has been shown to increase with latitude and that of lodgepole pine to decrease with elevation although both types could be found at all elevations. The report also considers the existence of genetic control of cone serotiny in jack pine. The serotinous cone class was suggested to be homozygous recessive and the open cone class to be heterozygous, with more than one gene governing the inheritance of serotiny. A possibility of cytoplasmic inheritance is also suggested. Genetic control of serotiny is also suggested in lodgepole pine. Lotan (1967) reported that cone serotiny appeared to decrease as elevation increased for lodgepole pine and also reported latitudinal cline differences in *Pinus banksiana.* Lotan (1967) also noted that stands in contiguous physiographic areas can vary widely in fruiting habit. This not only illustrates the wide variation in the fruiting habit but also emphasises the need for a better understanding of the basic factors that cause cone serotiny including the possibility of a strong genetic component influence.

The genetic control of cone serotiny is very important in nature since it allows cones to release seed at different times. Thus if seed is released when environmental conditions are not suitable for seedling establishment, there is always a reserve of seed in closed cones which could be released another time, providing a survival mechanism in natural forest dynamics.

2.6 Seed storability

Pinus radiata seed is known to remain viable for as long as 21 years in cold storage (Anonymous, 1946a). The same storage life was also reported by Mirov (1946), Schubert (1952) and Kartiko (1990). Seed moisture content should be between 5 and 10% before storage.

Beaufait (1960) reported that the seed germination for *Pinus contorta* deteriorated from 76% for 1-2 year old cones, to 66% for 3-4 year old cones and to 33% for cones over 5 years old. Seed moisture content should be between 5 and 10% before storage at about 5°C, for coniferous tree seed.

Rodin *et al* (1991) reported that ageing *Pinus sylvestris* seed caused a genetic imbalance, some seed families being more affected than others, with a trend for seed of fast growing groups to die out more quickly. They reported that testing seed by artificial ageing makes it possible to predict the change in the seeds of particular parent trees during storage, and allows screening of seedlots and extension of the storage period up to 18 years. Mercier *et al* (1991) noted that *Pinus banksiana* cones (which are serotinous) with a marked curve (8mm deviation from the axis) yielded the greatest number of seed, but that seeds extracted from curved cones had below-average fresh weight. The same authors also found that the seed numbers and quality were unaffected by cone age, but recommended that cones be harvested before they are 7 years old.

Lestander and Bergsten (1986) reported that seeds which float on water have greater germination capacity than sunken damaged seed. Downie and Bergsten (1991) reported that they successfully used the IDS (incubation, drying and separation) technique to distinguish between filled good seeds and filled dead seeds of *Pinus strobus* and *Picea glauca* (Downie and Wang, 1992) and that the IDS technique could be successfully used to upgrade germinability and vigour of conifer seed lots of *Picea glauca, Pinus contorta* and *P. banksiana.*

Bonner (1986b) reported that most tests for seed vigour were not successful, with only tetrazolium staining and leachate conductivity showing any correlation with germination. The report also suggested that a germination test and measure of germination rate are most accurate for assessing seed lot vigour. Bonner, (1991) reported that leachate conductivity can be successfully used to estimate seed quality of *Pinus taeda, P. elliottii, P. palustris, P. echinata* and *P. strobus*, and gave equations for estimating the germination of these species.

2.7 Seed extraction by microwave oven heating

2.7.1 The nature of microwaves

The term microwave is applied to the wavelengths from about 1mm to 300mm, bordering the far-infrared range (Copson, 1975). Microwave frequencies are part of the radiofrequency portion of the electromagnetic spectrum. Neither term, radiofrequency or microwave, is explicit, but radiofrequencies are generally considered to occupy that region of the electromagnetic spectrum between audiofrequencies and the infrared region, or the mean frequency range useful for radio transmission, roughly 10 kHz to 100 GHz (Nelson, 1987). Microwaves, on the other hand are considered loosely to consist of that portion of the radiofrequency region with frequencies above 1 GHz (Nelson, 1987). Nelson (1979) also notes that radiofrequency (RF) dielectric heating was developed earlier than microwave heating and this RF heating uses frequencies below the microwave region. Thus the terms can be confusing unless the frequencies or wavelengths are specifically stated. However it must be noted that the dielectric heating phenomenon is the same, in that energy is converted from electromagnetic form to heat in the process, whether we use frequencies in the microwave range or lower frequencies. Some materials will always behave the same whether subjected to dielectric heating at microwave or lower dielectric heating RF frequencies. The level to which heat is absorbed by an object depends on the dielectric and thermal properties of the material in question. Thus different objects subjected to the same strength of microwaves will heat to different temperatures (Bakker-Arkema and Hall, 1965).

2.7.2 The use of microwave heating in agriculture

It is well known that seeds are heat sensitive and that heating to excessive temperatures will result in loss of germination. Microwaves provide a quick heating mechanism as opposed to conventional heating ovens and will therefore result in a sudden increase in the seed temperature which may be detrimental to seed longevity and or viability (Casada and Walton, 1983). It must be noted that high drying temperatures are injurious to seed due to rapid removal of seed moisture which cools the seed at first but heats and damages the seed as the seed moisture falls and less moisture is readily available for evaporation.

The use of microwaves for domestic heating is very common whereas its use in industry is very limited especially in agricultural processing. Despite this, its importance in agriculture has been of increasing interest in potential areas such as weed and pest control for example (Nelson, 1987). However there is no literature on their use in seed extraction from pine cones.

Nelson, (1987) suggested that agricultural uses of microwave heating include grain drying, seed treatment, insect control, product processing, and measurement of moisture content (Becwar *et al*, 1977; Lee and Latham, 1976). He also suggested that microwave application to heating for domestic purposes has increased public awareness of this form of energy. In addition the mass production of magnetrons to serve as microwave energy sources for domestic and industrial microwave ovens has lowered the costs of energy sources so that other new applications of microwaves might be considered.

Early research on rice drying with 27 MHz dielectric heating showed promise for the continuous drying of rice from field conditions to safe storage levels without damaging milling quality (Nelson, 1987). Similarly experimental RF drying of grain and grass seed at frequencies from 1 to 12 MHz showed improvement in the drying process over conventional drying techniques, and that drying could be accomplished at lower temperatures with dielectric heating in combination with heated air drying (Fanslow and Saul, 1971).

In forestry the microwave method could be very important for the extraction of seeds from the serotinous cones. Such cones have resin which melts at high temperatures (at least 45°C) and therefore this would be a suitable method of extracting the seed. The temperature to which the seed can be exposed is likely to be very critical to the continued viability of the seed. It is therefore apparent that the power level and the duration of exposure of the seed within the cones be standardised to achieve repeatable and efficient results. The high costs of microwave and RF dielectric heating equipment with capacities great enough for practical scale drying of most agricultural commodities and the energy costs for operation have been too great for serious consideration to be given for practical use. The application of RF and Microwave energy for treatment of seed and extraction appears likely to be more practical than most other agricultural uses. This will entail use for the processing of small valuable seed quantities like that obtained from controlled crosses in plant breeding programs. This will have a very useful application in forestry where the value of improved seed is high due to the length of the seed production cycle, (for example, *Pinus radiata* seed takes about 22 months to harvest from the time of pollination).

2.7.3 The use of microwave heating in research and industry

In agriculture in general, and in seed technology in particular, the use of microwave heating could be important in seed moisture testing (Okabe *et al*, 1973), drying and seed extraction, particularly when small samples are involved. As examples, Nelson (1987) used microwaves to improve the germination of small seeded legumes (alfalfa, red clover and arrowleaf clover) which naturally have impermeable seedcoats. Shivhare *et al* (1992a) used microwave drying for maize with promising results. Nelson (1987) reported success in controlling storage and wood infesting insects using microwaves. The success was made possible because of differences between the dielectric properties of grain and insects which enables selective heating of insects to lethal temperatures without spoiling the grain. A similar possibility occurs in the use of microwaves to kill weed seeds.

Brusewitz (1984) and Farmer and Brusewitz (1980) reported that very good correlation in seed moisture levels which were never statistically different from the standard oven method could be obtained using the home microwave, although Noomhorm and Verma (1982) acknowledge that the microwave is more suitable for moisture determination at high moisture levels than at low moisture levels due to the burning effected experienced at low moisture contents. The time taken to attain results with microwaves is very much shorter than the vacuum oven or air oven moisture testing methods, that is, Baker and Walz (1985) compare 3 minutes with 8 hours in sunflower using the air oven, and Noomhorm and Verma (1982) compare 3 minutes with 24 hours in sweetcorn using the vacuum oven (Verma and Noomhorm, 1983).

2.7.4 Factors influencing microwave heating

Nelson (1979) found that the dielectric constant was the most reliable dielectric property to use as an indicator of moisture content since it increases with moisture content (Backer and Walz, 1985), bulk density and temperature and decreases with frequency. No differences in variability of the dielectric properties among corn seed lots were found at 3 different frequencies, indicating no inherent advantage of any one of the frequencies used (Nelson, 1979).

When grain is dried with hot air, there are two factors that tend to slow the process; one is the low thermal conductivity of grain, and the other is case hardening of the kernels. Since grain is a poor conductor of heat the seed heats up slowly whereas a microwave concentrates the heat in those parts of the kernel where the moisture content is highest, usually the centre part of the kernels. This therefore makes heat transfer easier within the grain and overcomes the problem of case hardening in that the outer part of the seed is least heated and therefore will remain permeable to the passage of moisture from the internal parts of the seed due to a lower vapour pressure which is the main property influencing moisture migration in the seed.

Drying of grain by microwaves is faster than by conventional methods; however scale-up and process optimisation with respect to energy efficiency and final product quality have yet to be achieved (Shivare *et al*, 1992a, 1992c). There is need for a better understanding of the heat and mass transfer mechanisms dominating the microwave-induced drying of grains. Shivare *et al*, (1992a) also stated that in a microwave field, kernel temperature is driven internally at a rate governed by the intensity of electromagnetic (EM) stimulation of water molecules. Above a certain level of EM intensity, kernel temperature is maintained at a higher level than that of the inlet air.

