# SUCCESSFUL REJUVENATION OF RADIATA PINE

Dale R. Smith[1]

Abstract:--Clonal forestry with radiata pine (and other pine species) has been constrained by the phenomenon of physiological ageing. Well-replicated trials in New Zealand have demonstrated that vegetative propagation from ortets older than three years results in volume loss in plantations compared to that expected from seedlings with the same genetic background. In order to take advantage of the benefits of clonal forestry, research and development has centred on techniques such as hedged cutting stool beds, cool-stored micropropagules, and cryopreserved embryogenic tissue from immature zygotic embryos. These methods, while all effective, require that a significant up-front investment is made to store material, much of which will give genetic gain no greater than that from seedlings. Data is presented which indicates that trees with superior growth and form, as well as a combination of valuable wood properties, are present at low frequency in a mature stand. Only 5 trees in a 15 hectare stand of *Pinus radiata* had diameters in the top 10 percent, no reaction wood, ring 16-20 basic density greater than 470 kg / $m^3$, and ring 20 mean tracheid length greater than 4.0 mm. This represents 0.03% of the trees initially planted.

Embryogenic tissue was induced from the apical meristems of vegetative buds of the 5 select trees. Mature somatic embryos formed in all 5 clones, and were converted to plants. Somatic seedlings were used as a source of rooted cuttings for use in field trials. This is the first demonstration of apparent rejuvenation of a commercial pine species, and the operational significance cannot be overstated. It is now possible to select mature trees in the field on the basis of their individual fiber properties, and through rejuvenation, make available to the forest establishment manager planting stock which will not show the growth rate penalty shown with cuttings were taken from old trees.

## INTRODUCTION

There is in the wood utilisation community within New Zealand a general and increasing (but difficult to document) disenchantment with some of the wood properties of fast-grown radiata pine. Unacceptable levels of dimensional instability of both sawn timber and mouldings, particularly of clear-wood resulting from pruned, heavily thinned stands, is frequently reported by saw-millers and manufacturers. Changes in kiln-drying schedules and processing plant design have helped alleviate the problem, but there are a number of new tree improvement and wood property research initiatives in train to find other solutions. Clonal forestry research and development is one possible route to improvement of the crop.

Enthusiasm for clonal pine forestry in New Zealand has waxed and waned over the last 50 years, and the evidence for greater economic gain through clonal forestry has been questioned, compared to a control-pollinated orchard strategy coupled with vegetative multiplication (Carson, 1986). Cutting techniques were first demonstrated by Field in 1934 (Field, 1934), and techniques for vegetative propagation of sexually mature radiata pine *(Pinus radiata* D. Don) were demonstrated over 30 years ago in New Zealand (Thulin and Faulds, 1968) but attempts at that time to establish commercial stands with ramets from elite, mature trees were not successful. Although clones displayed the expected improvements in form and intra-clonal uniformity in replicated field trials, stem volumes at ages 6-10 were more than 20% lower than seedling controls. In the mid-seventies Sweet and Wells published evidence that trees established from cuttings taken from ortets aged between 10 and 43 years showed incremental loss of ramet stem volume with advancing age of the ortets (Sweet and Wells, 1974). During the 1980's,

MetaGenet cs New Zealand, 93 State Highway 30, Rotorua, New Zealand. E-mail dalesmith@xtra.co.nz

Menzies and co-workers (Menzies *et al.,* 1991) established extensive ortet age effect field trials on a number of sites. Well-replicated trials were planted using ramets from ortets ranging in age from 1 to 5 years. These trials showed conclusively that cuttings from 4 and 5 year old ortets showed average stem volume losses of 8% and 19% respectively compared to seedling controls when measured five years after planting. Figure 1, which was constructed using published data (Sweet and Wells, 1974; Menzies *et al.,* 1991) illustrates the relationship between ortet age and ramet stem volume growth. This effect, often referred to as 'physiological ageing' has since been confirmed in a number of tree improvement trials in New Zealand (New Zealand Forest Research Ltd, unpublished data).

