Reprint No. 2829

RISK MANAGEMENT FOR CLONAL FORESTRY WITH PINUS RADIATA — ANALYSIS AND REVIEW. 2: TECHNICAL AND LOGISTICAL PROBLEMS AND COUNTERMEASURES

J. AIMERS-HALLIDAY and R. D. BURDON

New Zealand Forest Research Institute,
Private Bag 3020, Rotorua, New Zealand

(Received for publication 10 July 2003; revision 7 January 2004)

ABSTRACT

Risks, other than those associated with genetic uniformity and reduced genetic diversity, in implementing clonal forestry with *Pinus radiata* D.Don are often relatively predictable. They include technical and logistical difficulties in large-scale clonal propagation and clonal storage, and difficulties in accurate evaluation of clones. These problems not only impede capture of genetic gain, but they can also compromise the genetic diversity of deployed clonal material. Clones can be lost through failure in propagation and clonal storage systems. Such failure can occur early on, or it can occur through maturation in clonal storage during the clonal testing phase. As well as outright failure of clonal genotypes in clonal propagation and storage systems, general decline in clonal performance and unwanted intra-clonal variability can cause problems. The latter problem, which includes epigenetic effects and possible somaclonal variation, is sparsely reported but very insidious. Careful management of clonal material in large-scale propagation is therefore crucial, control of maturation being a key factor. Clonal testing, though costly, is important not only for testing the performance of individual genotypes, but also for testing clonal uniformity and the quality of clonal planting stock. Clonal testing can be complicated by genotype-environment interaction, and lack of clonal uniformity due to epigenetic effects, with imprecise evaluations compromising genetic gains. These risks can be minimised by fully researched and carefully managed propagation and clonal maintenance systems, together with stringent field testing.

Keywords: clonal propagation; clonal tests; maturation; epigenetic effects; rooted cuttings; *in vitro* propagation.

INTRODUCTION

Potential advantages of clonal forestry, and strategic issues centring around uncertainties and the need for risk spread, were explored by Burdon & Aimers-Halliday (2003) with prime reference to *Pinus radiata* in New Zealand. The risks considered included a range of biotic risks, especially the possibility of introduction of a new and serious fungal pathogen, in addition to risks associated with market uncertainties. Complementary risk issues, which are addressed in this paper, involve difficulties in large-scale propagation of

clonal material, difficulties in clonal storage during the clonal testing phase, and difficulties in accurate evaluation of candidate clones. Risks associated with these latter issues involve both incomplete capture of genetic gain and unwanted phenotypic variability of crops. Yet some of these same risks can involve loss of the genetic diversity that is a key element of risk spread in clonal forestry, which was addressed in the first paper.

A pre-eminent barrier to implementing clonal forestry with most conifers, including *P. radiata*, has been maturation of clonal material (Thompson 1984; Shelbourne 1991; Libby & Ahuja 1993; Ritchie 1994). Indeed, central to the success of clonal forestry is the maintenance of juvenility in clones during clonal testing, or, alternatively, the ability to restore juvenility at the end of clonal testing (Aimers-Halliday *et al.* 1997). Moreover, the interrelated issue of clonal testing poses its own problems, which are in part shared by testing of seedling progeny. All these risks relate, directly or indirectly, to problems that are no longer an outright barrier to clonal forestry but are not fully resolved. In that they involve known but ubiquitous problems that are generally amenable to specific countermeasures, they differ from the risks addressed in the preceding paper, which relate mostly to almost complete uncertainties.

This paper is aimed largely at readers who are directly involved in clonal propagation and storage, and at those who are responsible for deployment and management of clonal forests.

Alternative Clonal Propagation Technologies

Before reviewing the actual risks, it is appropriate to review briefly the alternative propagation technologies — namely field-collected cuttings, nursery stool-bed cuttings, classical tissue-culture systems, and somatic embryogenesis. The relative costs and benefits of these technologies are discussed by Menzies & Aimers-Halliday (in press). In *P. radiata*, use of cuttings appears to work for almost the entire population, provided the material is still juvenile, but multiplication rates remain a limiting factor, especially in the early stages of multiplication. Field-collected cuttings are too expensive for many routine commercial clonal forestry operations, but may have a role to play in specific circumstances, e.g., the deployment of stable planting stock for sites with high risk of tree topple. However, reliable nursery stool-bed systems are now used for clonal storage and for producing high-quality plants at quite low cost (*see* Menzies & Aimers-Halliday in press, Table 2).

