NURSERY SYSTEMS TO CONTROL MATURATION IN PINUS RADIATA CUTTINGS, COMPARING HEDGING AND SERIAL PROPAGATION

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ABSTRACT

Clonal forestry with *Pinus radiata* D.Don has been hampered by maturation (also termed physiological ageing) of clones during the clonal testing phase. In 1988, a long-term nursery trial was initiated to find the best treatment for delaying maturation in rooted cuttings. Clonal hedges were established, hedged annually, and subjected to five different cycles of serial propagation. Physiological age was estimated in the nursery using morphological markers. After 10 years, significant differences were observed between treatments, with the hedged treatment (no repropagation) recording the lowest physiological age of 2.24 years compared with 2.61 years for the treatment with the most frequent serial propagation. In contrast, results from a later assessment of the nursery hedges, and from a field planting of rooted cuttings harvested from the same hedges, yielded no statistically significant treatment effects, though significant differences were observed between families and between clones for physiological age, diameter at breast height (dbh), and height. There are some positive aspects of maturation, and this research demonstrated that nursery stool-beds can be managed using hedging to control maturation, keeping physiological age at optimal levels. Despite later non-significant results in physiological age for the different nursery treatments, a trend was still apparent and, therefore, hedging with minimal serial propagation of hedges is advised. There is a conflict between Australasian and North American researchers in terminology regarding "physiological age".

Keywords: rooted cuttings; nursery stool-beds; serial propagation; hedging; maturation; physiological age; clonal forestry; *Pinus radiata*.

INTRODUCTION

Clonal Forestry with Pinus radiata

Clonal forestry with *P. radiata* has been envisaged ever since it was recognised by tree breeders that cuttings could be reliably rooted (Fielding 1954, 1964; Thulin & Faulds 1968; Shelbourne 1991). Although a number of clonal propagation systems have been developed successfully (Menzies & Aimers-Halliday 1997), clonal forestry with *P. radiata* has been hampered by problems, especially physiological ageing or maturation of clones during the clonal testing phase (Aimers-Halliday *et al.* 1997; Menzies & Aimers-Halliday 1997). The

challenge has been to develop a propagation/clonal storage system that will work for most genotypes and allow for the rapid production of large numbers of uniform plants per clone, at an appropriate maturation state, *after* the assessment of clonal trials. Similar problems with maturation have hampered clonal forestry with other conifer species (Ritchie 1991; Russell 1993; Kleinschmit & Schmidt 1997; Mason *et al.* 2002).

Concept of Physiological Ageing — Conflicting Definitions

A particular physiological age refers to a developmental state (in the continuum from embryonic → juvenile → adolescent → mature → over-mature) as indicated by the presence of phase-specific characteristics. The apparent physiological age of a tree may be different from its chronological age (time taken to grow from seed) because of environmental influences and cultural practices. This definition of physiological age has been in use for over four decades in New Zealand (Sweet 1964), and is consistent with the concept discussed by Robbins (1957), Borchert (1976), and others, but is in conflict with more recent definitions published by North American authors.

Wareing (1959, 1987) used the term "maturation" to describe the transition from juvenile to mature phase, which is difficult to reverse, and the term "ageing" to indicate loss of vigour associated with increasing complexity in the plant, which is easily reversed through horticultural practices. Fortanier & Jonkers (1976) have referred to this loss of vigour as "physiological ageing" in contrast to the more persistent "ontogenetic ageing" or "maturation". This is in conflict with the concept of physiological ageing developed in New Zealand.

We make no attempt to separate apparent physiological age, which we define as a particular developmental state, into the phenomena of maturation (or ontogenetic ageing) and the easily reversible loss of vigour, which is largely associated with factors limiting nutrition. However, we have long been aware of the two distinct phenomena (Sweet 1964) and believe that most of the changes associated with physiological ageing in *P. radiata*, as described in this article, are due to ontogenetic ageing and, therefore, are very difficult to reverse. In New Zealand and Australia, the terms "physiological ageing" and "maturation" are often used synonymously, with "physiological age" used to define the particular developmental state.

In this paper, maturation is defined as progression of change from embryonic to mature state, due to ontogenetic ageing. This is generally related to the cumulative distance from the shoot-root interface, which increases as a plant grows. Physiological age is defined as the apparent maturation state of a tree, which is the result of ontogenetic processes that are largely irreversible, plus the more easily reversible loss of vigour associated with increasing age. Any reversible loss of vigour is recognised as a minor or negligible component in current forestry propagation practices with *P. radiata* in New Zealand. Physiological age can be quantified via morphological markers (Menzies *et al.* 2000).

Significance of Maturation

The negative effect of maturation in stock plants on rooting success of *P. radiata* cuttings was first recognised in the 1960s (Fielding 1964; Libby & Conkle 1966; Thulin &

Faulds 1968). However, the strategic importance of maturation did not become fully apparent until the 1970s (Brown 1974; Menzies *et al.* 1991; Forest Research Institute 1991).

A progressive decrease in the rate of diameter growth was observed with increasing physiological age of rooted cuttings, plus a decrease in the rate of height growth at older physiological ages (Sweet 1973; Sweet & Wells 1974; Menzies & Klomp 1988). However, there are advantages as well as disadvantages associated with maturation in *P. radiata* (Menzies & Klomp 1988; Menzies & Aimers-Halliday 1997). The positive effects on tree form were first noted by Thulin & Faulds (1968) and Fielding (1970). Libby and colleagues observed that several serious defects in form, associated with *P. radiata*'s juvenile and transitional stages, could be reduced by using planting stock with a greater maturation state (Libby *et al.* 1972; Tufuor & Libby 1973; Libby & Hood 1976; Hood & Libby 1978; Bolstad & Libby 1982).

Extensive field trials of rooted cuttings of various physiological ages were established by the New Zealand Forest Research Institute, in 1983 and 1984, throughout the North Island of New Zealand, with the objective of studying the effects of maturation. Morphological markers for physiological age were identified, based on *P. radiata* trees grown in the central North Island (Menzies *et al.* 2000). The main trends with increasing physiological age were identified as reduced early diameter and volume growth, but improved tree form. There is an optimal physiological age of 3 to 4 years, with the advantages of improved stem form associated with some maturation, but not the disadvantage of loss of early diameter growth associated with older physiological ages (Menzies *et al.* 1991; Forest Research Institute 1991; Holden 1995; Menzies & Aimers-Halliday 1997).

The control of maturation is important for family forestry and clonal forestry. If initial selection and later deployment of genotypes are with propagules of differing maturation states, then there will be inconsistency in performance of genotypes and loss of genetic gain for certain economic traits, particularly dbh and volume growth. Methods of maintaining an appropriate maturation state in clonal storage systems are, therefore, critical to successful family and clonal forestry systems.

Usually, a juvenile maturation state is desired. However, sometimes a degree of maturation is required, particularly on exposed, highly fertile, fast-growth, ex-farm sites where toppling and unacceptable form are generally a problem. Rooted cuttings with a physiological age of 3 to 5 years have been recommended for planting on topple-prone sites (Forest Research Institute 1999). They have sturdier roots that are less likely to be distorted at planting, and they develop crowns that are more open and permeable, with less wind resistance. Both these characteristics increase stability on sites prone to toppling. There are also situations where increased maturation in planting stock can confer a degree of disease resistance (Power & Dodd 1984; Zagory & Libby 1985; Power *et al.* 1994).