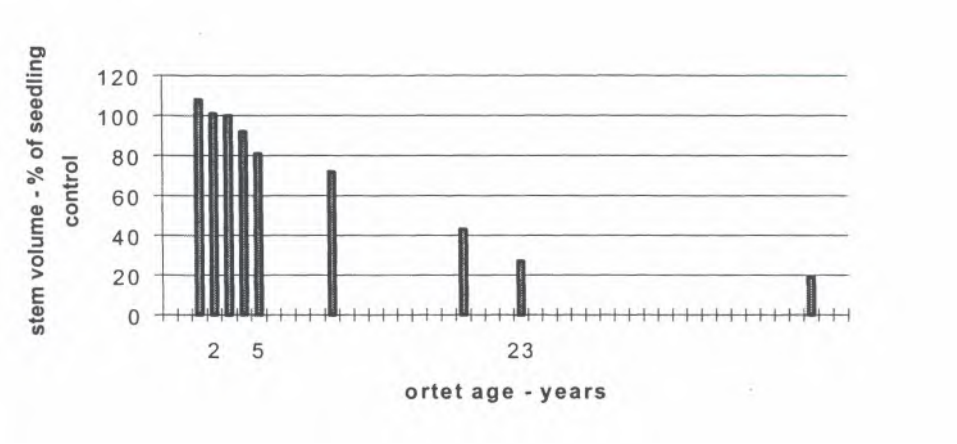


Figure 1. The effect of ortet age on the stem volume growth of ramets relative to seedlings. Data for years 1-5: ramets 5 years after planting (Menzies *et al.,* 1991). Data for years 10-43: ramets 6 years after planting (Sweet and Wells, 1974)

When the implications of physiological ageing became apparent, research effort and management practise in New Zealand was redirected towards the use of vegetative propagation from ortets aged 3 years or less. Stem cuttings from seedlings are currently used widely in New Zealand (1996, 10% of all plantings. Smith, 1997) to enable the realisation of genetic gain from limited amounts superior seed over a greater land area than possible if planted directly as seedlings. In most instances the cuttings are not used for clonal forestry, other than to enhance the efficiency of progeny testing. Research effort has also been directed towards developing techniques for maintaining the juvenile state of representative examples of each clone. The intention is that clonal forestry could eventually be possible with superior genotypes which became apparent from well-replicated clonal field trials (Smith, 1997). Pine micropropagation research initiated in the 1970s in New Zealand (Smith, 1986) was later adopted by industry, with more than 2 million radiata pine plantlets produced each year by Fletcher Forests in New Zealand (Nairn, 1992; Gleed, 1993). A general pine somatic embryogenesis protocol developed at the New Zealand Forest Research Institute (Smith, 1996) was subsequently licensed to Carter Holt Harvey Forests for radiata pine (Smith *et al.,* 1994) and has since been evaluated by forestry companies in the Southern USA for application to loblolly and slash pine. Pine somatic embryogenesis techniques depend upon capture of the zygotic embryo during its polyembryonic state, and like micropropagation, are effective only with juvenile clones which are untested genotypes with respect to their properties as crop trees. For a period, somatic embryogenesis enjoyed a comparative advantage over micropropagation. Samples of each embryogenic clone could be stored in liquid nitrogen in a juvenile state while clonal field trials were carried out with somatic seedlings regenerated from other samples of the same clones (Hargreaves and Smith, 1992; Smith *et al.,* 1994). This advantage has since been

negated by the development of protocols for cryopreservation of a high percentage (82%) of micropropagated clones (Hargreaves, *et al.,* 1999).

Although somatic embryogenesis and micropropagation are used by two major forestry companies in New Zealand, the total plantings represent less than 2% of the land area planted in 1996 (Smith, 1997). Micropropagation works for a wide range of genotypes, but the cost of production per plant is 5-7 times that of a seedling (Smith, 1986, 1997). This reflects the high labour cost, with approximately 50 people working part time to produce 2 million plants at Fletcher Forests in 1995 (B. Nairn, pers comm). While production of embryogenic tissue from immature zygotic embryos is effective for all radiata pine families tested, on average only 15 % of genotypes within a family will form plants from embryogenic tissue (Smith *et al.,* 1994). While somatic embryogenesis shows potential for automation through the use of bioreactor technology (Smith *et al.,* 1991), in practise, pine embryogenic cell lines typically have a finite life before losing plant regeneration capacity - currently a serious obstacle to industrial-scale plant propagation. Somatic embryogenesis currently offers the unique advantage of genetic transformation (Walter and Smith, 1995; Walter *et al.,* 1998).

Current uncertainty with respect to economic advantage over conventional tree breeding is perhaps the greatest obstacle to the widespread adoption of pine micropropagation and / or somatic embryogenesis in production-scale tree improvement operations. The most important economic questions are:

- How many juvenile clones must be tested in order to identify some that far outperform both their siblings, and the following breeding generations, with respect to both volume and added value (higher density, disease resistance, fiber quality, & etc)?
- What is the cost of maintaining many clones in a juvenile state for the years during which field trials are carried out?

Radiata pine differs somewhat from the case given for interior spruce in British Columbia. The testing of juvenile spruce clones from resistant families may well be economically justified in the case of insect resistance, where lack of resistance means that new plantings will fail (Sutton *et al.,* 1993). No such threat currently constrains radiata pine utilisation in New Zealand, and a new technology is judged only on the additional volume and / or value of wood produced. There is little data available from which an informed decision can be made, however computer models suggest that 5% or less of the top trees in any generation will outperform the mean of the generation that follows (King and Johnson, 1991). Kibblewhite and co-workers have recently shown in New Zealand that certain individual clones have fiber properties significantly better for Kraft pulp than run-of-the-mill radiata pine fiber (Evans *et al.,* 1999). There appear to be no published studies of a similar nature showing information on selection of superior pine phenotypes, based on wood properties, in stands old enough to produce commercial saw logs.