Heat is generated in a kernel when it is subjected to microwaves and the internal heat generation is fixed for a given microwave power level. The temperature of the kernels is usually higher than that of the inlet air especially at higher power levels. The heat loss from the kernels to the air will increase and the cooling of the grain bed will take place if the air flow rate through the bed is increased while keeping the power level constant (Shivhare *et al.* 1992b). When the inlet air velocity is increased, the drying rate also increases. Conversely, outlet air temperature increases as the air velocity is decreased.

Brusewitz (1984) noted that the microwave oven and the convectional oven produce similar results provided they attain the same temperature. The problem with the microwave system is that it varies according to the initial sample moisture content and therefore different time settings are needed for different seed moisture contents. The accuracy and repeatability of the microwave oven method is dependant on the sample weight (size). Longer periods of time are required for larger sample sizes. Brusewitz also noted that use of a 500-700 Watt microwave oven at a setting less than full power runs the risk of inadequate temperature rise and therefore results in residual moisture in the dried sample. He also found that there was a correlation between the temperature of the sample and the moisture determination error level.

2.7.5 Adverse effects of microwave heating

Despite the potential promise of effectiveness and speed offered by the use of microwaves, some adverse effects on seed quality can occur following exposure (Darrah *et al*, 1977). Although the higher kernel temperature, as indicated by the outlet air temperature results in greater mass diffusivity and a shortened drying time, higher kernel temperature can also result in lower germination and bulk density. There is a limit to the possible increase in drying rate without adversely affecting the soundness of the kernels; severe discolouration and cracking results from excessive power levels. Deleterious effects have been observed on product quality at power levels exceeding 1 W/g and it has been established that the absorbed power for corn drying should be less that 0.75 W/g wet corn.

Shivhare *et al.* (1992b) summarised his findings on the use of a continuous microwave drying system for corn as follows:

1. Drying time was reduced considerably when higher levels of microwave power were used.
2. The inlet air temperature did not affect drying behaviour, but the moisture content at any given instant increased with increasing velocities of air.
3. The diffusion coefficient increased with microwave power, decreased with inlet air velocity and was unaffected by inlet air temperature.
4. The diffusion coefficient, in microwave drying of corn, followed the Arrhenius law with respect to outlet air temperature.
5. Microwaves can be applied to corn drying for seed, food and animal feed. Greater than 90% germination was obtained when absorbed power at 0.25 W/g was used for corn drying.

CHAPTER 3

3. MATERIALS AND METHODS

3.1 Seed extraction by conventional electrical heating ovens

3.1.1 Preliminary studies

Cones of *Pinus radiata* were collected on the 7th of August 1996 from recently felled trees in an 18 year old plantation near Foxton on the west coast of the North Island of New Zealand. Only brown fresh cones were collected. All cones were subjected to a flotation test in water for specific gravity. Only those cones which floated were used in the experiment as these had a specific gravity of less than 1. Seed extraction was carried out about 60 days after cone collection. Specific gravity is defined as the ratio of the cone weight to the volume expressed as grams per cubic centimetre. Specific gravity and moisture content have also proved to be reliable and accurate indices of cone and seed maturity in white spruce (*Picea glauca* (Moench) Voss) (Cram and Worden, 1957). Cone specific gravity was determined by the water displacement method as described by Krugman and Jenkinson (1974).

The air oven extraction temperatures used were 40°C, 50°C, 60°C and 70°C. The durations were 3, 6, 12 and 24 hours. Half the cones were dipped in hot water (60-65°C) for 1-2 minutes to break the resin bonds and the other half put in the oven without wetting (dry). The experiment was set up as a balanced factorial design with 3 treatments per experimental unit or replication. This gave rise to 3 treatment combinations. Each replication was made up of 10 cones, and there were 4 replicates per treatment combination.

After heating the cones were tumbled in an indented cylinder seed separation machine and seed was separated from the cones. The same cones were then kept for 7 days in ambient conditions after which they were re-tumbled to allow a second extraction of seed. The two extracted portions were kept separate for quality tests. Seed moisture content and germination tests were carried out as described in sections 3.3.1 and 3.3.2.

3.1.2 Extraction studies from cones of different families

Cones from ten *Pinus radiata* families were collected on 22 November 1996 from Matakana Island Seed Orchard owned by Carter Holt Harvey Forests (CHHF). The seed came from open pollination of the 875 series of genetically improved material from the New Zealand Forest Research Institute (NZFRI) but grown by CHHF in their seed orchard on Matakana Island. The last 3 digits on the family identification number refer to the clone number of the mother tree (maternal parent).

The 10 families from the 875 series were numbered as follows:

875.066, 875.076, 875.088, 875.096, 875.204, 875.207, 875.210, 875.218, 875.222 and 875.250.

When referring to these families later, only the number after the dot will be used for lot identification.

The final studies were carried out in the same manner previously described except the two best treatments from the conventional heated air oven treatments were used. These were 60°C for 12 hours and 50°c for 24 hours. Five cone replications were used for this final study.

3.1.3 Quality tests

Germination and seed moisture content tests were carried out according to the ISTA Rules (1985, 1993) as described in sections 3.3.1 and 3.3.2. In the final experiment however moisture tests were not carried out, due to insufficient seed.

In each treatment seed was also extracted mechanically by cutting and chiselling the cones. This was done to exclude heat in the control treatment and therefore effectively examine the effects of heat on the seed. Seed moisture content and germination tests were also determined in this control treatment.

3.2 Extraction using a microwave oven

3.2.1 Preliminary Studies

A household microwave oven was used to heat the cones of *Pinus radiata* about 60 days after they had been collected from an 18 year old plantation near Foxton. These cones were from an open pollinated source. They were placed in the oven as 5 cone replicates and there were 4 replicates per treatment. The exposure time for the microwave heating ranged from 30 seconds to 200 seconds in intervals of 10 seconds. At each exposure time the cones were either heated dry or were first wetted for 1-2 minutes in hot water at 60-65°C. This temperature was chosen to avoid cooking the seeds at higher temperatures. After heating in the microwave oven the cones were tumbled to release the seed after they had cooled down after about 30 minutes. These same cones were then left for 7 days after which they were re-tumbled. The seed extracted in the first instance was kept separate from the seed that was extracted 7 days later. All seed extracted was weighed before and after de-winging and cleaning. De-winging was done by hand; by rubbing between the palms. Cleaning was done using an air-screen cleaner which was effective in separating all extraneous matter and empty seed from the good full seed.

3.2.2 Final studies

After the preliminary experiments the studies were streamlined and the treatments reduced. The dry treatment was discarded as it took too long to get the cones to open as compared to the wet treatment. The streamlined treatments chosen were 30, 40 50, 60 or 70 seconds in the microwave after dipping cones in water at between 60-65°C. The treatment for extracting seed immediately after heating was also discarded because microwave exposure up to 70 seconds did not result in immediate cone scale opening, and cones were only tumbled after 7 days storage at ambient temperature. The seed was then assessed for seed quality properties.

3.2.3 Extraction studies from cones of different families

Cones from ten *Pinus radiata* families were collected on 22 November 1996 from Matakana Island Seed Orchard owned by Carter Holt Harvey Forests (CHHF) and previously described (3.1.2) were used.

The best two microwave seed extraction procedures (30 and 40 seconds exposure) were used to extract the seed. All the cones satisfied the flotation screening test for maturity, which requires cones to have a specific gravity of less than 1 if they are to float on water. All cones were dipped in water at 60-65°C for about 1-2 minutes before they were subjected to the microwave treatments. The seeds so extracted were dewinged by hand, cleaned and then subjected to a standard germination test for quality assessments.

3.2.4 Quality tests

Germination and seed moisture content tests were carried out according to ISTA Rules (1993) as described in sections 3.3.1 and 3.3.2. In the final experiment no seed moisture tests were carried out, due to insufficient seed.

Seed was also extracted mechanically by cutting and chiselling the cones. This was done to exclude heat in the control treatment and therefore effectively examine the effects of heat on the seed. The moisture content and germination were also determined for seed from this control treatment.

3.2.5 Cone heating studies

Cones were heated in the microwave oven for varying periods after which temperature readings were taken using an infra-red thermometer to monitor the drop in temperature over time. Readings were taken every 20 seconds. This gave an indication of how much heat the cones and seed absorbed when heated by microwaves and an impression of the effect of heat on subsequent seed quality.

3.3 Seed quality assessment

3.3.1 Moisture content and seedling dry weight

Moisture determination was carried out using the low temperature air-oven method (103°C for 17 hours) as prescribed in the ISTA Rules (1993). The moisture content of seed was expressed as a percentage of the fresh weight of the seed, (fresh weight basis).

The moisture content of whole cones was also determined at 103°C for 24 hours. This was similar to the method used by Rimbawanto *et al* (1988a) and by Hellum and Barker (1980). The moisture content of the cones were expressed as a percentage of their fresh weight (wet basis).

3.3.2 Seed germination

The germination test is the most familiar method of determining the quality of seed. This is because it is an actual measure of growth and all other methods of seed quality are measured against the standard germination. The germination test was carried out as recommended in the ISTA Rules (1991).

Four replicates of 25 seeds each were used for each treatment or seed lot. Thiram dust was used as a seed dressing to curb fungal growth. Menzies *et al* (1991) have previously reported that the application of Thiram fungicide before a germination test is important.

Heit (1961) reported that the best method of detecting and judging abnormal versus normal germination in conifer seed during routine laboratory practice is to test the seed on top of blotter in artificial light rather than sand-box tests. This is in agreement with ISTA Rules (1993).

Seeds were placed in plastic boxes on top of moistened paper blotters. The boxes were then put into a germination room at 20°C with continuous low light. The test period was 28 days with a first count at 7 days and every 7 days thereafter until the 28th day.

The seed was not stratified because Menzies *et al* (1991) reported that stratification is only useful if the seed is to be sown in cold soil conditions. Rimbawanto *et al* (1989) also reported that stratification was not necessary for freshly harvested seed.

In final studies with 10 different families, 5 single cone replicates were also used for all the treatments and therefore 5 replicates were used in the germination tests. Thiram was applied as a dust seed dressing at the beginning of the experiment to curb fungal growth.

A seed was considered as a normal germinant when the radicle length was 4 times the length of the seed. Wang (1973) reported that this criterion for germination agrees well for medium to large seeds. However seedlings from small seeds were reported to appear to reach 4 times their seed length at the stage at which seedlings had moderately developed hypocotyl with cotyledons barely or not yet visible.