Nursery-based Clonal Maintenance Systems

For most *P. radiata* clonal forestry operations, clones must be maintained without major maturation (i.e., maintained at less than physiological age 3 years) for a minimum of 10 years, preferably much longer. This will allow sufficient time for accurate clonal assessment in field trials and bulking up of selected clones. The goal is a propagation/clone maintenance system that will work for most genotypes and allow rapid production of large

numbers of uniform plants per clone, with minimal additional maturation from the time clones have been established in storage until *after* the assessment of clonal trials. Different clone maintenance systems have been developed by the New Zealand Forest Research Institute and are now in operational use by the forest industry in New Zealand, including cool storage and cryogenic storage of tissue-cultured material, and nursery-based systems (Hargreaves & Smith 1992; Hargreaves *et al.* 2002; Horgan *et al.* 1997; Menzies & Aimers-Halliday 1997, in press).

Juvenile characteristics are retained at the base of the tree near the axis, in physiologically young tissue, while maturation occurs in the periphery of the tree in the physiologically older but chronologically younger tissues (Sweet 1964; Borchert 1976; Fortanier & Jonkers 1976; Kleinschmit 1977; Hackett 1985). Thus, juvenility is related largely to the distance of plant tissue from the root collar, and both hedging and serial propagation decrease this distance (Hackett 1985; St Clair *et al.* 1985; Bonga & von Aderkas 1993).

Hedging involves repeated pruning of the donor plant, forcing growth in meristems near the physiologically juvenile base. It generally induces long-shoot initiation from dormant short-shoot meristems below the point of hedging by removing the inhibition of IAA (indoleacetic acid, a natural auxin) from the terminal short-shoot meristems (W.J.Libby, pers. com.). Hedging has been used for many years as a method of both minimising the effects of maturation in conifers and providing a multiplication system to supply material for propagation (St Clair *et al.* 1985; Ritchie 1991; Bonga & von Aderkas 1993; Menzies & Aimers-Halliday 1997; Zhou *et al.* 1998).

Libby and colleagues (1972, 1976) found that hedging *P. radiata* effectively slowed the decline in rooting percentage, plant quality, and growth rate of cuttings, which were normally associated with the maturation of clones. Bolstad & Libby (1982) compared *P. radiata* cuttings of hedge- and tree-form origin and concluded that frequent hedging maintained a juvenile condition in the hedge-form donor plants, while the tree-form donors continued to mature. Hedging is used to maintain juvenility in a number of other conifer species, such as *Cunninghamia lanceolata* (Lamb.) Hook. (Chinese fir) (Zhou *et al.* 1998), *Chamaecyparis nootkatensis* (D.Don) Spach. (yellow cedar) (Russell 1993), and *Pinus elliottii* var. *elliottii* × *P. caribaea hondurensis* (hybrid Caribbean pines) (Walker *et al.* 1996).

The alternative nursery system for managing maturation is serial propagation from the previous cycle of cuttings or stool beds, starting with physiologically young material (St Clair *et al.* 1985; Kleinschmit 1992; Bonga & von Aderkas 1993). Serial propagation has been used to slow maturation in clones of Norway spruce (St Clair *et al.* 1985; Dekker-Robertson & Kleinschmit 1991; Kleinschmit 1992). After seven propagation cycles with 3 years per cycle (22 years from seed) there was no significant reduction in rooting, but in later cycles some changes in morphological traits became increasingly apparent.

There are few reported studies comparing the two nursery methods of controlling maturation. However, Mason *et al.* (2002) compared hedging and serial propagation as methods of controlling maturation in *Picea sitchensis* (Bong.) Carrière (Sitka spruce). The hedges were cut back annually to a height of 1 m. Using ease of rooting as an indicator of juvenility, the authors concluded that serial propagation was the better method for controlling maturation in Sitka spruce. The authors also had anecdotal observations of morphological differences between the different treatments, with cuttings from the hedges

having harsher needles, similar to those of mature trees. However, these morphological differences did not persist.

Few studies of maturation and the maintenance of juvenility have gone beyond the propagation stage to assess field performance (Ritchie 1991). Many workers consider ease of rooting to be a key indicator of juvenility. However, ease of rooting may be maintained, but maturation may continue in other characteristics (Kleinschmit 1977; St. Clair *et al.* 1985; Dekker-Robertson & Kleinschmit 1991) and this may have a significant impact on clonal performance. Indeed, juvenile characters are often not highly correlated with each other, suggesting relatively independent mechanisms for the control of maturation (Borchert 1976; Greenwood 1995). Also, it is not always certain to what degree ease of rooting is due to the actual slowing or arrest of maturation and how much is due to a temporary reinvigoration of the material (Fortanier & Jonkers 1976; Hackett 1985; St. Clair *et al.* 1985; Wareing 1987; Dekker-Robertson & Kleinschmit 1991; Bonga & von Aderkas 1993; Greenwood 1995).

In New Zealand, there is keen interest in the control of maturation in *P. radiata*, particularly where clonal forestry is becoming an established practice. In 1988, a long-term nursery trial was established at the New Zealand Forest Research Institute. The main objective was to determine the best combination of hedging and serial propagation for control of maturation. Clonal stool-beds have been hedged annually and subjected to five different nursery treatments, including four cycles of serial propagation and a treatment with hedging only. Results from the nursery phase of the research, and from a field trial established in July 1997, are reported below. This information is highly relevant to maintenance of physiological age (control of maturation) in current nursery stool-bed systems.

MATERIALS

Three clones from each of 10 polycross families were randomly selected and propagated by fascicle cuttings from an existing stool-bed management trial sown in 1988, and clonal hedges were established in 1990. There were two different controls. The first consisted of three clones of a "climbing select" seedlot (with minimal genetic improvement, seed collected by climbing selected trees), which were raised concurrently with the clones from the half-sib families. (A "family" is defined here as a group of individuals originating from the same parent tree, which has been selected as superior in the breeding programme). GF-14 seedlings, planting stock with a well-characterised level of genetic improvement for growth and form, were a second control in the field trial. This gave a standard baseline comparison for the performance of the cuttings raised under the five different nursery regimes.

METHODS

Nursery Propagation Treatments

(1) Seed was sown in 1988 and clonal hedges were established from fascicle cuttings, in 1990, at a spacing of 30 × 50 cm. Four ramets were established per clone. The raised open beds were fertiliser treated with three applications of Nitrophoska Blue at a rate of 10 g/m^2. All beds were sprayed with a pre-emergence weedicide (propazine) at a rate of 0.6 kg active ingredient/ha, and this spray regime was repeated at about

6-weekly intervals to prevent weed germination. Fungicides and insecticides were applied as necessary. All clonal hedges were hedged every summer (January-February) to a height of 50 cm, using a rotary slasher.

(2) The clonal hedges were serially repropagated either every year or every second, third, or fourth year to evaluate the effect of serial propagation on physiological age (Fig. 1). For one treatment there was no serial propagation (until 1996) and cuttings were propagated directly from the original hedges. From 1990 to 1995 inclusive, topped cuttings were set directly into stool-beds at a 50 × 50 cm spacing for the 3- and 4-year cycles, and at 30 × 50 cm spacing for the 1- and 2-year cycles. From 1996 onwards, cuttings were set into BCC 150-cc containers (a standard container used in New Zealand) and subsequently lined out the following April at 30 × 50 cm. This change to containers was for ease of operation and to counteract any negative herbicide effects.

(3) From 1990 to 1995 the cuttings collected were 10 to 12 cm in length and 4 to 5 mm in diameter, and were direct set in open beds in June to a depth of 4 cm. From 1996 onwards, cuttings were 7 to 8 cm in length and were set in containers to a depth of 2.5 cm. No rooting hormones were applied, but irrigation was given for the 3 weeks immediately after setting and then as required. Rooting was normally completed by early summer (November-December) and three foliar applications of Yates Thrive® were given to the container cuttings (in February, March, and April) to counteract yellowing of the cuttings.

(4) The original clonal hedges and all subsequent propagations were planted with clones completely randomised within two replications.