Assuming that superior clones can be detected in a mature stand of pines, due to the "physiological ageing" effect, those clones will be of little economic value unless "rejuvenated" to a state equivalent to the vigorous growth habit of natural seedlings. Rejuvenation of conifers has been an elusive goal, compared to angiosperms and monocotyledonous plants. There have been two claims of rejuvenation of spruce in recent years (Westcott, 1994; Paques *et al.,* 1997), and one claim for pines (Smith, 1997). In the present paper the author reports on the screening of a 20 year old stand of radiata pine for unusual wood properties, and the rejuvenation of a select few clones to collect information on field performance in the future.

## METHODS

### Operational strategy

Having access to rejuvenation technology (MetaGenetics New Zealand, unpublished data), this project was founded on exploring the concept of selecting individual trees from a 20-year-old stand of radiata pine, based on their stem diameter and wood properties. The trees were selected according to the specifications of a client, based on their experience with machining radiata pine timber. Clones selected for propagation were chosen by following these specifications:

- Measure all stem diameters to identify largest 10% of trees with required form, sample wood from these trees.
- Eliminate all trees with reaction wood.
- Eliminate all trees with ring 16-20 basic wood density less than 470 kg / m3.
- Eliminate all trees with ring 20 tracheid mean length less than 4.0 mm.
- Rejuvenate all remaining trees to produce experimental plantings.

### Wood property sample collection

Screening was carried out in 15 hectares of twenty-year-old radiata pine growing in the coastal Eastern Bay of Plenty, at an altitude of 100 metres. The trees were planted at 3m x 3m spacing on a farm site in 1977 using open pollinated progeny of the '268' series seed orchard. Trees were pruned to 3.4 metres, and thinned to waste at 5 and 11 years, giving a final crop density of 330 trees / hectare (thinning ratio 1:3). All trees in the stand were measured (diameter at breast height) and scored for stem and branch form. Trees were ranked based on stem diameter, and all large trees with butt sweep, or with branch size estimated to be greater than 25 mm in the first unpruned log were excluded from further sampling. After exclusion of those trees, two 5mm core samples were taken from each of the 33 trees in each hectare with greatest diameter, a total of 495 trees. The core samples were taken at right angles to the downhill side (if any) of each tree. The whole 5mm cores were assessed for reaction wood according to the method of Harris (Harris, 1977), with each core being rated from 0 (none) to 5 (severe). A further 10mm X 10 mm sample was cut with a sharp chisel from the outermost ring of a sub-sample of 70 trees, at breast height on the uphill side.

Basic wood density was assessed gravimetrically for each tree using the mean of two 5 mm cores. The maximum moisture content method (Smith, 1954) was used for lengths comprising 2 sets of consecutive growth rings (1-5, and 16-20) after Soxhlet extraction in methanol. Wood samples (10mm x 10mm) from the outermost growth ring of 70 trees (Table 2) were macerated in 50:50 glacial acetic acid: hydrogen peroxide. Fiber sample preparations were assessed for tracheid length according to the method of Harris (Harris, 1966) using a 0.8 objective on a Reichert Diavar microscope. The mean for each tree was determined from 20 randomly selected fibers from each of 5 sub-samples of the macerate.

### Vegetative propagation

Vegetative bud samples were taken in May 1997 from 5 trees selected after determination of their wood properties. Some of the buds were grafted to seedling rootstocks as a future "gene bank". The remaining vegetative buds were used as a source of apical meristems. Following an induction treatment on meristem fragments or protoplast preparations, (MetaGenetics, proprietary technology), tissue containing immature somatic embryos was observed to arise from each clone. Mature somatic embryo production was achieved using a protocol reported for *P. strobus* (Klimeszewska and Smith, 1997), and

plants from each of the 5 clones were eventually established in a greenhouse in September 1997 Somatic seedlings in Spencer Lemaire rootrainers were transferred to a Rotorua nursery in Decembe 1997 where they were subsequently used as stool-beds. Shoot sections 120 - 150 mm long were set in June 1998 into a friable pumice soil augmented with sedge peat and containing magnesium ammonium phosphate. Rooted cuttings will be used to plant field trials in July - August 1999.

Gene fingerprinting

Needle samples from 2 of the 5 original ortets, and from somatic seedlings from each clone were used as a source of DNA. The extracted DNA was amplified by the Gene Mapping research group at New Zealand Forest Research Institute Ltd., using 8 RAPD primers for '268' series seed orchard clones that are used routinely in NZFRI commercial seed orchard fingerprinting services.