Abnormal germination was also noted where it was exhibited. The categories identified as abnormal seedlings were as follows (Heit, 1961):

1. reverse germination - where cotyledons emerge first and radicle remains in the seed;
2. albinos - seedlings without pigment;
3. watery seedlings - seedlings translucent in appearance;
4. seed split open but with only short blunt extension of endosperm and radicle;
5. stunted and weak seedlings.

The last category of seedlings is the most difficult to clearly define and interpret yet it is the group where the most serious and greatest number of abnormal seedlings were found in the studies done by Heit (1961).

3.3.3 Germination progress and seedling dry weight

Germination speed was also measured with seed germination assessments being made every 7 days throughout the 28 day test period. At the final count, 10 seedlings (plate 1) were taken from each replicate and seedling dry weight was determined. This was done by drying seedlings in weighed metal containers at 80°C for 48 hours before re-weighing.

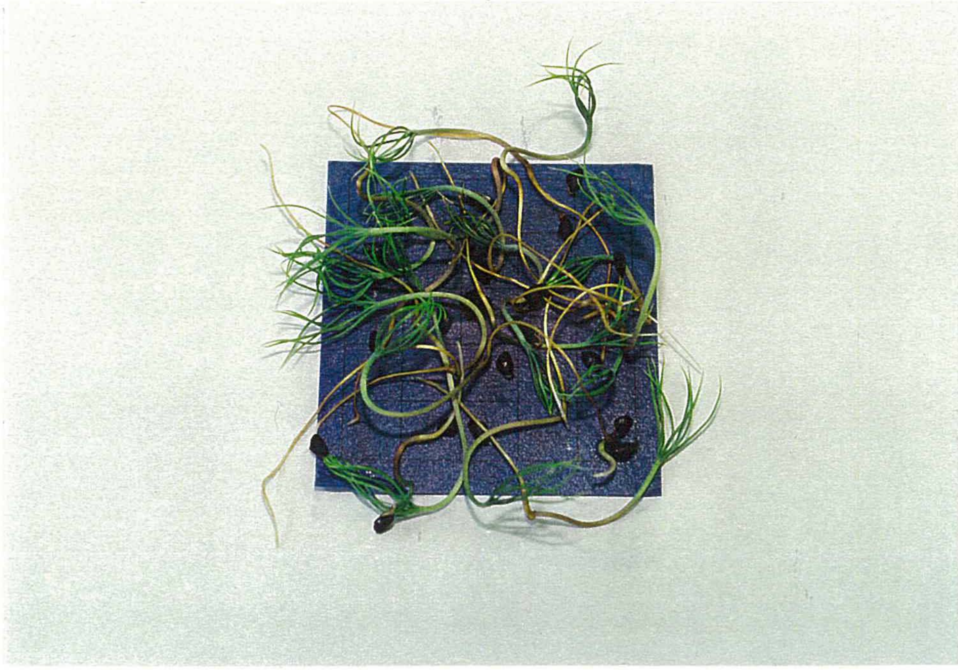

Plate 1. Seedlings of *Pinus radiata* after a germination test period of 28 days.

CHAPTER 4

4. RESULTS

4.1 PRELIMINARY STUDIES.

4.1.1 Heated air oven extraction

4.1.1.1 General

An analysis of variance (ANOVA) was performed on the data obtained from the conventional heating oven extraction and germination experiments. The data was analysed according to the method developed by Hellum and Pelchat (1979), where the amount of seed extracted was expressed as a percentage of the highest possible of the treatments used. Since in this study there were two extraction times, the percentage of extraction was expressed as a proportion of the highest at each of the extraction times, that is, immediately after oven heating or after 7 days, these were 44.1 grams and 34.2 grams respectively. The two percentages should therefore not be added directly. This percentage was then multiplied by the percentage germination of the seed from the different treatments. The product of the two percentages gave what is termed the 'seed value'. This optimum seed release and seed quality measure can only attain a maximum value of 100 or a minimum of zero just like a percentage, and is a 'super character' or 'merit character' which can be useful in discriminating between the treatments since the figure is based on actual measurements of performance. Seed value figures facilitate easier discrimination of treatments since a good treatment combination should give both high extraction values as well as high germination performance. This concept is based on the fact that a loss in the amount of seed extracted due to inadequate opening of the cone scales is just as bad as a loss in seed germination due to use of excessive extraction temperatures.

The initial treatment of immersing cones in hot water at 60-65°C for about 2 minutes showed that the resin bonds could readily be broken. The flexing of the cone scales however required the application of more heat to remove further moisture from the woody scales. Care was taken to prevent cones from absorbing too much water as this increased the time taken to dry the cones during heating before they released seed.

4.1.1.2 Seed extraction efficiency immediately after heating

Most complete seed release was obtained from dry cones that had been exposed to 60°C and heated for 12 hours, (table 1),for the seed extracted immediately after heating (denoted as zero days). Wetting was not beneficial at this temperature. This was therefore used to describe full seed release. The other treatments were expressed as percentages of this treatment. The second best results were given by wetted cones heated at 50°C and 24 hour duration, (table 1). For the best treatments, very little seed remained to be extracted after a 7 day delay with almost all seed being tumbled out of the cones immediately after heating in the oven. There was therefore no advantage of leaving the cones in these treatments for re-extraction after a further 7 days, (table 1). The 3 hour heating duration was not effective in any of the temperatures. However, even in the best treatment some residual seed was left in the woody cone as shown by the results of extraction after a 7 day delay. It was also observed that some seed was retained in the cone after heating at 70°C due to some cone scales reflexing to such an extent that they trapped the seed in the scales behind them - a situation which might be referred to as "scale reflexing seed entrapment" (Plate 2C).

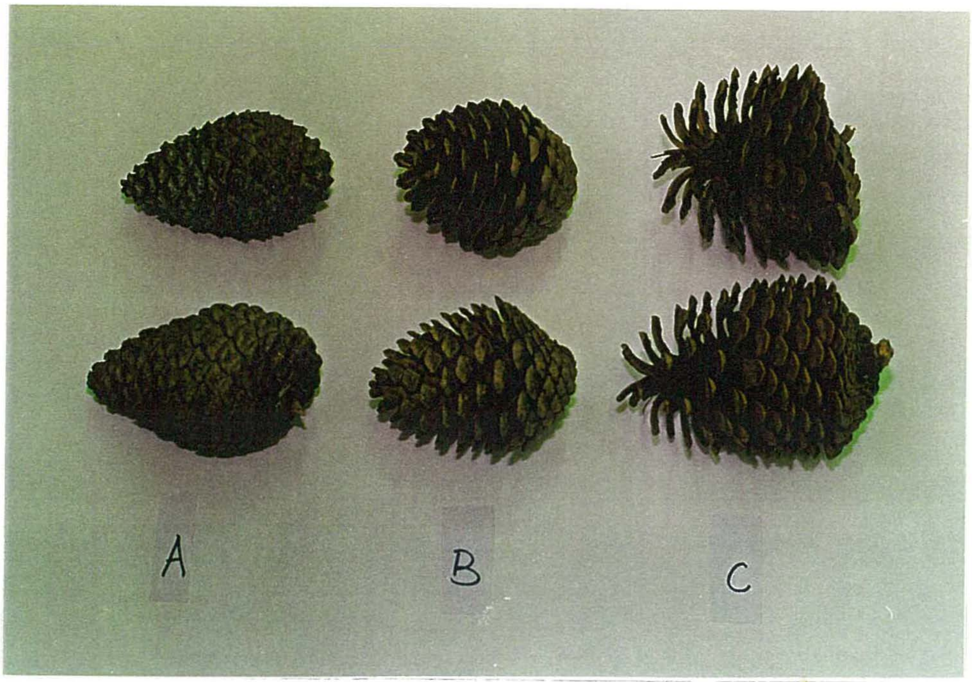

Plate 2. *Pinus radiata* cones after varying degrees of heating.

A - insufficient cone opening which does not allow enough seeds to be released because of low temperature and/or low heating duration.

B - sufficient cone opening due to adequate heating allowing complete seed release.

C - excessive cone opening due to excessive heating by high temperatures and/or long heating durations which is deleterious to seed viability.

The higher the temperature and the longer duration the more seed was released. The wetting treatment was generally not effective in improving extraction and the effects of wetting became generally negative for good extraction treatments. It should be also be noted that wetting did not improve extraction immediately after oven heating for the best temperatures and durations (table 1). Wetting was therefore discarded in further extraction experiments.

4.1.1.3 Seed extraction efficiency after a 7 day delay

Wetting was beneficial at lower temperatures (namely 40°C) and shorter durations (notably 6 hours) when extraction was done 7 days after oven heating (table 1). After 7 days the extraction of the lower temperatures and wetted treatments was higher than the higher temperatures and dry treatments, (table 1). Of the 7 day extraction treatments, the best treatment was 60°C and a 6 hour duration although it was about 10 grams lower than the best treatment when seed was extracted immediately after oven heating. However the 50°C and 6 hour duration and 70°C and 3 hour duration also produced acceptable extraction results. The 7 day extraction was, however, discarded as it took longer to extract the seeds and further experiments excluded this treatment. Normally in commercial pine seed production operations it would be more costly to keep cones for a further 7 days before tumbling due to the extra cost of sheltered storage space that is required to store a large bulk of cones.

The results show that temperature is no longer important in facilitating seed release after the cone scales open. This means that as soon as the resin bonds are broken, the cone scales can continue opening without further heating, although the rate of opening without further heating would be determined by the ambient temperature and relative humidity of the storage facility. These extraction results mirror those obtained when extraction was done immediately after oven heating since those treatments that produced high extraction percentages at the first extraction produced low extraction percentage at the second extraction after 7 days. This is because there was little or no seed left in the cones after the first extraction (table 1).

The results from this study show that within limits an increasing temperature has an effect on seed release that is comparable with the effect of a longer exposure time at a lower temperature. Similar results were reported by Hellum and Pelchat (1979) who reported that in general for every 10°C increase in temperature, exposure time could be shortened by nearly 2 hours and still allow comparable seed release.

Table 1. Effect of extraction temperature and duration of heating for wet and dry cones on mean extraction percentage for seed extraction immediately after heating or after extraction following seven days post-heating storage.