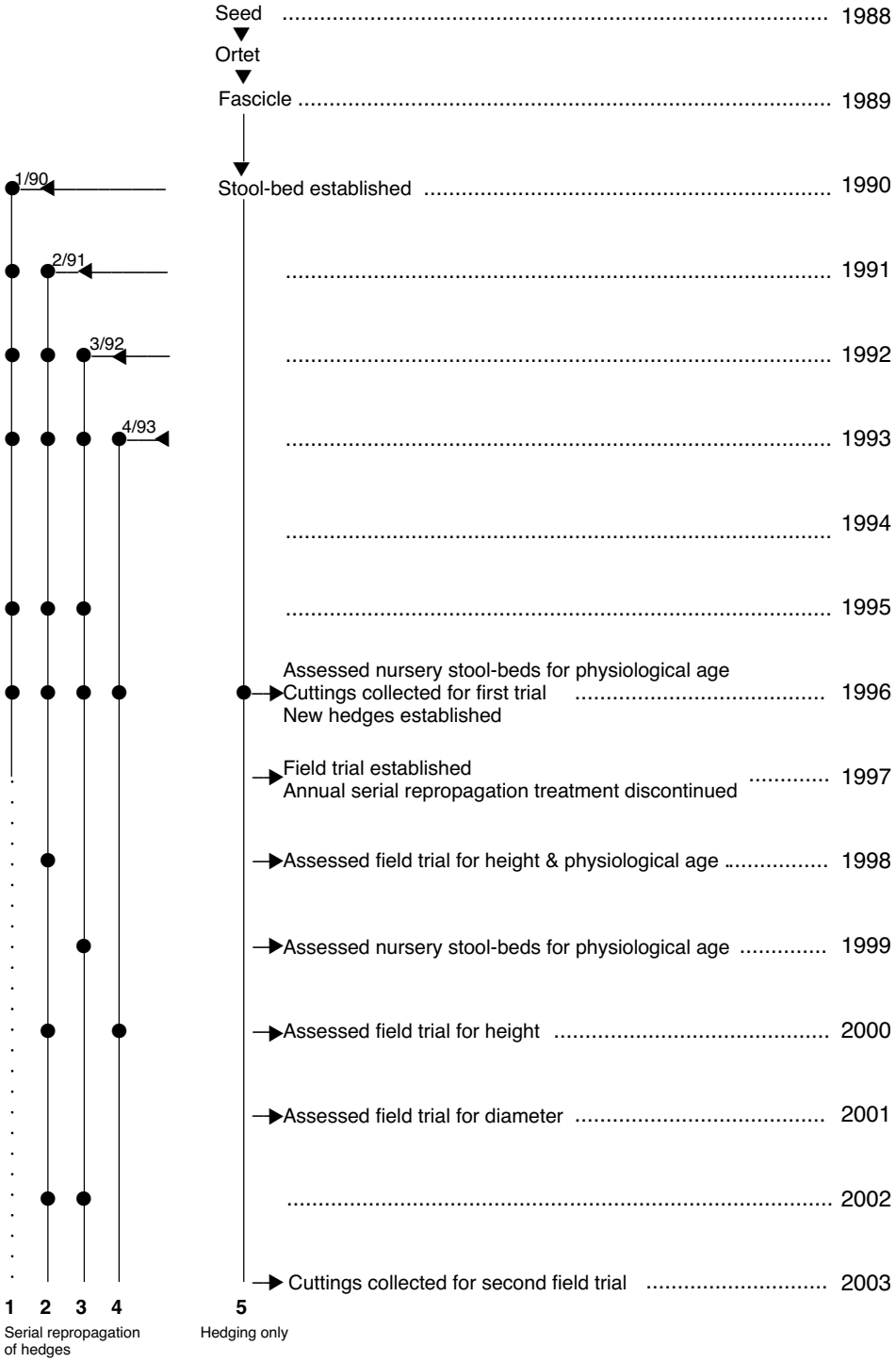
(5) In 1994 a year was skipped in the annual serial-repropagation treatment due to loss of vigour with such an intense propagation treatment.

(6) Cuttings of similar diameter and length were collected from all treatments in the winter of 1996 (June) for establishment of a field trial, and were set in rootrainers with bark/pumice/peat media. Uniformity in size was achieved by topping selected cutting material 6 weeks prior to collection. The cuttings were all in a similar physiological state within each treatment, with small (approximately 1-cm) fascicle buds present at setting. This avoided the problem, seen in standard cuttings, of considerable differences in size, type, and condition of cuttings between and within clones. Also, care was taken to collect at least one cutting from each ramet per clone to minimise any potential bias due to variation between ramets within clones.

(7) Because of decline in vigour due to root rot, and demand for nursery-bed space, the original clonal hedges were repropagated in 1996 for the establishment of new clonal hedges in 1997. Although there is no standard practice, *P. radiata* clonal hedges are generally repropagated every 3–5 years, depending on their health.

(8) The annual serial-repropagation treatment was discontinued in 1997 because it proved impractical due to loss of vigour in the stool-beds. This was caused by the annual

FIG. 1 (*facing page*) –History of the nursery propagation trial. Note—black dots represent propagation events and solid lines represent the five different nursery treatments. Cuttings were collected from all treatments in 1996 for a field trial and, for health reasons, the original hedges were repropagated for establishment of new clonal hedges.


Seed
Ortet
Fascicle
Stool-bed established
1/90
2/91
3/92
4/93
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
Assessed nursery stool-beds for physiological age
Cuttings collected for first trial
New hedges established
Field trial established
Annual serial repropagation treatment discontinued
Assessed field trial for height & physiological age
Assessed nursery stool-beds for physiological age
Assessed field trial for height
Assessed field trial for diameter
Cuttings collected for second field trial
1 2 3 4
Serial repropagation of hedges
5
Hedging only
Nursery treatments

propagation shortening the growing season (only November until June — rooting to collection). The plants were barely large enough to provide adequate material for collection of cuttings.

Assessment of Physiological Age in the Nursery

In the winter of 1996, all clonal hedges were visually assessed ("blindly", i.e., the treatment information was coded) for morphological markers of physiological age by two experienced nursery researchers, working independently. The characteristics observed were the presence of sealed buds, length of primary needles, and length of fascicle needles, as described by Menzies *et al.* (2000). The hedges were allowed to grow *unhedged* for 1 year from 1998, and then reassessed for physiological age in July 1999, to check if the hedging treatment had masked the physiological age of the hedges.

Analyses of Nursery Data

Analysis of variance (ANOVA) was done using the SAS general linear model procedure (SAS Institute 1989) to determine if there were any differences in the physiological age scores of the clonal hedges for the five different nursery treatments. Differences in the physiological age of the different families, plus the interaction between the families and treatments, were also tested. Where appropriate, the data were transformed to stabilise the variances and satisfy the ANOVA assumptions. The data were analysed as a fixed effects model. Tukey's Honestly Significant Difference (HSD) multiple comparison test was used to compare the family and treatment means at the 5% level. Rooting of the cuttings for the field trial, from the different serial propagation treatments, was also analysed using ANOVA and Tukey's HSD test. This could possibly give a further indication of maturation, as rooting of cuttings declines with increasing maturation of the donor material (Forest Research Institute 1991).

Pearson's correlation coefficients were calculated for the two sets of physiological age scores for the stool beds (1996 and 1999 assessments), and the correlation between the 1996 data for physiological age and rooting percentage.

Field Trial Design and Establishment

The trial (FR 311) was established in July 1997 in Cpt 156 of Woodhill Forest, north of Auckland, on land owned by Carter Holt Harvey Forests Ltd. The cut-over site had sandy-loam soils and flat to gently undulating topography with short slopes up to 15°. No site preparation was necessary, but releasing with herbicide was done 1 month after planting, using standard practices.