## RESULTS AND DISCUSSION

Stand and wood properties

The mean breast-height diameter over bark (DOB) for the whole stand was 480 mm (Table 2). The mean DOB of the top 10% trees was 595 mm, which was significantly higher ($p<0.01$) than the whole stand mean. From the 5 mm core samples, it was determined that the breast-height diameter under bark (DUB) of the top 10% of trees was 535 mm (Table 2). The whole stand DOB indicates an average ring width of 10.1 mm (using a bark thickness of 30 mm) and this value is 11% higher than that previously published for the '268'' families at age 20 (Cown *et al.*, 1992). The greater values for the stand described in the current paper will be due to the higher site fertility, and the lower altitude of 100 metres compared with more than 300 meters for trees in the Cown *et al.* study.

Of the top 495 trees 68.7% were rejected on the basis of reaction wood in the 5 mm core samples, leaving 155 trees (31.3%) with no observed reaction wood Table 1). The mean value for the 495-tree sample was 1.75%, somewhat higher than the value of 1.52 reported for similar material in the Central North Island (Cown *et al.*, 1992).

From the 5 mm core samples, it was determined that the mean outer 5 growth ring density of the trees sampled was 420 kg /$m^3$. Of the 495 trees sampled, there were 70 tree with a ring 16-20 basic core density equal to or greater than 470 kg / $m^3$ Samples of the outermost growth ring (ring 20) were taken from these 70 trees, and the mean fibre length determined. The mean tracheid length for these 70 trees was 3.65 mm. This is close to the mean of 3.70 mm reported by Cown and co-workers for the '268' families in the Central North Island. Of the 70 trees samples in the Eastern Bay of Plenty site, only 5 were found to have ring 20 mean tracheid length equal to or greater than 4 mm (mean 4.13, Table 2).

Table 1. Stand description

| | |
|---|---|
| Stand area - hectares | 15 |
| Initial stocking - total number of trees | 16667 |
| Final stocking - total number of trees | 4957 |
| Number of trees in top 10% DOB | 495 |
| Number of trees in top 10% with no reaction wood | 155 |
| Number of trees with ring 16-20 basic density >470 kg/m3 | 70 |
| Number of trees with ring 20 mean tracheid length > 4.0 mm | 5 |

Table 2. Wood properties

| Sample | DOB mm +/-SD | DUB mm +/-SD | Reaction wood | Ring 20 density kg / $m^3$ | Ring 20 mean tracheid length mm |
|---|---|---|---|---|---|
| Whole stand | 480 | - | | | - |
| Top 10% of trees | 595 | 535 | 1.75 | 420 | 3.65 (1) |
| 5 select trees | 570 | 510 | 0 | 476 | 4.13 |

DOB - breast-height diameter over bark.
DUB - breast-height diameter under bark.
(1) From 70 trees with ring 16-20 basic density. 470 kg / m3.

Propagation and molecular verification

Embryogenic tissue was established from less than 5% of the vegetative bud explants placed in vitro from the 5 selected 20-year-old trees. Despite the low response, however, embryogenic tissue was established for all 5 clones, with meristem fragment samples and protoplast preparations giving similar results (data not shown). Mature somatic embryos were harvested from this tissue following transfer to maturation medium, and more than 50% of these converted to plants established in soil. Between 17 and 47 plants were established in soil for each of the 5 clones. The somatic seedlings had stems containing whorls of primary needles identical with that of seedlings, and, after 1 year in the nursery bed, their form continues to be similar to that of seedlings. Stem cuttings established from 10 somatic seedlings of each clone, set directly in the nursery bed, all formed adventitious roots and are identical in form with stem cuttings from seedlings 1 year old or younger.

DNA gels run using the products of 8 different primers were used to compare two ortet and two ramet samples for two different clones. Results for all 8 primers indicate that the ortet and ramet samples are probably genetically identical (Fig 2).

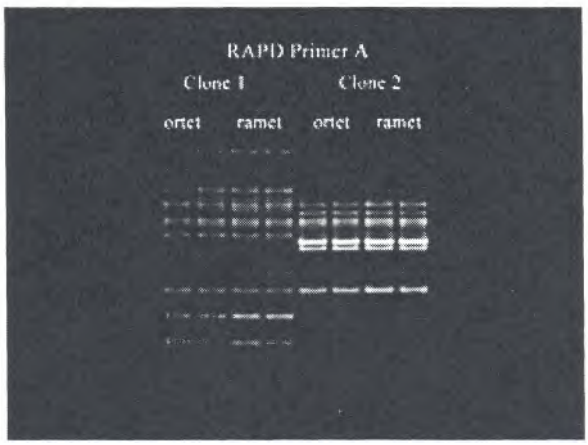


Figure 2. Comparison of somatic seedling needle tissue (ramet) with source tree (ortet) for two clones.