Temperature.	Duration	WET or DRY cones	mean extraction % zero days	7 days
40	6	D	-	22.8ef
40	6	W	-	19.4ef
40	12	W	5.8e	70.9b
40	24	D	1.3e	8.4f
40	24	W	10.6e	44.7d
50	3	D	0.0e	20.9ef
50	6	D	40.5d	61.1c
50	6	W	5.1e	79.8b
50	12	D	61.7c	32.0de
50	12	W	52.8cd	25.8e
50	24	D	93.1ab	1.1f
50	24	W	95.3ab	2.5f
60	3	D	0.0e	6.7f
60	6	D	40.8d	50.6cd
60	6	W	5.7e	100.0a
60	12	D	100.0a	8.2f
60	12	W	86.6ab	9.8ef
60	24	D	90.0ab	4.9f
60	24	W	91.8ab	5.2f
70	3	D	0.0e	53.3cd
70	3	W	0.0e	66.7bc
70	6	D	79.1b	25.3e
70	6	W	51.3cd	24.1ef
70	12	D	96.7a	6.5f
70	12	W	81.3b	6.3f
70	24	D	81.2b	11.1ef
70	24	W	86.3ab	9.2f

Probability of the difference (0.05)

Means with the same letter are not significantly different. Comparisons are within each column.

4.1.1.4 Effects of extraction treatments on seed quality of seed extracted immediately after heating.

It was observed that seed quality generally decreased with an increase in cone heating temperature and/or with an increase in duration of extraction (table 2). The results on seed quality were mirrored to those of extraction in the sense that the highest temperatures and durations produced inferior seed quality despite superior extraction results, (for example, 70°C and 12 hours). The moderate temperatures (50°C and 60°C) were more effective in producing both high extraction percentages and high seed quality. These deleterious effects of temperature have been reported in past research (Beaufait 1960, Writ 1931). Whether cones were heated dry (D) or after dipping in hot water (W) had no obvious effect on seed germination. This suggests that these treatments, while affecting seed extraction efficiency by initially breaking cone scale resin bonds, were not responsible for further cone scale opening and did nothing to affect seed quality.

4.1.1.5 Effects of extraction treatments on seed quality after a 7 day delay

The temperature and the duration affected germination capacity and there was an interaction between the two factors (table 2). This is not different to the first sample where this interaction was also significant. This means that the 70°C treatment was too hot and the 40°C temperature was too cool unless the heating period was 24 hours and tumbling was then delayed for 7 days. The 60°C and 50°C temperature produced good germination for most durations (table 2). The fact that duration was significant, and hence the duration by w/d interaction, would be expected since duration is complimentary to temperature. This also means that after 7 days the deleterious effects of temperature and heating duration had already affected the seed, for example, the low germination produced by the 70°C and 24 hour treatment (table 2). The w/d treatment did not have any effect on the germination percentage of the seed as was the case with seed extracted immediately after oven heating since that this treatment was only responsible for resin bond breakage.

The 70°C and 3 hours dry cones produced the similar results together to the 40°C and 24 hour duration for dry cones (table 2). This further demonstrates that extraction temperature and duration are complimentary (Hellum and Pelchat, 1979), that is, a low temperature can be compensated by increasing the heating duration. Cones exposed to these heating treatments did not release any seed in the first extraction sample and hence all the good seed still remained in the cones. Other treatments which performed well in seed obtained from the first extraction produced moderately inferior germination results in the second sample. This is due to the fact that there was little seed left in these cones, thereby reducing the probability of getting good seed. Some seedlots, for example the 60°C with 6 hours (W) heating treatment, which had the best extraction results after the 7 day delay and produced good germination from both extraction times.

Table 2. Effect of extraction temperature and duration of heating of wet or dry cones on mean germination for both extraction immediately after heating or after extraction following seven days post-heating storage.

Temperature	Duration	WET or DRY cones	mean germination % zero days	7 days
40	6	D	-	51b
40	6	W	-	60ab
40	12	W	69b	76ab
40	24	D	24c	77a
40	24	W	77a	74ab
50	3	D	-	70ab
50	6	D	54bc	62ab
50	6	W	40c	61ab
50	12	D	73ab	73ab
50	12	W	76ab	64ab
50	24	D	60b	34bc
50	24	W	69b	38bc
60	3	D	-	65ab
60	6	D	61b	64ab
60	6	W	62b	73ab
60	12	D	86a	55b
60	12	W	77a	67ab
60	24	D	77a	51b
60	24	W	66b	71ab
70	3	D	0d	77a
70	3	W	-	46b
70	6	D	68b	17c
70	6	W	55bc	60ab
70	12	D	64b	56ab
70	12	W	55b	47b
70	24	D	2d	9c
70	24	W	2d	7c

Probability of the difference (0.05)

Means with the same letter are not significantly different. Comparisons are within each column.

4.1.1.6 Effects of extraction treatments on seed value for seed extracted immediately after heating.

Seed value is the product of the germination and the extraction percentages and can therefore not exceed a value of 100. This concept was developed by Hellum and Pelchat (1979) who used it to give a realistic value to seed. The idea is based on the fact that a loss in the amount of seed extracted due to a lower temperature and therefore inadequate flexing of cone scales was just as bad as a loss in germination due to excessive heating temperatures. It would be thought that the best seed value might be obtained where average seed release percentage equals average germination percentage. This was not so because the seed value is a product (multiplication) of germination percentage and percent seed release. The 60°C and 12 hour treatment gave the best results on dry cones. This was followed by the 60°C for 24 hours treatment of dry cones and the 60°C for 12 hours treatment of wetted cones which were both not significantly different from the 50°C and 24 hour duration for wetted cones (table 3). This shows that 60°C temperature can be tolerated by pine seed for various heating durations without any detrimental effect on seed germination. The same relationship was reported by Hellum and Pelchat (1979) for *Pinus contorta*. The seed value was used as the final parameter for choosing the best treatments.

4.1.1.7 Effects of extraction treatments on seed value after a 7 day delay

For the 7 day extraction, the 60°C and 6 hour duration for wetted cones was the best (table 3). All treatments produced significant differences. This is due to the fact that the seed value is a parameter derived from two measured characters, the germination percentage and the extraction percentage. Thus significance is enhanced by one of the characters being significant even if the other is not. It is therefore apparent that while there may be no obvious differences when looking at the germination percentage or the extraction percentage as single characters, overall there would be a difference (table 3). Generally more seed values were poor as compared to the first seed samples obtained when cones were tumbled immediately after heating.

Table 3. Effect of extraction temperature and duration of heating of wet and dry cones on mean seed value for both seed extraction immediately after heating or after extraction following 7 days post-heating storage.

Temperature	Duration	WET or DRY cones	mean seed value zero days	mean seed value 7 days
40	6	D	-	10.1de
40	6	W	-	10.3de
40	12	W	3.9e	52.3b
40	24	D	0.5e	6.6de
40	24	W	8.7de	32.4c
50	3	D	-	14.7de
50	6	D	22.3d	39.2bc
50	6	W	2.0e	49.5b
50	12	D	45.0c	25.0cd
50	12	W	38.9c	15.9de
50	24	D	56.1bc	0.4e
50	24	W	65.2b	1.5e
60	3	D	-	4.9de
60	6	D	26.2d	29.6cd
60	6	W	4.1e	73.3a
60	12	D	86.2a	4.5de
60	12	W	66.5b	6.0de
60	24	D	69.5ab	2.7e
60	24	W	61.9bc	3.7de
70	3	D	0.0e	40.8bc
70	3	W	-	29.9c
70	6	D	55.3bc	5.6de
70	6	W	28.4cd	14.8de
70	12	D	60.4bc	3.8de
70	12	W	44.4c	2.9e
70	24	D	1.4e	0.8e
70	24	W	1.7e	0.7e

Probability of the difference (0.05)

Means with the same letter are not significantly different. Comparisons are within each column.

4.1.1.8 Effects of extraction treatments on seedling dry weight for seed extracted immediately after heating

The seedling dry weight is not very sensitive to extraction temperature and duration, (table 4). It would therefore appear that even though some treatments reduce germination they have little effect on seedling dry weight. The same was reported by Nath *et al* (1991). The effects of temperature were significant, only due to the fact that the highest temperatures reduced seedling dry weight (table 4), but lower temperatures (40 to 60°C) generally did not. Seedling dry weight was similar in 14 of the 16 treatments on seed extracted immediately after heating, and in 23 of the 24 treatments on seed extracted seven days after heating.

There was a significant temperature by duration interaction. This means that some seed extraction temperatures and duration treatments caused a considerable drop in seedling dry weight, (for example 60°C and 24 hours) (table 4). Thus a high temperature and long duration would be likely to have a detrimental effect on seedling vigour.

Some treatments did not produce enough seedlings to make up 10 seedlings per replication for the seedling dry weight comparisons. Notice the much lower seedling dry weight at lower temperatures and/or duration. These lower seedling dry weights at the lower temperatures, for example at 40°C and 12 hours or 50°C for 6 hours, are probably due to cone scales opening a little and thus only allowing smaller and inferior seed that produce lower vigour seedlings to be released (plate 2A). Very little seed was released from these low temperature treatments, thus lowering the probability of getting good seed.

4.1.1.9 Effects of extraction treatments on seeding dry weight after a 7 day delay

None of the factors lowered seedling dry weight, but there was a significant interaction between duration and wet or dry cone treatments. While this parameter is not easily influenced by extraction treatments (table 4), the interaction may reflect the fact that wetting the cones more readily breaks the scale resin bonds and thereby exposes seed in wetted cones to longer desiccation time in prolonged heating treatments that in dry cones where scale opening is slower. This desiccation is likely to be responsible for reducing seedling dry weight.

Table 4. Effect of extraction temperature and duration of heating of wet and dry cones on mean seedling dry weight following extraction immediately after heating or after a seven day delay.