The design was a split plot, with the main plot being the family component (and clones nested within families). For the sake of simplicity, the "climbing select" seedlot is viewed as a "family". The subplot was the method of propagation — that is, the four cycles of serial propagation plus the hedged treatment. There were six replications each with 11 main plots, representing the 11 families, randomly positioned within each replication. Each main plot had 16 trees: three clones from that family were included within each of the subplots — the five different nursery treatments, plus a seedling control. The design is summarised as 11 families × 3 clones × 5 nursery treatments in each replication. The six ramets per clone were included in each of the six replications.

Each replication contained a total of 176 trees and the area was 0.282 ha, with 4 × 4 m spacing. The total number of trees in the trial was 1056 and the total area was 1.69 ha, plus a two-row buffer planted around the trial.

Assessments of the Field Trial and Data Analyses

The heights of all the trees were measured immediately after planting. Height, survival, and health were recorded in the winters of 1998 and 2000. Physiological age was scored in June 1998. The indicators of physiological age that were recorded included the presence of a tuft of primary needles at apex, transitional buds (semi-sealed) or sealed buds in winter, and the presence of female flowers or pollen catkins (Menzies *et al.* 2000). Diameters were recorded in 2001, 4 years after the trial was planted.

An analysis of covariance (adjusting for initial height as a covariate) was done using the SAS GLM procedure, to determine if there were any differences in physiological age, height, and diameter for the five different nursery treatments. All the variables were analysed as fixed effects. The data were also analysed as a mixed effects model using the SAS MIXED procedure, with random family and clone effects and fixed treatment effects. Tukey's HSD test was used to compare the treatment means (at the 5% level). The data from the field trial included a clonal component, allowing for evaluation of clonal differences in physiological age, height, and diameter, as well as family differences, and the presence or absence of any clone-by-treatment interaction. Where necessary, the data were transformed to stabilise the variances and satisfy the ANOVA assumptions and the correct F-tests were specified. Trees assessed as having markedly poor health were excluded from all analyses.

Pearson's correlation coefficients were calculated to determine relationships between different variables.

RESULTS

Physiological Age and Rooting in the Nursery

The results presented here are from the analysis of the general linear model, with all variables analysed as fixed effects. Analysis of variance for physiological age of the different nursery treatments is presented in Table 1. The differences in physiological age

TABLE 1–Analysis of variance for the physiological age scores of the clonal hedges from the five nursery propagation treatments, July 1996 assessment.
(The data were transformed by $1/\sqrt{x}$)

Source of variation	Degrees of freedom	Mean squares	F values	Probability
Family	9	0.01011942	5.45	<0.0001 ***
Treatment	4	0.00885069	4.77	0.0015 **
Family × Treatment	36	0.00119470	0.64	0.9326
Error	100	0.001885600		
Total	149			

* Significant at the 0.05 level
** Significant at the 0.01 level
*** Significant at the 0.001 level

between the 10 families, and for the different serial propagation treatments, were both highly significant. The mean physiological age varied from 2.13 years for Family 10 to 2.70 years for Families 1 and 2. The climbing-select control had a mean age of 2.37 years (Table 2).

The mean physiological age in July 1996, for the five different nursery treatments, varied from 2.24 years for the hedged treatment (no serial propagation) to 2.61 years for hedges that had been serially propagated annually (Fig. 2) with an overall mean of 2.44 years. Therefore, increasing the number of cycles of serial propagation in the stool-

TABLE 2–Mean physiological age in the nursery (assessed 1996 and 1999) and rooting percentages of the families (assessed 1996) from all the nursery treatments.

Families	Physiol. age (yr) 1996	Significant differences	Rooting percentage 1996	Significant differences	Physiol age (yr) 1999	Significant differences
1	2.70	A	96.7	AB	3.14	AB
2	2.70	A	72.9	C	3.10	AB
3	2.57	AB	92.9	AB	3.04	AB
4	2.57	AB	92.1	ABC	3.13	AB
5	2.50	ABC	91.3	ABC	3.21	A
6	2.43	ABC	95.8	AB	3.00	AB
7	2.40	ABC	83.8	BC	3.08	AB
Climbing select	2.37	ABC	92.5	AB	3.21	A
8	2.27	BC	91.7	AB	2.75	AB
9	2.23	BC	82.9	ABC	2.79	AB
10	2.13	C	97.1	A	2.68	B
Overall mean	2.44		90.0		3.01	

Note:
(1) Means followed by the same letter are not significantly different at the 5% level with the Tukey's HSD test.
(2) The means presented here have not been transformed (although the analysis was done on transformed data).

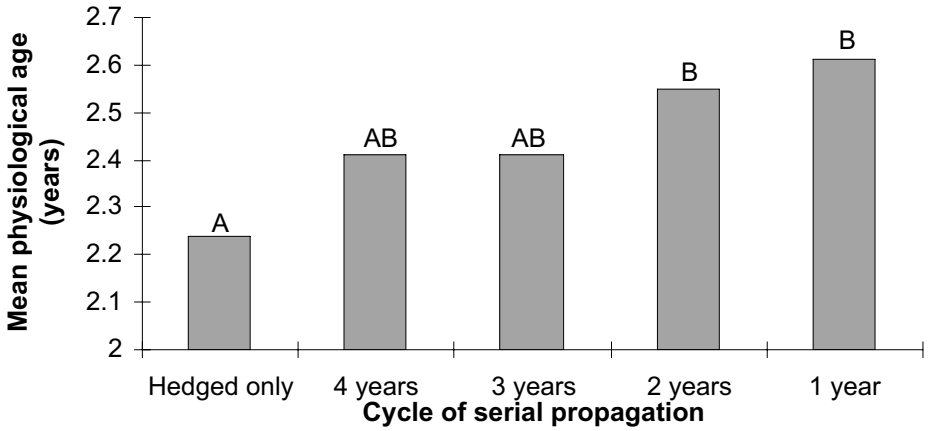


FIG. 2–Physiological age of stool-beds after nursery propagation treatments. Bars with the same letter are not significantly different at the 5% level with the Tukey's multiple comparison test.

beds increased physiological age, but even the degree of physiological ageing in the annual repropagation treatment was not great. There was no difference in the physiological age of the treatments that were serially repropagated every 3 and 4 years, which probably reflects the small difference in the number of actual cycles of serial propagation at the time of assessment (Fig. 1).

The hedges were assessed again for physiological age in July 1999 after they were allowed to grow unhedged for one year. This time there was no significant difference in physiological age for the nursery treatments (Table 3), with means varying only from 2.98 years for the hedged treatment (no serial propagation) to 3.03 years for hedges that had been serially repropagated every 2 years. The overall mean was 3.01 years. The annual serial propagation treatment, which was discontinued in 1997, was not included in this assessment and that may have influenced the results.

For the 1999 data, the differences in physiological age between the families were again highly significant (Table 3). Interestingly, for some families the rankings for physiological age in 1999 were similar to those in 1996 (e.g., Family 10), yet others changed in ranking (e.g., Family 5 and the "climbing select"). It appeared that some of the families were showing different rates of maturation. However, the correlation in physiological ages for the 10 different families (excluding the "climbing select") between the 1996 and 1999 nursery data was 0.75 and highly significant (Pearson's correlation coefficient, $p = 0.007$).

The analysis of variation for the rooting of cuttings from the five different nursery treatments is presented in Table 4 (1996 rooting percentage). The effects of families and treatments on rooting were highly significant. Rooting varied from 73% (Family 2) to 97% (Families 1 and 10) (Table 2). Eight families had rooting percentages over 90%, two were in the range 83–84%. There was little difference in the rooting percentage between the different nursery treatments, with only the 2-yearly serial propagation treatment significantly different from the others at 83% (Fig. 3). There was no obvious explanation for the lower rooting for the biennial serial propagation treatment. The highest rooting percentage was for the hedged-only treatment (94%), with 89% for the hedges that had been serially repropagated every 4 years, 91% for hedges that had been serially repropagated every 3 years, and 92% for hedges that had been serially repropagated annually. The overall mean percentage rooting was 90%.