Significance and application of results

Two novel contributions to pine improvement technology are explored in this paper. The first of these is the demonstration of rejuvenation of 5 twenty-year-old *Pinus radiata* clones, selected for their wood properties and apparent high growth rate. This rejuvenation, when verified by field plantings which will be established this year, will offer a new tool in the quest for establishing stands of pines with known wood properties.

The second feature of this study is a preliminary investigation of the concept of phenotypic selection in a final crop once the growth and form, as well as the wood properties have become apparent. From the viewpoint of stem diameter, there is a risk in selecting unreplicated genotypes, as the genetic superiority of a tree cannot be taken for proven on the basis of single tree performance. However it is unlikely that inferior genotypes would survive two thinnings-to-waste and be represented in the top 10% of stem diameters after 20 years of growth. The risk inherent in selecting trees on the basis of the wood properties of individual trees is small, since studies in New Zealand have shown that ramet-to-ramet variation in wood properties is negligible ( C.J.A. Shelbourne and R.P. Kibblewhite, New Zealand Forest Research Institute Ltd., pers comm).

There appear to be no published studies of fast-grown radiata pine sawn timber that pinpoint the cause of dimensional instability increasingly experienced with this species. There is, however, anecdotal evidence from saw-millers in New Zealand, which indicates that dimensional instability is often associated with severe reaction wood. On the other hand, reaction wood is rarely seen in pinewood, which saws and machines well. On this basis, only 31.3% of the sample in the present study would be expected to have high dimensional stability following sawing.

Owing to the number of interacting factors, there are few definitive studies of the relationships between wood characteristics and product values (Cown *et al.*, 1992). Wood density is a predictor of hardness, ease of drying and machining, strength, and stiffness (Panshaw and de Zeeuw, 1980; Megraw, 1985). For New Zealand radiata pine wood chips, the basic density has been shown to be strongly correlated with pulp coarseness and handsheet properties (Kibblewhite, 1985; Kibblewhite and Bawden, 1992). In the present study, the mean basic density of rings 16-20 of the 10% of trees with greatest diameter was 420 kg / $m^3$, which is lower than the mean value of 435 kg / $m^3$ reported elsewhere for the same families (Cown *et al.*, 1992). The low altitude Bay of plenty site sampled in the present is warmer than that sampled by Cown and co-workers, and a higher outer ring wood density would have been expected, based on the very substantial surveys carried out by Cown's group (Cown, 1980). The lower than expected result may be a consequence of limiting density measurements to the 10% of trees with largest diameter, but this explanation implies a negative relationship between density and diameter. There is ongoing debate in New Zealand on the relationship between diameter and wood density. Burdon and Low reported a "negative association (both genetic and within-family phenotypic correlations) with ring width" (Burdon and Low, 1993). On the other hand, Cown and co-workers interpreted their study of the `268' families as showing that there is "no indication of change in average wood density" of the '268' families compared to the control population (Cown *et al.*, 1992). Close analysis, however, reveals a negative correlation (-0.6) when their data for mean ring width of 30 individual families (their Figure 1) is analysed against growth ring 16-20 density (their Figure 4b).

The present study does not resolve the question of the relationship between diameter and wood density in radiata pine, nor add information to the role played by reaction wood. However this study does provide new information on intensive clonal selection. King and Johnson have calculated that intensive clonal selection (top 5% of trees in a stand, selected on the basis of form and volume) may give

substantially greater gains than seed orchard options King and Johnson, 1991). The present study indicates that, when other phenotypic characteristics are also taken into consideration, the selection intensity needs to be much greater than that indicated by King and Johnson. In the present study only 5 trees met the criteria set at the beginning of this study. This represents 0.1% of the crop trees (4957 trees) or 0.03% of the trees originally planted (16667 trees)

Economic comparison of propagation methods

The total cost of assaying wood properties in this study was less than NZ $45,000. This cost is actually quite low when compared with the alternative of screening trees using propagation technologies. Embryogenesis and micropropagation are expensive and may be totally impractical when very intensive clonal selection is required. Somatic embryogenesis and micropropagation of radiata pine have both been available for over 10 years, and the costs of plant production are well established.

Table 3. The cost of juvenile planting stock for clonal field trials (1)

| | **Cost of Propagation Option NZ$ per Production Clone** | |
|---|---|---|
| | **Embryogenesis** (2) | **Micropropagation** |
| Cost of 5 ramets at nursery gate (3) | $245 | $65 |
| **Clone selection intensity** | | |
| 5% | $4,900 | $1,300 |
| 1% | $24,500 | $6,500 |
| 0.03% | $816,683 | $21,6671 |

(1) Cost to produce one production clone at indicated selection intensity.
(2) Using immature zygotic embryos and cryopreservation.
(3) Includes cost of cryostorage for 5 years.