Temp.	Duration	WET or DRY cones	mean seedling dry weight zero days	7 days
40	6	W	-	0.138b
40	12	W	0.120b	0.145ab
40	24	D	0.085c	0.168ab
40	24	W	0.150ab	0.195a
50	3	D	-	0.137b
50	6	D	0.140b	0.163ab
50	6	W	0.125b	0.173ab
50	12	D	0.162ab	0.183ab
50	12	W	0.153ab	0.155ab
50	24	D	0.168ab	0.180ab
50	24	W	0.165ab	0.160ab
60	3	D	-	0.163ab
60	6	D	0.160ab	0.162ab
60	6	W	0.145ab	0.185ab
60	12	D	0.182a	0.168ab
60	12	W	0.175ab	0.152ab
60	24	D	0.135b	0.155ab
60	24	W	0.135b	0.160ab
70	3	D	-	0.150ab
70	3	W	-	0.170ab
70	6	D	0.135b	0.155ab
70	6	W	-	0.140ab
70	12	D	-	0.170ab
70	12	W	-	0.160ab
Probability of the difference (0.05)				

Means with the same letter are not significantly different. Comparisons are within each column.

4.1.1.10 Second conventional oven extraction experiment

A second extraction experiment was carried out using the best two temperature and two duration air oven heating combinations previously considered to be of most use in terms of seed extraction efficiency and seed quality. The two temperature (50°C and 60°C) and duration (12 and 24 hours) combinations were reassessed using the same conventional oven methods previously described, that is, 10 cone samples and dry cones, but with the percentage of seed extracted being determined immediately following removal from the oven only. The results in table 5 clearly show that all four treatment combinations gave high (89-100%) seed extraction from cones and resulted in seed of high germination (95-99%). As a result seed values were in all cases high (88-98). Seedling dry weight was only reduced at a temperature 60°C and 24 hours duration compared with 60°C and 12 hours, again illustrating the adverse effects of prolonged desiccation.

It is interesting that in this second experiment seed germination percentages were considerably higher (95% plus) than those obtained in the preliminary experiment (table 2, 60%-86%). This suggests that the storage of cones improved the germination while extraction was only improved for the lower temperature and shorter duration (50°C and 12 hours, 98% compared with 53-61% in table 1). This also suggests that the two treatment combinations (50°C and 24 hours or 60°C and 12 hours) are likely to be most reliable when the age or collection date of the cones is not known. These two treatment combinations were therefore selected for the next experiment where cones from different families were evaluated.

Table 5. Results of the second oven extraction trial showing the extraction percentage, germination percentage, seed value and seedling dry weight.

Temp	Duration	Extraction%	Germ%	Seed value	Sdlg dry weight
50	12	97.9a	95a	93.3a	0.190ab
50	24	98.4a	99a	97.5a	0.190ab
60	12	100.0a	96a	96.2a	0.195a
60	24	88.6a	99a	87.8a	0.165b
Probability of the difference (0.05)					

Means with the same letter are not significantly different, in each column.

4.1.1.11 Seed and cone moisture studies

Seed moisture content

The seed moisture content (smc) was measured for all seed lots in the experiments reported in section 4.1.4, (table 6). There was a significant but small difference in moisture content at the temperatures 50°C and 60°C and for the durations 6, 12 and 24 hours of oven heating. Immediately after cone scale resin release cone flexing allows heat to penetrate directly to the seed enclosed within the scales. The longer seed remains in the oven the lower the seed moisture content. As a result the weight of seed extracted is likely to be less with longer treatment durations and higher temperatures. For this reason all weighed seed values were adjusted to a common 7% seed moisture content.

Table 6. Seed moisture content variation with extraction temperature and duration.

Temperature (°C)	Duration (hours)	Mean moisture content (%)
50	6	7.09a
50	12	6.86b
50	24	6.42c
60	6	6.95ab
60	12	6.55c
60	24	6.02d
Probability of the difference (0.05)		

Means with the same letter are not significantly different.

Cone Moisture content

Immediately after harvest individual cones were separated according to their ability to float or sink when placed in water, that is, had a specific gravity less or greater than 1. This water displacement method allowed a distinction to be made between cones which floated (mean cone moisture content of 14.5%) and those which sank (mean cone moisture content 22.9%) .

Following cone storage in jute bags for five months under ambient conditions seed moisture content in all cones had equilibrated to about 14.6%. In all cases these stored cones, irrespective of whether they had been classed as 'floaters' or 'sinkers' by the water displacement method immediately after harvest were subsequently all classed as 'floaters'.

It was noted that germination improved following storage of cones previously classed as 'sinkers'. This could be due to the fact that there is continued seed maturation in cones after collection (Rimbawanto, 1989) resulting in more mature seed after a five month storage period. It has also been reported by Rimbabwanto *et al* (1989), that moisture loss switches the seed from the developmental phase to the germinative mode. Dormancy is also overcome by cone storage which might also result in lower seed moisture.

Cone opening seems to be influenced not only by cone moisture but also by the strength of resin bonds between the cone scales. These resin bonds seem to 'weather' or weaken with time, irrespective of whether the cone is attached to the parent tree or not. Weathering however, is thought to occur faster when cones are detached from the parent tree and stored in a sheltered place, possibly because this prevents constant re-wetting of the cones. Dry conditions certainly seem to enhance the weathering of the resin bonds.

4.1.2 Preliminary microwave oven seed extraction studies

4.1.2.1 Initial screening experiments

There is no literature available on the use of microwave heating in seed extraction from *Pinus radiata* cones or any other coniferous tree species. Therefore an initial screening experiment was carried out (Appendix 1). From this, the preliminary microwave treatments were chosen. Most of the treatments in the screening produced very good extraction results but germination was poor. Only those treatments in which germination performance was good were therefore selected for further evaluation.

An analysis of variance (ANOVA) was performed on data obtained from the microwave oven heating extraction and germination experiments. The data was analysed according to the mean seed value method developed by Hellum and Pelchat (1979), (See section 4.1.1). This is based on the fact that a loss in the amount of seed extracted due to inadequate opening of the cone scales is just as bad as a loss in seed germination due to the use of excessive microwave extraction duration/temperature and/or power. The control seed lot was mechanically extracted.

4.1.2.2 Extraction using various cone numbers in the microwave oven.

4.1.2.2.1 Germination percentage and seedling dry weight

Table 7. Effects of cone number on extraction percentage and seed quality following microwave heating at two different durations (seconds).

Cone number	mean germination %		mean seedling dry weight (grams)	
	30sec.	60sec	30sec.	60sec
1	96a	0c	0.205a	-
2	95a	57b	0.170b	0.185ab
3	94a	94a	0.155b	0.170b
4	91a	84a	0.185ab	0.195ab
5	98a	88a	0.193ab	0.170b

Probability of the difference (0.05)

Means with the same letter are not significantly different for each column.

When various cone numbers were used in the microwave extraction procedure, there was a significant difference in germination between seed from different cone after 60 seconds exposure but not after 30 seconds (table 7). When one or two cones were used, results were significantly different from those obtained when 3, 4 and 5 cones were used for the 60 second duration. The 3, 4 and 5 cone treatments were not significantly different among themselves. However when one or two cones were used the germination results were significantly different from one another. The seedling dry weight did not seem to be affected by any of these extraction treatments (table 7).

4.1.2.2.2 Seed extraction percentage and seed value

Table 8. Effects of microwave oven seed extraction duration (seconds) on mean seed extraction percentage after 2 days, 4 days, 7 days and the cumulative mean total, and effects on mean seed value.

Cone	2-days mean		4-days mean		7-days mean		total mean		seed value	
no.	30 sec	60 sec	30 sec	60 sec	30 sec	60 sec	30 sec	60 sec	30sec	60sec
1	0.0c	73.2a	51.1bc	25.6c	36.4a	0.0b	87.5a	98.8a	84.2a	0.0c
2	0.0c	43.2b	95.5a	31.3c	0.0b	0.0b	95.5a	74.5a	90.4a	46.4b
3	0.0c	15.6c	63.1b	53.8bc	21.5ab	16.3ab	84.6a	85.7a	79.4a	81.0a
4	0.0c	0.0c	64.8b	89.2ab	18.4ab	10.8ab	83.2a	100.0a	75.2ab	83.7a
5	0.0c	0.0c	65.8b	67.6ab	20.6ab	16.6ab	86.4a	84.2a	84.4a	73.4ab
Probability of the difference (0.05)										

Means with the same letter are not significantly different.

Comparisons are made for each extraction day.

Overall table 8 shows that a higher microwave heating duration yields more seed in a shorter time (days) than a lower microwave exposure time. This confirms earlier results obtained at different days after microwave heating. However the total amount of seed extracted was the same after 7 days.

The results also show that a 30 or 60 second exposure in the microwave oven while sufficient to heat cones to the stage where scale resin bonds were broken, was not (particularly in the case of 30 seconds) sufficient to cause scale opening for seed release until at least 4 days after removal from the microwave oven. A similar situation occurred when 4 or 5 cones were exposed for 60 seconds. Nevertheless by 7 days after exposure seed extraction percentages ranged from 75-100% across both treatments and seed values of 73-90 were obtained except in the 1-2 cone 60 seconds treatment where seed values were low due to reduced seed germination.

When one cone is used seed is released earlier than when 2, 3, 4 or 5 cones were used at 60 seconds microwave heating duration (table 8). However more seed is released after the fourth day from the other treatments. The cumulative total for all the treatments is however not significantly different. The low number of cones used therefore only influenced the extent of seed release for early extraction at 2 days and 4 days after heating but not for the total extraction after 7 days. The results clearly suggest that the cone number or weight of cones is important in microwave extraction, as opposed to heating ovens where it would not make a difference.

4.1.2.3 Extraction using single cone samples

4.1.2.3.1 Seed extraction

Generally the extraction efficiency was acceptable with little differences occurring (table 9). The extraction efficiency was not ordered according to microwave heating duration, but can be related to duration, since the 30 second treatment gave the least and the highest duration gave the most extraction.

4.1.2.3.2 Seed germination percentage

Germination performance fell drastically from the control to the highest duration of microwave heating (table 9). This means that the temperatures reached by the seed were lethal in longer microwave heating durations.

4.1.2.3.3 Seed value and seedling dry weight

The combination of extraction percentage and germination percentage as shown by 'seed value' again reflected the major impact of increasing microwave exposure times on loss of germination (table 9). The detrimental effects of even short (30-40 seconds) microwave exposure times on seedling dry weight are also evident.

Table 9. Effects of microwave oven extraction on seed extraction percentage, germination performance, seed value and seedling dry weight using single cones in the microwave oven.