TABLE 3–Analysis of variance for the physiological age scores of the clonal hedges, subjected to five propagation treatments, after they were allowed to grow unhedged before assessment in July 1999. (The data were transformed by $1/\sqrt{x}$.)

Source of variation	Degrees of freedom	Mean squares	F values	Probability
Family	9	0.00445912	2.68	0.0093 **
Treatment	3	0.00015935	0.10	0.9622
Family × Treatment	27	0.00061161	0.37	0.9977
Error	76	0.00166535		
Total	115			

* Significant at the 0.05 level
** Significant at the 0.01 level
*** Significant at the 0.001 level

TABLE 4–Analysis of variance for rooting percentage (in 1996) of cuttings from clonal hedges subjected to five different propagation treatments. (The data were angular transformed.)

Source of variation	Degrees of freedom	Mean squares	F values	Probability
Family	9	692.3547	4.13	0.0001 ***
Treatment	4	711.8563	4.25	0.0031 **
Family × Treatment	36	100.2348	0.60	0.9668
Error	100	167.4883		
Total	149			

* Significant at the 0.05 level
** Significant at the 0.01 level
*** Significant at the 0.001 level

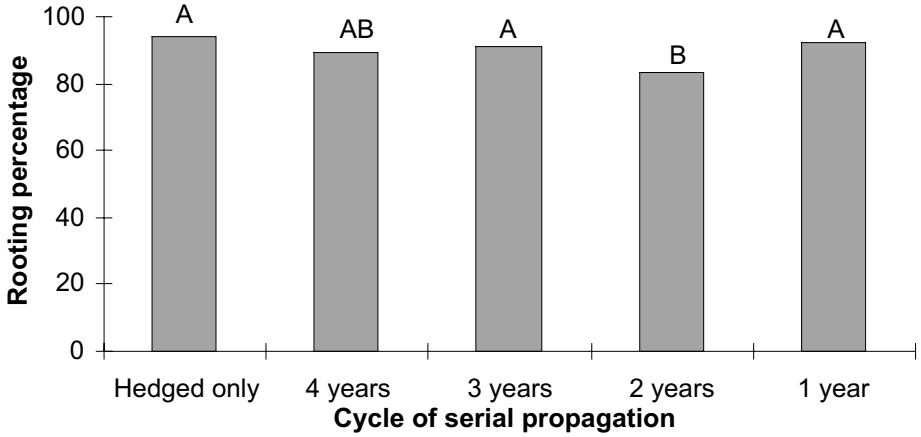


FIG. 3–Rooting percentage of cuttings from the nursery propagation treatments. Bars with the same letter are not significantly different at the 5% level with the Tukey's multiple range test.

There were no significant treatment-by-family interactions for any of the data analyses for the nursery phase of the experiment.

Physiological Age and Growth in Field Trials

The results presented here are from the analysis of the general linear model, with all variables analysed as fixed effects. The data were also analysed as a mixed effects model using the SAS MIXED procedure, with random family and clone effects, and fixed treatment effects. The results for the mixed effects model gave very similar results with the same interpretations as for the fixed model, and are not presented in this report.

Analysis of variance for physiological age of the rooted cuttings from the nursery treatments, 1 year after field planting, is presented in Table 5. The differences in the scores for physiological age among the 10 families (excluding the "climbing select") and for the clones within families were both highly significant. The mean physiological age varied from 2.76 years for Family 10 to 3.23 years for Family 4 (Table 6). Rooted cuttings from the "climbing-select" seedlot were the next most mature, with a mean physiological age of

TABLE 5–Analysis of variance for physiological age of rooted cuttings in field trial, July 1998. The cuttings originated from hedges subjected to different propagation treatments. (Data were transformed by $1/\sqrt{x}$, and correct F-tests specified).

Source of variation	Degrees of freedom	Mean squares	F values	Probability
Initial height	1	0.006769	1.65	0.1991
Replication	5	0.004387	0.75	0.5887
Family	9	0.016755	2.90	0.0077 **
Replication × Family	45	0.005884	1.44	0.0363 *
Clone (Family)	20	0.012395	3.03	<0.0001 ***
Treatment	4	0.008952	2.19	0.0694
Family × Treatment	36	0.004609	1.13	0.2866
Clone (family) x Treatment	80	0.003845	0.94	0.6280
Error	524	0.004095		
Total	724			

* Significant at the 0.05 level
** Significant at the 0.01 level
*** Significant at the 0.001 level

TABLE 6–Family means (including "climbing select" control) for the rooted cuttings in the field trial. Height measurements, physiological age scores, and diameter at breast height are listed.

Families	Physiol.age in 1998 (yr)	Significant differences	Height in 2000 (m)	Significant differences	Dbh in 2001 (cm)	Significant differences
1	2.80	A	2.49	A	5.55	A
2	2.91	AB	2.27	A	5.19	A
3	3.00	AB	2.20	A	5.24	A
4	3.23	B	2.19	A	5.25	A
5	3.12	AB	2.44	A	5.73	A
6	3.04	AB	2.45	A	5.40	A
7	2.86	AB	2.62	A	6.37	A
Climbing select	3.13	AB	2.17	A	4.99	A
8	3.09	AB	2.39	A	5.65	A
9	2.85	A	2.50	A	5.78	A
10	2.76	A	2.19	A	5.31	A
Overall mean	2.99		2.33		5.46	

Note:
(1) Means followed by the same letter are not significantly different at the 5% level with the Tukey's HSD test.
(2) The physiological age means presented here have not been transformed (although the analysis was done on transformed data).

3.13 years. The overall mean for all the clonal material across all treatments was 2.99 years, which is almost half a year older than the overall mean of 2.44 years for the 1996 nursery assessment of the clonal hedges. The seedling controls in the field trial (GF-14 bare-root seedlings) were assessed as having a mean physiological age score of 2.40 years when they were at a chronological age of 2 years, indicating more rapid maturation than observed at central North Island sites.

It is interesting that some of the families had similar rankings for physiological age throughout the experiment (e.g., Family 10 with the youngest physiological age score in the nursery and field assessments) yet others changed in ranking (e.g., Family 1 and the "climbing select"). It appears that the families (and the "climbing select" seedlot) were showing different rates and patterns of maturation. Some families matured at faster rates early on and then maturation slowed, while others initially matured at a slower rate and then matured at faster rates later on. Differences between clones, within families, were highly significant. Clonal means for physiological age in the field trial are shown in Table 7. For

TABLE 7–Clone means (including "climbing select" control) for the rooted cuttings in the field trial. Height measurements, physiological age scores, and diameter at breast height are listed with year of assessment.