The production cost for 5 ramets each of somatic seedlings or micropropagated plants are NZ$245 and NZ$ 65 respectively, including the additional cost of cryogenic storage for 5 years while clonal tests are carried out (Table 3; MetaGenetics New Zealand - unpublished data). The cost of producing enough clones to lead to the identification of just one elite tree, at the selection ration used in the study reported in this paper (0.03%), would be NZ$ 816,683 using somatic seedlings, or NZ$ 216,671 using micropropagated plants (Table 3). Added to this would be the considerable cost of establishing clonal field trials, and still having to screen for wood properties at the end of the exercise. When a cost comparison of this type is carried out, it becomes evident that rejuvenation of select clones, following the screening of individual mature trees for desirable wood properties, becomes an extremely attractive option.

## CONCLUSION

Tissue culture techniques have been used to rejuvenate five 20-year-old clones of radiata pine following screening of a stand of trees for wood properties. Of the 495 trees selected for superior diameter in a 15 hectare stand, only 5 met the specification of no reaction wood, ring 16-20 wood density equal to or greater than 470 kg / $m^3$, and ring 20 mean tracheid length greater than 4.00 mm. This is a preliminary report from an ongoing study of rejuvenation and clonal propagation in radiata pine, but indicates that

Significance and application of results

Two novel contributions to pine improvement technology are explored in this paper. The first of these is the demonstration of rejuvenation of 5 twenty-year-old *Pinus radiata* clones, selected for their wood properties and apparent high growth rate. This rejuvenation, when verified by field plantings which will be established this year, will offer a new tool in the quest for establishing stands of pines with known wood properties.

The second feature of this study is a preliminary investigation of the concept of phenotypic selection in a final crop once the growth and form, as well as the wood properties have become apparent. From the viewpoint of stem diameter, there is a risk in selecting unreplicated genotypes, as the genetic superiority of a tree cannot be taken for proven on the basis of single tree performance. However it is unlikely that inferior genotypes would survive two thinnings-to-waste and be represented in the top 10% of stem diameters after 20 years of growth. The risk inherent in selecting trees on the basis of the wood properties of individual trees is small, since studies in New Zealand have shown that ramet-to-ramet variation in wood properties is negligible ( C.J.A. Shelbourne and R.P. Kibblewhite, New Zealand Forest Research Institute Ltd., pers comm).

There appear to be no published studies of fast-grown radiata pine sawn timber that pinpoint the cause of dimensional instability increasingly experienced with this species. There is, however, anecdotal evidence from saw-millers in New Zealand, which indicates that dimensional instability is often associated with severe reaction wood. On the other hand, reaction wood is rarely seen in pinewood, which saws and machines well. On this basis, only 31.3% of the sample in the present study would be expected to have high dimensional stability following sawing.

Owing to the number of interacting factors, there are few definitive studies of the relationships between wood characteristics and product values (Cown *et al.*, 1992). Wood density is a predictor of hardness, ease of drying and machining, strength, and stiffness (Panshaw and de Zeeuw, 1980; Megraw, 1985). For New Zealand radiata pine wood chips, the basic density has been shown to be strongly correlated with pulp coarseness and handsheet properties (Kibblewhite, 1985; Kibblewhite and Bawden, 1992). In the present study, the mean basic density of rings 16-20 of the 10% of trees with greatest diameter was 420 kg / $m^3$, which is lower than the mean value of 435 kg / $m^3$ reported elsewhere for the same families (Cown *et al.*, 1992). The low altitude Bay of plenty site sampled in the present is warmer than that sampled by Cown and co-workers, and a higher outer ring wood density would have been expected, based on the very substantial surveys carried out by Cown's group (Cown, 1980). The lower than expected result may be a consequence of limiting density measurements to the 10% of trees with largest diameter, but this explanation implies a negative relationship between density and diameter. There is ongoing debate in New Zealand on the relationship between diameter and wood density. Burdon and Low reported a "negative association (both genetic and within-family phenotypic correlations) with ring width" (Burdon and Low, 1993). On the other hand, Cown and co-workers interpreted their study of the `268' families as showing that there is "no indication of change in average wood density" of the '268' families compared to the control population (Cown *et al.*, 1992). Close analysis, however, reveals a negative correlation (-0.6) when their data for mean ring width of 30 individual families (their Figure 1) is analysed against growth ring 16-20 density (their Figure 4b).