Duration (seconds)	Mean extrac percentage	Mean germ percentage	Mean seed value	Mean seedling dry weight (grams)
control	100.0a	90a	90.6a	0.158a
30	76.9b	39b	29.6b	0.130b
40	93.0ab	23bc	19.4bc	0.140ab
50	87.6ab	6cd	6.0cd	-
60	82.9ab	1d	1.5d	-
70	98.8a	0d	0d	-
LSD(0.05)	20.8	17	16	0.024

Means with the same letter are not significantly different.

4.1.2.4 Extraction using 5 cone samples.

Table 10. Effects of microwave oven seed extraction on extraction percentage, germination percentage, seed value and seedling dry weight when 5 cones were used.

Duration (seconds)	Mean extrac. percent	Mean germ. percent	Mean seed value	mean seedling dry weight (grams)
control	99.0a	95a	94.1a	0.208a
30	94.3a	96a	90.4a	0.184ab
40	78.6a	95a	75.1a	0.186ab
50	83.5a	97a	80.6a	0.160b
60	92.8a	89b	81.7a	0.166ab
70	100.0a	92ab	92.0a	0.162ab
LSD(0.05)	28.94NS	5.1	26.8NS	0.047

Means with the same letter are not significantly different.

The effects of microwave oven extraction on extraction and germination percentage and mean 'seed value' and seedling dry weight when cone numbers were increased to five per sample are shown in table 10. While there was no effect on mean extraction percentage the main effect of increased cone numbers was on seed germination which was maintained at high levels (89-97%) irrespective of heating duration up to 70 seconds. As a result seed values were also high, although there was a trend for seedling dry weight to fall slightly as exposure duration was increased.

The 30 second and 40 second treatments were therefore chosen for use in further extraction studies using cones from different genetic families. This was because there was no advantage in using longer microwave seed extraction durations. Five cone samples were used in all future tests.

4.1.2.5 Microwave oven studies on cone heat gain.

The differences in seed extraction efficiency and germination which occurred when different numbers of cones were exposed to microwaves is presumably associated with internal cone temperature during the time cones are in the oven. In addition however there is likely to be a continuation of this heating effect as cones cool after they have been removed from the oven. To examine this situation, five cones of different weights were heated in a microwave oven for 2 minutes. After removal from the oven cone temperature was measured using an infrared thermometer for up to 7 minutes and 20 seconds and the cooling rate was determined (figure 1). An infrared thermometer was used due to the difficulty of using a glass thermometer to measure the internal temperature of the cones and this type of device gave better assessment of cone temperature. The temperature of the cones reached about 100°C. The cooling rate was about 8.5°C per minute, but the rate of cooling dropped as cone temperature approached ambient temperature.

The amount of heat retained in the centre of the cone is however not easy to measure. Since wood is a poor heat conductor, the temperature drop is expected to be slower as temperatures approach ambient levels. Due to limited understanding of how microwave heating changes the structure of the woody cells of cone scales, it is not easy to explain the sudden opening of cone scales after heating for only 2 minutes in a microwave. Certainly an air oven does not achieve the same results in such a short time at 100°C.

Differences in the weight of the cones had no appreciable effect on cone cooling rate. It is generally known that the mechanism that causes the cones scales to open operates much the same as a bi-metallic strip, suggesting that microwaves have an effect which causes cone scales to exhibit this phenomenon in a shorter time without reaching the excessive temperatures as would be the case during conventional heating.

From figure 1, it can be seen that for each cone, the temperature drop was not a consistent loss. Variation in cone temperature could be explained by the fact that the hotter core of the cone is at a higher temperature and therefore continues to dissipate heat to the exterior by conduction. The fact that wood is a poor heat conductor makes this process take an extended time. There could also be some residual activity of microwaves in the cones. Seed obtained from heating cones in a microwave oven for 2 minutes and extracted after allowing them to cool to ambient temperature was dead.

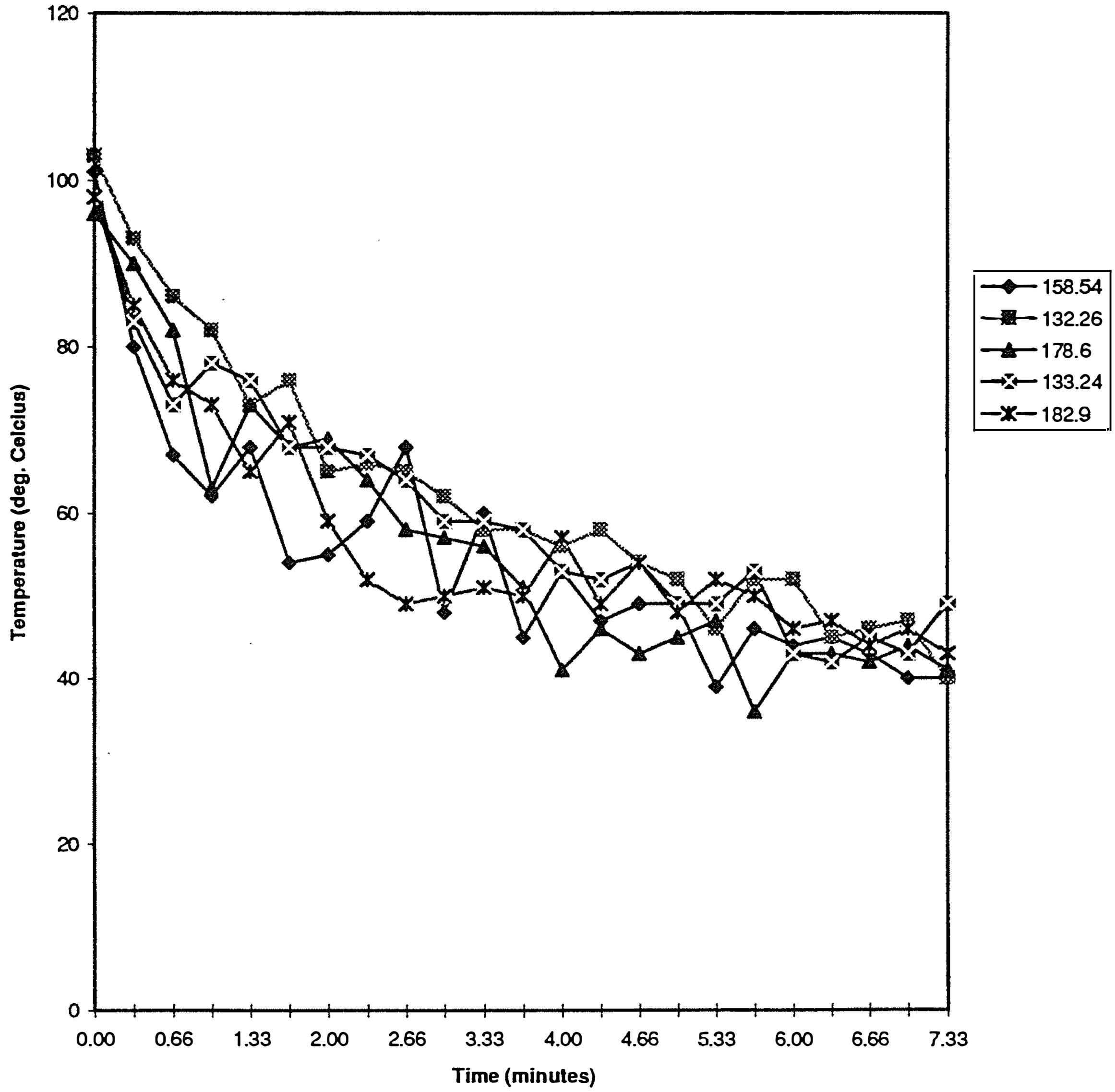

Figure 1. Cooling curves of 5 cones of various weights following removal from a microwave oven after heating for 2 minutes.

In a further study, five cone samples were dipped in hot water at 60-65°C for 1-2 minutes after which they were heated in the microwave oven for periods varying form 30 to 60 seconds. Thereafter their temperatures were measured using an infrared thermometer. These were the durations and condition of cones used in the microwave extraction experiment. Although the rate of cooling of the cones following removal from the oven is similar (0.3-0.5°C/minute), it is interesting that cone temperatures are not greatly different when they are exposed to microwaves for 30 to 60 seconds (figure 2). Figure 2 shows the cooling patterns of cones heated for the same durations used in the preliminary microwave seed extraction experiments. It is apparent that these different durations of heating raised the temperature of the cones to different levels.

The shortest microwave exposure duration of 30 seconds produced the lowest cone temperature of 38°C. This was followed by the 40 second and then the 50 second treatment with peak temperatures of 40°C and 42°C respectively. The 60 and 70 second duration did not differ much in terms of peak temperatures reached (45-46°C). This is perhaps surprising since much of the heat generated in cones is accumulated early in the microwave exposure period. In a 30 second exposure cone temperature rose by 19°C (0.63°C/second) while in a 70 second exposure cone temperature rose by only 26°C (0.37°C/second). This would be expected since cooler objects tend to gain heat quicker than hotter ones.