Family	Clone	Number of trees	Physiological age in 1998 (yr)	Height in 2000 (m)	Dbh in 2001 (cm)
6	5	23	3.00	2.70	6.00
	19	25	3.30	2.52	5.76
	29	23	2.90	2.12	4.41
Climbing	5	24	3.10	2.14	4.96
select	11	27	3.20	2.31	5.37
	17	25	3.10	2.06	4.60
3	5	26	2.90	2.03	4.71
	17	23	3.00	2.28	5.55
	19	25	3.20	2.32	5.52
7	7	26	2.90	2.43	6.08
	9	26	3.10	2.82	6.73
	11	25	2.60	2.60	6.30
9	5	23	2.90	2.50	5.73
	7	27	2.70	2.41	5.76
	17	22	3.00	2.62	5.85
5	5	25	3.00	2.31	5.63
	7	26	3.10	2.26	5.08
	39	28	3.30	2.72	6.41
1	11	27	2.60	2.46	5.11
	17	25	2.90	2.47	5.61
	19	28	2.90	2.52	5.92
8	5	24	3.00	2.44	6.13
	7	30	3.20	2.46	5.61
	9	24	3.00	2.26	5.21
10	5	26	2.50	2.02	5.11
	7	22	3.00	2.57	6.51
	17	25	2.90	2.02	4.46
2	5	20	2.70	2.19	4.52
	7	21	3.20	2.40	5.84
	11	29	2.90	2.24	5.17
4	5	25	3.30	2.28	5.41
	11	27	3.30	2.13	5.20
	17	25	3.10	2.15	5.14

some families, clonal means for physiological age were very similar, but within other families there were large differences in clonal means, though there were only three clones per family, which limits the value of comparisons.

The mean physiological ages for the serial propagation treatments varied from 2.90 years for the hedged treatment (no serial propagation) to 3.10 years for hedges that had been serially repropagated annually, with the overall mean of 2.99 years for all the treatments (Table 8). There appeared to be a persistence of the trend observed in the nursery, i.e., increasing the number of cycles of serial propagation in the stool-beds increased physiological age in the subsequent rooted cuttings, but this difference was marginally non-significant in the analysis of variance ($p = 0.069$) (Table 5).

TABLE 8–Treatment means for the rooted cuttings in the field trial. Height measurements, physiological age scores, and diameter at breast height are listed for the five different serial propagation treatments and for the seedling control.

Serial propagation treatment	Number of trees	Initial height 1997 (cm)	Height in 1998 (cm)	Physiological age in 1998 (yr)	Height in 2000 (m)	Dbh in 2001 (cm)
Propagated every year	161	19.7	38.5	3.10	2.35	5.42
Propagated every 2 years	162	19.6	37.1	3.00	2.31	5.35
Propagated every 3 years	174	21.1	38.8	3.00	2.38	5.56
Propagated every 4 years	161	19.9	37.9	3.00	2.35	5.57
Hedged control	169	21.7	40.7	2.90	2.39	5.59
Seedling control	61	23.8	47.5	2.40	2.52	6.05
Means for all trees	888	20.6	39.2	2.90	2.37	5.54

Note: there were no significant differences between means

For the 1998 and 2000 height assessments, and the 2001 diameter assessment, there were no significant treatment effects or treatment interactions in the analyses of variance (results not presented). For the height assessment in 1998, the family effect was statistically significant ($p = 0.006$), but the effect of clones (within families) was not significant. However, for the height assessment in 2000, the family effect was marginally non-significant ($p = 0.0960$) and the clones-within-families effect was highly significant ($p \leq 0.0001$). For the 2001 diameter assessment also, the family effect was non-significant, but the clones-within-families effect was highly significant ($p \leq 0.0001$). There were no significant correlations between the mean physiological age of the rooted cuttings in the field trial and the means for the various growth assessments.

Results for height and diameter were very similar for the different propagation treatments and the GF-14 seedling controls in the field trial. The small differences that were evident between the rooted cuttings and the seedling controls were proportional to the small differences in size at planting.

Pearson's correlation coefficients for the relationship between the treatment means for the initial physiological age scores of the clonal hedges (from the nursery phase of the experiment), and means for the dbh and physiological age assessments in the field trial, were –0.86 and 0.64, respectively. This could indicate a negative trend between increasing

physiological age scores in the hedges and dbh in the subsequent cuttings, and a positive relationship between subsequent age assessments, as could be expected, but neither correlation was significant ($p = 0.1395$ and $p = 0.3637$, respectively). For individual-tree data, the correlation between the initial physiological age scores in the clonal hedges and dbh in the subsequent rooted cuttings was weakly negative but significant ($r = –0.080$, $p = 0.03$). This negative relationship might have been stronger if there were greater differences in initial physiological age in the nursery for the various treatments.

DISCUSSION

After 8 years from the initial sowing of seed for this experiment, significant differences in physiological age were observed between the nursery treatments, with the hedged treatment having the lowest physiological age score. In 1998, 1 year after field planting and 10 years after the seed was sown, the overall mean physiological age in the rooted cuttings was 2.99 years. The seedling controls in the field trial had a mean physiological age score of 2.40 years. These results are encouraging, as previous research indicates that if physiological age in *P. radiata* rooted cuttings is maintained below age 3 years, early loss of diameter growth can be avoided (Menzies & Klomp 1988; Forest Research Institute 1991).

The difference in the field trial between the mean score for physiological age and the chronological age for the seedling controls was approximately 0.40 years. This indicates either a degree of inaccuracy or bias in the physiological age assessments or, more likely, that maturation was occurring at a faster rate on this warm northern site. (The morphological markers described by Menzies *et al.* (2000), which were used to score physiological age, were based on *P. radiata* grown in the central North Island.)

The rooting results were expected to give a further indication of physiological age, as rooting of cuttings declines with increasing maturation of the donor material. Based on physiological age, we would have expected the annual serial-propagation treatment to have the lowest rooting percentage, but this treatment mean was not significantly different from the means for the hedged treatment and 3- and 4-yearly serial propagation treatments. Also, there was no significant correlation between the rooting percentage of the families and their physiological age scores. This is in agreement with Borchert (1976) and Greenwood (1995) who contended that juvenile characters are often not highly correlated with each other, suggesting relatively independent mechanisms for the various manifestations of maturation. However, the range of physiological age scores between the nursery propagation treatments in 1996 was only 2.24 to 2.61 years, and this was probably too small to give any consistent treatment effects for rooting. Also, as stated above, rooting may be influenced by re-invigoration of plant tissue with each propagation cycle.

The relative lack of significant correlations between different variables gives further indications of independence between growth variables and morphological markers for physiological age, but this may be due in part to the small (but often highly significant) differences in physiological age among families and treatments.

Our results contrast with results obtained by Mason *et al.* (2002) with Sitka spruce, where the best overall rooting was obtained from serial propagation every 2 years, from the

previous cycle of cuttings. Their rooting success for the hedged treatments was lower in most years, but varied greatly from year to year. The authors noted that there were indications that some of the poor rooting results for the two hedged treatments may have been due to the hedge management regime, particularly the maintenance of nutrient status. It is possible that the better rooting in the Sitka spruce cuttings for the serially propagated treatment was due to better nutrition and invigoration of the cuttings rather than maintenance of juvenility *per se*. It is also possible that the differing morphology and physiology of Sitka spruce were responsible for the different nursery results, compared with the results reported here for *P. radiata*.

In addition to this, our *P. radiata* experiment did not have a true test of the comparison between hedging and serial propagation treatments, because the clonal hedges from all treatments were hedged every summer to a height of 50 cm or less. In hindsight, it would have been useful to have included a serial propagation treatment without any hedging, as with the Sitka spruce experiment (Mason *et al.* 2002). Also in hindsight, it would have been useful to have included a seedling control in the nursery phase of the experiment.

For Sitka spruce, the difference in rooting performance between the serially propagated and two hedged treatments was more pronounced after 10 chronological years from the initiation of the experiment. This was possibly because their hedges were maintained at 1 m and, therefore, cuttings harvested from them were more distant from the roots than in the serially propagated cuttings treatment. All our *P. radiata* plants were hedged at 0.5 m, and so all the harvested cuttings would have been a similar distance from the root systems.