The present study does not resolve the question of the relationship between diameter and wood density in radiata pine, nor add information to the role played by reaction wood. However this study does provide new information on intensive clonal selection. King and Johnson have calculated that intensive clonal selection (top 5% of trees in a stand, selected on the basis of form and volume) may give

substantially greater gains than seed orchard options King and Johnson, 1991). The present study indicates that, when other phenotypic characteristics are also taken into consideration, the selection intensity needs to be much greater than that indicated by King and Johnson. In the present study only 5 trees met the criteria set at the beginning of this study. This represents 0.1% of the crop trees (4957 trees) or 0.03% of the trees originally planted (16667 trees)

Economic comparison of propagation methods

The total cost of assaying wood properties in this study was less than NZ $45,000. This cost is actually quite low when compared with the alternative of screening trees using propagation technologies. Embryogenesis and micropropagation are expensive and may be totally impractical when very intensive clonal selection is required. Somatic embryogenesis and micropropagation of radiata pine have both been available for over 10 years, and the costs of plant production are well established.

Table 3. The cost of juvenile planting stock for clonal field trials (1)

| | **Cost of Propagation Option NZ$ per Production Clone** | |
|---|---|---|
| | **Embryogenesis** (2) | **Micropropagation** |
| Cost of 5 ramets at nursery gate (3) | $245 | $65 |
| **Clone selection intensity** | | |
| 5% | $4,900 | $1,300 |
| 1% | $24,500 | $6,500 |
| 0.03% | $816,683 | $21,6671 |

(1) Cost to produce one production clone at indicated selection intensity.
(2) Using immature zygotic embryos and cryopreservation.
(3) Includes cost of cryostorage for 5 years.

The production cost for 5 ramets each of somatic seedlings or micropropagated plants are NZ$245 and NZ$ 65 respectively, including the additional cost of cryogenic storage for 5 years while clonal tests are carried out (Table 3; MetaGenetics New Zealand - unpublished data). The cost of producing enough clones to lead to the identification of just one elite tree, at the selection ration used in the study reported in this paper (0.03%), would be NZ$ 816,683 using somatic seedlings, or NZ$ 216,671 using micropropagated plants (Table 3). Added to this would be the considerable cost of establishing clonal field trials, and still having to screen for wood properties at the end of the exercise. When a cost comparison of this type is carried out, it becomes evident that rejuvenation of select clones, following the screening of individual mature trees for desirable wood properties, becomes an extremely attractive option.

## CONCLUSION

Tissue culture techniques have been used to rejuvenate five 20-year-old clones of radiata pine following screening of a stand of trees for wood properties. Of the 495 trees selected for superior diameter in a 15 hectare stand, only 5 met the specification of no reaction wood, ring 16-20 wood density equal to or greater than 470 kg / $m^3$, and ring 20 mean tracheid length greater than 4.00 mm. This is a preliminary report from an ongoing study of rejuvenation and clonal propagation in radiata pine, but indicates that

powerful new tools will soon be available to the industry for the improvement of wood quality of radiata pine, without sacrificing wood volume production.

## ACKNOWLEDGEMENTS

J. Ropati, F. McKay, and T. Callender are thanked for their technical support in the field collection and wood property studies. Dr. T. Richardson is thanked for advice on gene fingerprinting of clones. Staff of the TTT Timber Company are thanked for access to trees and for their contributions to defining the criteria for clonal selection.

## LITERATURE CITED

Burdon, R.D. and C.B. Low. 1993. Genetic survey of *Pinus radiata*. 6: Wood properties: variation heritabilities, and interrelationships with other traits. New Zealand journal of Forestry Science 22(2/3); 228

Carson, M.J. 1986. Advantages of clonal forestry for *Pinus radiata* - real or imagined? New Zealand journal of Forestry Science 16(3): 403

Cown, D.J. 1980. Radiata pine: Wood age and wood property concepts. New Zealand Journal o Forestry Science 10:504

Cown, D.J., G.D. Young, and R.D. Burdon. 1993. Variation in wood characteristics of 20-year-old half-sib families of *Pinus radiata*. New Zealand Journal of Forestry Science 22(1): 63

Evans, **R., R.P.** Kibblewhite, and S. Stringer. 1997 Kraft pulp property prediction from wood properties in eleven radiata pine clones. Appita 50(1): 25

Field, J.F. 1934. Experimental Growing of Insignis Pine From Slips, New Zealand Journal of Forestry 3(4): p. 185

Gleed, J.A. 1993. Development of Plantlings and Stecklings of Radiata Pine, Clonal Forestry 11, Genetics and Biotechnology. Ed Ahuj a, M.R., and Libby, W.J. Springer-Verlag. P. 149.

Hargreaves, C.L. and D.R. Smith. 1992. Cryopreservation of Pinus radiata Embryogenic Tissue, The International Plant Propagators Combined Proceedings, 2: p. 63.

Hargreaves, C.L, M.N. Foggo, D.R. Smith, and M.E. Gordon. 1999. Development of protocols for the cryopreservation of zygotic embryos of *Pinus radiata* and subsequent plant regeneration. New Zealand journal of Forestry Science. In Press.