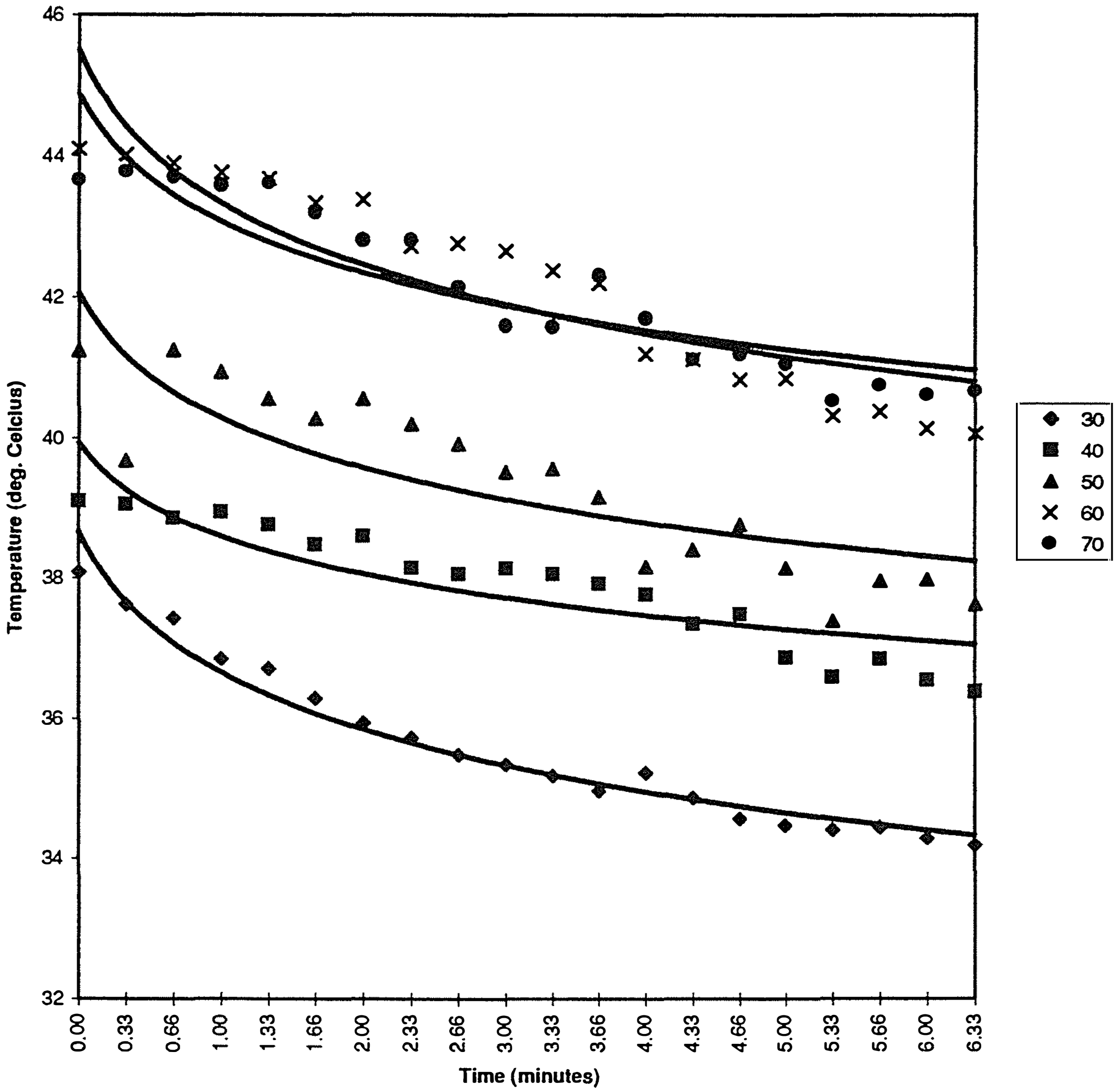

Figure 2. The mean cooling rates of various cones exposed to different durations in the microwave.

4.2 FINAL STUDIES

All the results described so far were obtained using pine cones from one source, that is, a mature *Pinus radiata* stand sited at Foxton. To test whether similar extraction and quality results could be obtained with cones representing a wider genetic range, cones from 10 different families were obtained from an open pollinated seed orchard owned by Carter Holt Harvey Forests Ltd. Although it is acknowledged that controlled pollination cones would have been more appropriate for this study, such cones were not available. Nevertheless cones from each of the 10 families were exposed to the same 'best' heating treatments used previously (air oven 50°C/24 hours, 60°C/12 hours; microwave oven 30 and 40 seconds). Unheated (control) seeds were obtained by manual extraction from cones.

4.2.1 Conventional heated air ovens

4.2.1.1 Effects on germination

There were significant differences between families in germination performance, following oven heating (table 11), but no family by temperature interactions. The fact that the cones are from an open pollinated seed orchard perhaps confounds the issue. This means that while the cones have a known maternal parent the pollinator is not known. This results in variation of seed genetics from cones from the same mother tree and even variation in seed from the same cone. However, there are differences in family performance with some having very consistent performance, (for example, family numbers 222 and 218). The 60°C and 12 hour heating treatment produced better overall germination performance than the 50°C and 24 hours treatment. Both treatments were however, no better than the control.

Table 11. Comparison of germination percentage performance for the conventional and microwave oven treatments and the control.

Family	Microwave oven (seconds)		Conventional oven		Control
	30	40	50oC+24hrs	60oC+12hrs	
066	64.8b	62.4bc	45.6bc	66.4ab	76ab
076	61.6bc	62.4bc	53.6bc	61.6bc	80.8ab
088	69.6ab	76.8ab	48.8bc	64.8b	83.2ab
096	80.0ab	44.0bc	39.2c	48.0bc	73.6ab
204	54.4bc	67.2ab	48.8bc	80.0ab	71.2ab
207	68.0ab	42.4bc	40.8bc	69.6ab	87.2ab
210	53.6bc	76.8ab	42.4bc	69.6ab	91.2a
218	80.8ab	70.4ab	59.2bc	76.8ab	76.0ab
222	84.8ab	84.0ab	82.4ab	82.4ab	84.8ab
250	51.2bc	65.6b	62.4bc	48.0bc	71.5ab
Probability of the difference (0.05)					

Means with the same letter are not significantly different. Comparisons were made for all means shown in the table.

4.2.1.2 Effects on seedling dry weight

It seems that seedling dry weight is not very sensitive to the effects of different extraction temperatures, (table 12) but is dependent more on the family. There were significant differences between families but not between treatments except for the families 076 and 250. This uniformity in seedling dry weight could be related to thousand seed weight (TSW) (table 13), since with smaller seed producing a lower seedling dry weights. No temperature and family interactions were found which makes the suggestion of a link between the TSW and seedling dry weight even more likely.

Table 12. Comparison of overall seedling dry weight (grams) performance for the conventional and microwave oven treatments and the control.

Family	Microwave oven (seconds)		Conventional oven		Control
	30	40	50oC+24hrs	60oC+12hrs	
066	0.192ab	0.194ab	0.188ab	0.180bc	0.182bc
076	0.147cd	0.190ab	0.150cd	0.180bc	0.168bc
088	0.150cd	0.130cd	0.140cd	0.140cd	0.168bc
096	0.206ab	0.200ab	0.200ab	0.218a	0.196ab
204	0.167bc	0.168bc	0.162bc	0.148cd	0.186b
207	0.192ab	0.173bc	0.170bc	0.180bc	0.184bc
210	0.142cd	0.188ab	0.166bc	0.172bc	0.178bc
218	0.164bc	0.150cd	0.156c	0.166bc	0.134cd
222	0.154cd	0.154cd	0.144cd	0.132cd	0.126d
250	0.197ab	0.172bc	0.182bc	0.148cd	0.153cd
Probability of the difference (0.05)					

Means with the same letter are not significantly different. Comparisons were made for all means shown in the table.

4.2.2 Microwave oven

4.2.2.1 Effects on germination

There were significant differences in germination between families, (table 11). The control had better results than both microwave oven treatments which were not significantly different from each other. As in the conventional oven method the families 222 and 218 are consistently high in germination for all the treatments and the control. However there is no definite trend between the 2 microwave treatments, that is, neither of these treatments were consistently better than the other as was the case with the heated air oven method. This suggests a complex mechanism in microwave heating since each cone is likely to have different dielectric properties, which may affect the heating effect of microwaves. Another possible explanation is the differences in individual cone weights between families.

4.2.2.2 Effects on seedling dry weight

Seedling dry weight varied significantly between families, (table 12). There was also a significant interaction between family and microwave oven exposure duration, suggesting that some families responded to microwave heating (for example, 066 and 207) more than others. These results are similar to those obtained from the heated air oven and supports the suggestion that seedling dry weight is linked to seed size which in turn is related to family genetics. Kusmintarjo (1992) also suggested that smaller seed produces smaller seedlings which are of lower vigour.

4.2.3 Conventional and microwave oven treatment comparisons.

4.2.3.1. Effects on germination.

The results (table 11) clearly show germination differences between the two oven heating and two microwave treatments. However there are also major differences in germination between families, but no significant interaction between family and treatment. A comparison of the two oven heating treatments shows that the 60°C and 12 hour heating treatment is generally superior in terms of subsequent seed germination to the 50°C and 24 hour treatment. This suggests that the advantages of cooler temperatures is more than offset by the longer heating duration. The 30 second microwave treatment resulted in seed with generally superior germination than seed heated for 40 seconds, for example, families 096 and 207. Despite this, none of the microwave heating extraction treatments matched the germination results obtained from seed in the unheated control. Of the 10 families used in this study only 222 and 218 showed comparable germination results to the control across all extraction treatments. In other families (for example, 210) major inconsistencies occurred.

Generally the microwave treatment was better than the oven method (table 12), in terms of germination percentage. However, differences are not obvious in some families. Again the families 222 and 218 stand out from the rest in terms of consistently high germination for all the treatments. When compared to the control, there is a larger drop in germination percentage for the heated air oven than for the microwave oven method.

4.2.3.2 Effects on seed and seedling weight

The seedling dry weight showed significant differences between families, (table 12). There were few significant differences between treatments. This shows that differences are due mainly to family genetic differences. There were also significant differences between families in TSW (table 14). The trends in the TSW and the seedling dry weight are linked. In most cases the larger seeded families had the highest seedling dry weight. This is a logical connection since seeds which are heavier are more likely to produce larger seedlings because their endosperm (megagametophyte) is larger and the germination test conditions support little photosynthesis, making seedling growth dependent on seed endosperm size.

4.2.4 Thousand-seed weight (TSW) comparisons by family.

Table 13. Mean thousand seed weight (grams) shown by family.

FAMILY	Mean 1000-seed weight
210	36.60a
207	35.2ab
066	32.2bc
250	32.0bc
204	30.0cd
096	29.6cd
088	28.3cd
076	26.1de
222	24.0e
218	23.7e
LSD	4.1

Means with the same letter are not significantly different.

The results in table 13 clearly show that major differences in seed weight occurred between families. Perhaps surprisingly, however, the two most consistently performing (germination) seed lots (218 and 222) were the two lightest seedlots with TSW of 23.7 and 24.0 grams respectively. These values are about one third lighter than the TSW of the heaviest seeded families (207 and 210). Seed from these latter two families were markedly and adversely affected by heat extraction in terms of germination despite their high control germination values (87 and 91% respectively). Perhaps lighter (smaller) seeds are less affected by the temperatures imposed by the heat treatments used, that is, microwave 30 seconds (39°C), 40 seconds (40°C) and conventional oven temperatures of 50°C and 60°C.