In the Sitka spruce experiment, field trial results for 11 years of plantings yielded some significant differences in height growth, but there was no consistency in the differences between treatments (Mason *et al*. 2002). With *P. radiata*, the growth differences were not significant between treatments, probably reflecting the small variation recorded in physiological age. Mason and colleagues report only one diameter assessment in the Sitka spruce experiment, made 15 years after the first field trial was planted (in 1982, 6 chronological years after seed was first sown). The diameters for the serially propagated treatment were significantly below those for cuttings taken from the original ortet, but this may have been due to anomalous height growth for the 1982 planting, which was not observed in other years of planting.

Previous research with *P. radiata* showed that height growth is similar for seedlings and cuttings (Menzies & Klomp 1988; Menzies *et al*. 1991; Forest Research Institute 1991). Early diameter growth up to the low pruning stage is similar for seedlings and cuttings up to a physiological age of 3 years, but may be markedly less for cuttings with a physiological age of 5 years or more (Menzies & Klomp 1988; Menzies *et al*. 1991; Forest Research Institute 1991).

McGranahan *et al.* (1999) quantified the effect of stock plant age on rooting and early growth of cuttings of *P. radiata*, plus genetic control of propagation effects. Performance of rooted cuttings declined with increasing stock plant age, but this effect was less than the genetic effects. However, the interaction between genotype and stock plant age was highly significant, and larger than the effect of age alone for all measured traits — that is, the decline in growth associated with propagules of increasing physiological age was not consistent across genotypes.

In this trial, no trends in treatment means for dbh and height were evident, and there were no significant correlations between the mean physiological age of the rooted cuttings in the field trial and the means for either growth variable. The only statistically significant main effect in the analysis of variance for dbh was the clone-within-families effect. The correlation between the initial age assessment in the clonal hedges and dbh in the subsequent rooted cuttings was weakly negative and marginally significant, indicating a slight decrease in dbh associated with increasing physiological age. This negative relationship would probably have been stronger if there were greater differences in initial physiological age in the nursery.

The field trial was on a sandy loam site, with slower growth than on many of the fertile farm sites currently being planted. It would be appropriate to plant a second trial on a fertile, faster growth site, as such a site would likely result in bigger growth differences between the different nursery treatments. It would also be useful to observe the effectiveness of the maintenance of juvenility over a longer time period, which would be more in line with operational, nursery-based, clonal forestry practices. Such a field trial is planned for 2004.

CONCLUSIONS

Methods of consistently maintaining maturation state in clonal storage systems are critical to successful family and clonal forestry systems. There will be loss of genetic gain if there is significant deviation from the optimum maturation state for the desired traits. Results from this experiment indicate that nursery-based hedging systems are a reliable method for controlling maturation.

In the nursery phase of this propagation trial, more frequent serial propagation (the annual treatment) resulted in slightly more maturation, while the treatment with hedging only was the most effective in minimising maturation. However, the treatment differences for physiological age, though highly significant statistically, were not large, and they became non-significant in the field trial of rooted cuttings, which were derived from the treated hedges. In the field trial, the difference between the mean physiological age of the rooted cuttings and the seedling controls (1 year after planting the trial, 10 years from sowing of seed for the stool-beds) was not large: 2.99 years and 2.40 years, respectively.

In the nursery, there were significant differences among families in both physiological age scores and rooting percentage, although no relationship was detected between the two variables. Indeed, the overall results for physiological age, rooting, and growth indicate relatively independent mechanisms for the control of maturation, but this may be due in part to the small differences in physiological age scores for the different families and treatments.

Overall, this research demonstrates that nursery stool-beds can be managed using hedging to control maturation, keeping physiological age at optimal levels. It appears that the best option for minimising physiological age would be to maintain low hedges (approximately 30–50 cm high) and replace them by serial propagation every 4 years, or possibly even less frequently depending on the health of the clonal hedges. Even though the results for the different nursery treatments in the field trial were not significant, the trend was still evident and it would be better to err on the side of caution and minimise propagation events until further field trial results are obtained. If further field trials confirm that the

number of serial propagation cycles has a negligible effect on maturation, then greater flexibility would be possible in management of stock plants in nursery stool-beds.

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REFERENCES

AIMERS-HALLIDAY, J.; SHELBOURNE, C.J.A.; HONG, S.O. 1997: Issues in developing clonal forestry with *Pinus radiata*. Pp. 264–272 *in* Burdon, R.D.; Moore, J.M. (Ed.) "IUFRO '97 Genetics of Radiata Pine". Proceedings of NZ-IUFRO Conference 1–4 December and Workshop 5 December, Rotorua, New Zealand. *FRI Bulletin No. 203*.

BOLSTAD, P.V.; LIBBY, W.J. 1982: Comparisons of radiata pine cuttings of hedge and tree-form origin after seven growing seasons. *Silvae Genetica 31(1)*: 9–13.

BONGA, J.M.; VON ADERKAS, P. 1993: Rejuvenation of tissues from mature conifers and its implications for propagation *in vitro*. Pp. 182–199 *in* Ahuja, M.R.; Libby, W.J. (Ed.) "Clonal Forestry I, Genetics and Biotechnology". Springer-Verlag, Berlin Heidelberg.

BORCHERT, R. 1976: The concept of juvenility in woody plants. *Acta Horticultura 56*: 21–36.

BROWN, A.G. 1974: Comparison of early growth in radiata pines raised by cuttings from parents of different ages with that of seedling trees. *Australian Forest Research 6(3)*: 43–47.

DEKKER-ROBERTSON, D.L.; KLEINSCHMIT, J. 1991: Serial propagation in Norway spruce (*Picea abies* (L.) Karst): Results from later propagation cycles. *Silvae Genetica 40*: 202–214.

FIELDING, J.M. 1954: Methods of raising Monterey pine from cuttings in the open nursery. *Australian Forest and Timber Bureau Bulletin No. 32*. 29 p.

———1964: The possibility of using cuttings for the establishment of commercial plantations of Monterey pine. Proceedings of World Consultation Forest Genetics and Tree Improvement, Stockholm (FAO), Vol. II: 5/10. 7 p.

———1970: Trees grown from cuttings compared with tree grown from seed (*Pinus radiata* D. Don). *Silvae Genetica 19*: 54–63.

FOREST RESEARCH INSTITUTE 1991: Promising future for radiata pine cuttings. *New Zealand Forest Research Institute, What's New in Forest Research No. 212*.

———1999: Soften the blow — Plant aged radiata pine cuttings on sites. *New Zealand Forest Research Institute, What's New in Forest Research No. 248*.

FORTANIER, E.J.; JONKERS, H. 1976: Juvenility and maturity of plants as influenced by their ontogenetical and physiological ageing. *Acta Horticultura 56*: 37–44.

GREENWWOD, M.S. 1995: Juvenility and maturation in conifers: current concepts. *Tree Physiology 15*: 433–438.

HACKETT, W.P. 1985: Juvenility, maturation, and rejuvenation in woody plants. *Horticulture Reviews 7*: 109–155.

HARGREAVES, C.; SMITH, D. 1992: Cryopreservation of *Pinus radiata* embryogenic material. *International Plant Propagators Society Combined Proceedings 42*: 327–333.

HARGREAVES, C.L.; GRACE, L.J.; HOLDEN, D.G. 2002: Nurse culture for efficient recovery of cryopreserved *Pinus radiata* D. Don embryogenic cell lines. *Plant Cell Reporter 21*: 40–45.

HOLDEN, D.G. 1995: Field use of cuttings. Pp. 71–73 *in* Hammond, D. (Ed.) "NZIF 1995 Forestry Handbook". New Zealand Institute of Forestry (Inc.).

HOOD, J.V.; LIBBY, W.J. 1978: Continuing effects of maturation state in radiata pine and a general maturation model. Pp. 220–232 *in* Hughes, K.W.; Henke, R.; Constantin, M. (Ed.) Proceedings of International Symposium on "Propagation of Higher Plants Through Tissue Culture." USDOE Conf. 7804111.