Harris, J.J. 1966. A method of minimizing operator bias in measuring tracheid length. Journal of the Royal Microscopical Society 86(1): 81

Harris, J.M. 1977. Shrinkage and density of radiata pine compression wood in relation to its anatomy and mode of formation. New Zealand Journal of Forestry Science 7: 91

Kibblewhite, R.P. 1985. Qualities of Kraft and thermo-mechanical radiata pine papermaking fibres. In Punton, V. The Raw Materials of Papermaking and Their Effects Upon the Papermaking Process and the Properties of the Paper. Transactions of the Bch Research Symposium, Oxford. p95

Kibblewhite, R.P. and A.D. Bawden. 1993. Kraft fibre properties on *Pinus radiata* toplogs, thinnings, and slabwood, and a "genetic misfit". New Zealand journal of Forestry Science 22(1): 96

King, J.N. and G.R. Johnson. 1991. Computer Simulation of Clonal Selection Gains, *New Zealand* Forest Research Institute Bulletin No. 160, p. 51.

Klimaszewska, K. and D.R. Smith. 1997. Maturation of somatic embryos of *Pinus strobus* is promoted by a high concentration of gellan gum. Physiologia Plantarum, vol.100. p 949

Megraw, R.A. 1985. Wood Quality Factors in Loblolly Pine: The Influence of Tree Age, Position in Tree, and Cultural Practice on Wood Specific Gravity, fiber Length, and Fibril Angle. Tappi Press, Atlanta, 345p.

Menzies, M.I., B.K. Klomp, and D.G. Holden. 1991. Optimal Physiological Age of Propagules for use in Clonal Forestry, New Zealand Forest Research Institute Bulletin, *No.* 160: p. 142.

Nairn, B.J. 1992. Commercial Micropropagation of Radiata Pine, Micropropagation of Woody Plants. Kluwer, P. 383.

Panshin, A.J. and C.E. de Zeeuw. 1980. Textbook of Wood Technology. McGraw-Hill, New York, 643p

Paques, M., J. Berceteche, and L. Harvengt. 1997. Somatic embryogenesis: a way to recover clones from needles of selected Norway spruce. Abstract. Joint Meeting of the IUFRO Working Parties 2.04-07 and 2.04-06 Somatic Cell Genetics and Molecular Genetics of Trees. Quebec, August 1997.

Smith, D.M. 1954. Maximum moisture method for determining the specific gravity of small wood samples. USDA Forest Service Forest products Laboratory Report No 2014. 8p.

Smith, D.R. 1986. Radiata Pine (Pinus radiata. D. Don.), in Biotechnology of tree improvement. Ed. Y.P.S.Bajaj. Springer- Verlag, p. 274.

Smith D.R. 1996. Growth Medium. US Patent 5,565,355.

Smith, DR. 1997. The role of *in-vitro* methods in pine plantation establishment: the lesson from New Zealand. Feature article in Plant Tissue Culture and Biotechnology, Vol. 3. No.2. p63

Smith. DR., L.A Donaldson, and A.A Wan. 1991: Bioreactor Technology for Mass Propagation of *Pinus radiata* by Somatic Embryogenesis. Abstract, 1991 World Congress on Cell and Tissue Culture. Anaheim, USA.

Smith, D.R., C, Walter, A.A. Wan, C.L. Hargreaves, and L.J. Grace. 1994. Somatic embryogenesis joins the plantation forestry revolution in New Zealand. Proceedings, TAPPI Biological Sciences Symposium, Minneapolis, 1994. p. 19.

Sutton B.C.S, S.C. Grossnickle, D.R. Roberts, J.H. Russell, and G.K. Kiss. 1993. Somatic Embryogenesis and Tree Improvement in Interior Spruce, Journal of Forestry, 91(10): p. 34.

Sweet, G.B. and L.G. Wells. 1974. Comparison of the Growth of Vegetative Propagules and Seedlings, New Zealand Journal of Forestry Science 4(2): p. 399

Walter, C. and D.R. Smith. 1995. Transformed Pinus radiata now growing in greenhouses at the New Zealand Forest Research Institute. Dendrome, September 1995, vol. 2, no 2. p. 1.

Walter, C., L.J. Grace, A. Wagner, D.W.R. White, A.R. Walden, S.S. Donaldson, H. Hinton, R.C. Gardner and D.R. Smith. 1998. Stable transformation and regeneration of transgenic plants of *Pinus radiata* D. Don. Plant Cell Reports, 17:460

Westcott, R.J. 1994. Production of embryogenic callus from nonembryogenic explants of Norway Spruce *Picea abies (L.)* Karst. Plant Cell Reports 14:47

Thulin, I.J. and T. Faulds. 1968. The use of cuttings in the breeding and afforestation of *Pinus radiata*. New Zealand Journal of Forestry. 13:66