CHAPTER 5

5. DISCUSSION

5.1 General

The most appropriate oven and microwave heating systems identified in this study were effective in facilitating high levels of seed release from pine cones and of allowing the harvest of seed with high germination. These treatments involved either a conventional air oven at 60°C for 12 hours or a microwave oven for 30 seconds. The major difference in these systems was that although both were effective in breaking the resin bond between the scales of the serotinous cones, scale flexing to allow seed release was not complete in microwave treated cones until up to seven days after heat treatment.

The question of moistening cones prior to heat treatment to assist resin bond breakage and cone scale reflexing and seed release when heat is applied has been considered by other workers (Krugman and Jenkinson, 1974). They suggest that heat alone does not lead to complete seed release in the same time that heat and water does. In the present study wetting cones by dipping them in water at 60-65°C for 2 minutes was effective in improving ultimate seed release. However it was only of value if it was used in combination with oven heating of cones at lower temperatures, for example, 40°C for longer durations, for example, 24 hours or with microwave oven heating. Since the advantage of heating dipped or wet cones compared to dry cones was not obvious in the higher oven temperature treatments (50°C, 60°C, 70°C) which included those ultimately chosen as the 'best' treatments for seed release, the continuation of comparison of 'wet' and 'dry' cone performance in air oven work was discontinued after the preliminary study.

Seedling dry weight was found to be closely related to seed size, as previously reported in this species by Kusmintardjo (1994, 1992). Since the metabolic process determining seedling growth rate are distinct from those controlling germination

(Argerich *et al*, 1979; Scott and Jones, 1985; Alvarado *et al*, 1987; Alvarado and Bradford, 1988; Odell and Cantliffe, 1986) this may explain why the germination percentage was high in some families which produced seedlings of low dry weight, for example, 222 while the reverse situation occurred in others (for example, 096, 204).

Following heat treatment, whether this was in an air oven or in a microwave oven, cones in the preliminary study were stored under ambient conditions for 7 days before further seed was extracted. This allowed further drying, reflexing of cone scales and seed release. It was interesting, however, that only cones which had received sufficient heat exposure to break the scale resin bonds were capable of scale opening during this 7 day storage period. Natural drying alone was not sufficient to allow subsequent cone scale opening. This situation was seen in the higher quantities of seed extracted immediately after oven heating in some treatments (for example, air oven 60°C, 70°C) and the large quantities of seed which were removed after 7 days post heating storage in other treatments (for example, microwave treatments).

5.2 Conventional oven

The overriding question which dominated this study was, how can we obtain maximum seed release from closed scale cones of *Pinus radiata* without an unacceptable loss of seed quality (germination and seedling dry weight)? Hellum and Pelchat's (1979) study on lodgepole pine (*Pinus contorta* var *latifolia*) considered a similar problem. They concluded that both temperature and duration are important in explaining why seed is damaged by the extraction process. Their use of 'seed value' comparisons was adopted in this study and proved useful in identifying those treatments more likely to be of value. This resulted in the exclusion of 40°C and 70°C oven treatments after the preliminary study, - since the former was insufficient to allow seed release and the latter was deleterious to seed germination.

Another interesting feature of heat extraction of seed and seed release was the effect of excessive temperature and duration combination on scale reflexing. Under these conditions cone scales continued to dry and reflex to the point where they prevented seed release from opened scales below them. This over-reflexing of scales resulting in entrapment of seed in adjacent reflexed scales was most apparent in the lower third of cones towards the tree attachment point (plate 2C).

There was considerable variation in the response to both heat extraction temperature and duration in terms of seed germination. This was not surprising and has been previously well documented by Thompson (1969) and Teich (1979). However cones of some different families were very tolerant to varying extraction temperatures and durations, (for example, 222, 218). Others were not. This suggests that no one single heat extraction combination can be expected to give consistently identical results for seed from different families. However whether this reflects differences in cone serotiny, seed thermosensitivity differences, cone wood density, resin bond strength, or is related to seed size and/or moisture content is not known. The deleterious effects of high temperatures on seed have also been reported by various researchers (Arisman and Powell, 1986; Edwards, 1980; Hellum, 1980; Hellum and Pelchat, 1979; Thompson, 1969; Heit, 1961; Beaufait, 1960; Wright, 1931).

One surprising aspect of this study was the improvement in seed germination capacity when cones were dry stored at ambient temperature for 5 months between the first (preliminary) and second air oven experiments. Seed germination percentage rose from approximately 70% in freshly harvested cones to 95-99% when the cones were dry stored. Rimbawanto (1987) also reported a similar improvement in seed germination with cone storage in *Pinus radiata*. Seed after-harvest maturation and dehydration are likely to be features of this effect. At harvest, *Pinus radiata* cones contained seed of approximately 17% moisture content. After 5 months dry storage air dry cones contained seed of about 7% moisture content. These features may also have been important as possible reasons for the highly consistent performance of some families (218, 222) during seed extraction compared with the inconsistent performance of seed from others (for example, 096).

One of the major problems in this study was the considerable variability among cone lots. This variability was expressed in differences in response to extraction temperature and duration and was also obvious when seed was extracted from cones of different families in the final study. This makes a precise prescription of optimum heating temperature and duration impossible. Despite this, not all cones of different families behaved identically in terms of released seed quality. Whether this variation in response was related to seed size, moisture content or was genetically controlled was not determined, although the two families which showed most uniform heat resistance and highest seed values were those with the lowest seed weight. This question of variability has also been considered by Hellum and Pelchat (1979) in lodgepole pine where standard deviations showed extreme differences among cone lots exceeding 25% at the 1% standard deviation level.

5.3 Microwave oven

The 'wet' treatment was, however, very useful in improving both seed release and germination when cones were heated in the microwave oven. Dipping cones in hot water prior to microwave treatment lowered the exposure time needed to break resin bonds compared to dry cone exposure. The dry cone treatment was discarded after the preliminary study since it proved to be lethal to seeds.

Acceptable seed values were obtained after microwave heating of wetted cones for 30 or 40 seconds. Greater duration resulted in poor seed germination. Microwave exposure was effective in breaking scale resin bonds but did not result in scale reflexing immediately after treatment. A cone storage period of up to 7 days was necessary to allow sufficient scale dehydration to ensure full scale reflexing and seed release.

Seed quality following microwave heating was greatly affected by the number of cones used. Although cone number did not influence subsequent seed extraction percentage, seed germination was adversely affected unless at least 3 cones, and preferably 4 or 5 cones were used in each sample. This presumably reflects the lower maximum temperature of each cone with increase in cone numbers as suggested by Nelson (1987, 1979. Despite this there is a continuation of cone and seed drying during the rather prolonged cooling which occurs after cones are removed from the microwave oven. Loss of cone temperature of approximately 8°C per minute frequently occur.

Cone density, synonymous to bulk density reported by Nelson (1987) and Shivhare *et al* (1992b) to be a major factor in microwave drying of corn, is likely to be important in affecting microwave heating sensitivity. Similarly, seed and cone moisture levels and variation between and within cones are likely to affect seed germination after microwave exposure; simply because higher moisture tissues attain higher temperatures during microwave heating (Baker *et al*, 1991). Despite this, cone temperature measurements, in the microwave oven, obtained using an infrared thermometer were relatively low (39-45°C) during 30 or 60 seconds exposure provided 3-5 cones were used. When only 1 or 2 cones were heated temperatures rose to levels lethal to seed viability. Copson (1975) has reported that the quantity of material to be heated in a microwave oven or the power level applied are unimportant. It is the final temperature to which the material is heated and the duration of exposure which determines ultimate effectiveness.

CHAPTER 7

7. CONCLUSIONS

Variation in seed viability between cones of *Pinus radiata*, even when they were collected from the same source, had a major effect on the interpretation of results. The considerable variation between and among cone lots in response to extraction temperature and duration does not make it possible to prescribe one general extraction process suitable for all cone lots. Despite this, best seed value (that is, most complete extraction of best quality seed) was generally obtained in this study when cones were exposed to either 60°C for 12 hours in an air oven or for 30 seconds in a microwave oven (using at least 3 and preferably 5 cones). Seed values dropped at longer durations in the air oven and longer exposure times in the microwave oven. The advantage of the air oven extraction system is that seed release is almost complete by the end of the treatment. Cones heated in the microwave oven for 30 seconds do not show immediate scale reflexing and full seed release is only possible after treated cones have been air-stored for up to 7 days.

Scope for further research

1. Examine the efficacy of intermittent microwave heating on seed extraction using various microwave power levels.

2. Examine the effects of microwave treatment on seedling dry weight or seed vigour.

3. Examine the effects of reducing the duration of heating at 50°C and 60°C on the extraction efficiency and quality of seed obtained from heating closed cones in a conventional heating oven.

4. Determination of the relationship or response of seedling dry weight to various seed sizes and stress conditions. Also the determination of the threshold level after which increased seed size ceases to be an advantage.

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APPENDICES

Appendix 1. Means for different durations (seconds) of microwave oven heating for the weight of seed extracted, the percentage of seed extracted, germination perfomance and seed value for the initial treatment screening, using 5 cones in the microwave.

Wet or Dry	Duration (seconds)	Wght of seed extracted (grams)	Extraction percentage	Germination percentage	Seed value
dry	40	0.00	0.000	-	-
dry	60	6.89	18.25	68	12.4
dry	80	1.72	4.56	88	4.0
dry	120	21.55	57.07	56	32
dry	160	33.52	88.77	12	11
dry	180	37.76	100.00	16	16
dry	200	35.47	93.94	4	4
wet	40	21.95	59.13	76	44
wet	50	31.87	84.40	100	84
wet	60	23.14	61.28	88	54
wet	70	34.29	90.81	84	76
wet	80	25.69	68.03	72	49
wet	90	24.39	64.46	72	46
wet	100	21.82	57.79	72	42
wet	110	24.18	64.04	48	31
wet	120	27.39	72.53	84	61
wet	130	32.37	85.73	64	55
wet	140	29.94	79.29	24	19
wet	150	28.72	76.06	44	33
wet	160	29.33	77.67	4	3
wet	170	19.54	51.75	12	6
wet	180	28.00	74.15	0	0
wet	190	27.35	72.43	0	0
wet	200	28.65	75.87	0	0