HORGAN, K.; SKUDDER, D.; HOLDEN, D.G. 1997: Clonal storage and rejuvenation. Pp. 273–280 *in* Burdon, R.D.; Moore, J.M. (Ed.) "IUFRO '97 Genetics of Radiata Pine". Proceedings of NZ-IUFRO Conference 1–4 December and Workshop 5 December, Rotorua, New Zealand. *FRI Bulletin No. 203*.

KLEINSCHMIT, J. 1977: Problems of vegetative reproduction. Pp. 784–798 *in* "Third World Consultation on Forest Tree Breeding". 21–26 March, FAO / IUFRO Canberra, Australia.

———1992: Use of spruce cuttings in plantations. Pp. 1–10 *in* Rook, D.A. (Ed.) "Super Sitka for the 90s". *Forestry Commission Bulletin 103*.

KLEINSCHMIT, J.; SCHMIDT, J. 1997: Experiences with *Picea abies* cuttings propagation in Germany and problems connected with large scale application. *Silvae Genetica 26(5-6)*: 197–203.

LIBBY, W.J.; CONKLE, M.T. 1966: Effects of auxin treatment, tree age, tree vigor and cold storage on rooting young Monterey pine. *Forest Science 12*: 484–505.

LIBBY, W.J.; HOOD, J.V. 1976: Juvenility in hedged radiata pine. *Acta Horticultura 56*: 91–98.

LIBBY, W.J.; BROWN, A.G.; FIELDING, J.M. 1972: Effects of hedging radiata pine on production, rooting and early growth of cuttings. *New Zealand Journal of Forestry Science 2(2)*: 263–283.

McGRANAHAN, M.F.; BORRALHO, N.M.G.; GREAVES, B.L. 1999: Genetic control of propagation effects and the importance of stock plant age and source on early growth in cuttings of *Pinus radiata*. *Silvae Genetica 48(6)*: 267–272.

MASON, W.L.; BIGGIN, P.; MENZIES, M.I. 2002: A comparison of hedging and repeated cutting cycles for propagating clones of Sitka spruce. *Forestry 75(2)*: 149–162.

MENZIES, M.I.; AIMERS-HALLIDAY, J. 1997: Propagation options for clonal forestry with radiata pine. Pp. 256–263 *in* Burdon, R.D.; Moore, J.M. (Ed.) "IUFRO '97 Genetics of Radiata Pine". Proceedings of NZ-IUFRO Conference 1–4 December and Workshop 5 December, Rotorua, New Zealand. *FRI Bulletin No. 203*.

———: Propagation options for clonal forestry with conifers. *In* Carson, M. J.; Walter, C. (Ed.) "Plantation Biotechnology for the 21st Century". Research Signpost, Trivandrum, Kerala, India (in press).

MENZIES, M.I.; KLOMP, B.K. 1988: Effects of parent age on growth and form of cuttings, and comparison with cuttings. Pp. 18–39 *in* Menzies, M.I.; Aimers, J.P.; Whitehouse, L.J. (Ed.) "Workshop on Growing Radiata Pine Cuttings, May 1986". *New Zealand Ministry of Forestry, FRI Bulletin No. 135*.

MENZIES, M.I.; KLOMP, B.K.; HOLDEN, D.G. 1991: Optimal physiological age of propagules for use in clonal forestry. Pp. 142–145 *in* Miller, J.T. (Ed.) "Proceedings of FRI/NZFP Forests Ltd Clonal Forestry Workshop, 1–2 May 1989, Rotorua, New Zealand". *New Zealand Ministry of Forestry, FRI Bulletin No. 160*.

MENZIES, M.I.; DIBLEY, M.J.; FAULDS, T.; AIMERS-HALLIDAY, J.; HOLDEN, D.G. 2000: Research Note: Morphological markers of physiological age for *Pinus radiata*. *New Zealand Journal of Forestry Science 30(3)*: 359–364.

POWER, A.B.; DODD, R.S. 1984: Early differential susceptibility of juvenile seedlings and more mature stecklings of *Pinus radiata* to *Dothistroma pini*. *New Zealand Journal of Forestry Science 14*: 223–228.

POWER, A.B.; DODD, R.S.; LIBBY, W.J. 1994: Effects of hedging on maturation in radiata pine: western gall rust susceptibility. *Silvae Genetica 43*: 1–7.

RITCHIE, G.A. 1991: The commercial use of conifer rooted cuttings in forestry: A world overview. *New Forests 5*: 247–275.

ROBBINS, W.J. 1957: Physiological aspects of aging in plants. *American Journal of Botany 44*: 289–294.

RUSSELL, J.H. 1993: Clonal forestry with yellow-cedar. Pp. 188–201 *in* Ahuja, M.R.; Libby, W.J. (Ed.) "Clonal Forestry II, Conservation and Application". Springer-Verlag, Berlin Heidelberg.

St CLAIR, J.B.; KLEINSCHMIT, J.; SVOLBA, J. 1985: Juvenility and serial vegetative propagation of Norway spruce clones (*Picea abies*). *Silvae Genetica 34(1)*: 42–48.

SAS INSTITUTE 1989: "SAS/STAT Users Guide, Release 6.04 edition". SAS Institute Inc., Cary, NC, USA.

SHELBOURNE, C.J.A. 1991: Clonal testing of *Pinus radiata* in New Zealand. Pp. 25–31 *in* Miller, J.T. (Ed.) "Proceedings of FRI/NZFP Forests Ltd Clonal Forestry Workshop, 1–2 May 1989, Rotorua, New Zealand". *New Zealand Ministry of Forestry, FRI Bulletin No. 160*.

SWEET, G.B. 1964: The effect of physiological age of scion on growth of grafts in *Pinus radiata*. *New Zealand Forest Service, Forest Research Institute, Forest Research Note 37*. 8p.

———1973: The effect of maturation on the growth and form of vegetative propagules of radiata pine. *New Zealand Journal of Forestry Science 3*: 191–210.

SWEET, G.B.; WELLS, L.G. 1974: Comparison of the growth of vegetative propagules and seedlings of *Pinus radiata*. *New Zealand Journal of Forestry Science 4*: 399–404.

THULIN, I.J.; FAULDS, T. 1968: The use of cuttings in the breeding and afforestation of *Pinus radiata*. *New Zealand Journal of Forestry 13(l)*: 66-77.

TUFUOR, K.; LIBBY, W.J. 1973: First-lift pruning times of radiata pine seedlings and rooted cuttings in a small California experiment. *New Zealand Journal of Forestry 18*: 124–132.

WALKER, S.; HAINES, R.; DIETERS, M. 1996: Beyond 2000: Clonal forestry in Queensland. Pp. 351–354 *in* Dieters, M.J.; Matheson, A.C.; Nikles, D.G.; Harwood, C.E.; Walker, S.M. (Ed.) "Tree Improvement for Sustainable Tropical Forestry". Proceedings of QFRI-IUFRO Conference, October-November, Caloundra, Queensland, Australia.

WAREING, P.F. 1959: Problems of juvenility and flowering in trees. *Journal of the Linnaean Society London (Botany) 56*: 282–289.

———1987: Phase change and vegetative propagation. Pp. 263–270 *in* Abbott, A.J.; Atkin, R.K. (Ed.) "Improving Vegetatively Propagated Crops". Academic Press, London.

ZAGORY, D.; LIBBY, W.J. 1985: Maturation-related resistance of radiata pine to western gall rust. *Phytopathology 75*: 1443–1447.

ZHOU, T.; ZHOU, J.; SHELBOURNE, C.J.A. 1998: Clonal selection, propagation, and maintenance of juvenility of Chinese fir, and afforestation with monoclonal blocks. *New Zealand Journal of Forestry Science 28(3)*: 275–292.