In vitro culture systems, namely tissue culture and somatic embryogenesis*, have the advantages of high potential multiplication rates, amenability to cool storage or cryopreservation systems for clonal maintenance, and amenability to genetic modification. However, there are major disadvantages in that the techniques may not work for a considerable proportion of genotypes, plant quality may be poor, and costs are currently high. The costs of *in vitro* systems, particularly somatic embryogenesis, may decrease significantly through automation, particularly if artificial seeds become available (Sutton 2002), and plant quality continues to improve with further development of the technology.

---

* Such embryogenesis, while being somatic in that it involves a post-zygotic phase (i.e., after fusion of the pollen nucleus and the egg nucleus), is feasible only with explants of seed embryos that are in early stages of development.

It should be noted that for many decades mature clones of superior *P. radiata* have been vegetatively propagated by grafts and cuttings for the establishment of genetic archives and clonal seed orchards. However, such mature material which readily produces pollen and seed, is inappropriate for clonal forestry. Grafts, while they may grow rapidly on seedling rootstocks, are very costly to produce, and are subject to delayed graft incompatibility. Cuttings of such material are not only difficult and costly to produce, but they also grow much more slowly than seedlings, at least during the early years.

Categorisation of Risks

The risks addressed in this paper are listed below in terms of generating factors, with co-factors and impacts summarised.

- Risks associated with failure in part of a population in propagation and clonal storage systems, causing
  - Wastage of resources invested in selection and testing of clones
  - Loss of genetic gain, and/or
  - Loss of genetic diversity in the production population;
- Risks associated with mislabelling and other clonal misidentification, causing direct losses of genetic gain;
- Risks associated with unwanted variation within clones
  - Somaclonal variation
  - Systemic infections
  - Epigenetic effects, especially differential maturation effects,

  which will compromise commercial acceptability of planting stock and generally lead to clonal under-performance;
- Climatic risks, leading to clonal under-performance or even outright crop failure;
- Risks of inadequate evaluation
  - Inherently inadequate testing programme
  - Foreshortening the duration of clonal tests
  - Lack of clonal buffering against genotype-by-environment interaction (G×E)
  - Interaction between cultivar-decline effects and growing environment, which will lead to both some loss of genetic gain and clonal under-performance;

A summary statement of these categories of technical risk, co-factors, and potential impacts, extending to appropriate countermeasures, is given in Table 1.

RISKS RELATED TO VARYING SUCCESS OF GENOTYPES IN PROPAGATION AND CLONAL STORAGE SYSTEMS

Propagation failure in its various forms is the most obvious type of cultivar decline that can affect clonal forestry with *P. radiata*. It can erode both the genetic gain, by reducing effective selection intensity (Burdon 1989; Haines & Woolaston 1991), and the genetic diversity of commercial crops. It need not take the form of outright failure, since even

TABLE 1–Summary of risks of clonal forestry with *Pinus radiata* in New Zealand, including cofactors, potential impacts, and appropriate management countermeasures*.

Risk category	Cofactors	Potential impacts	Management approach	Specific countermeasures*
Propagation failure of some clones, or whole families	• Propagation method • Clonal storage technique • Length of time in storage • Number of propagation cycles	• Loss of genetic gain • Increased cost • Loss of genetic diversity in production population • Loss of valuable, well-known clones	Active countermeasures (risk spread)†	• Increase numbers and diversity of genotypes initially propagated • Choice of propagation &/or clonal storage method • Improvement of propagation and storage protocols • Rejuvenation (if possible)
Mislabelling and clonal misidentification	• Propagation method • Clonal storage technique • Record keeping • Length of time in storage • No. of propagation cycles • Label durability and resistance to tampering	• Loss or confusion of clonal identity and/or pedigree • Loss of genetic gain • Incorrect deployment • Loss of clonal uniformity and predictability	Active countermeasures	• Labelling protocols • Carefully developed clonal database • Check for archegonial polyembryony • Culling "off types" • Clonal certification process (including fingerprinting)
Unwanted variation within clones Maturation effects Somaclonal variation Systemic infection	• Propagation method • Clonal storage technique • Length of time in storage • Number of propagation cycles • Disease problems	• Under-performance‡ • Loss of clonal uniformity and predictability • Marketability problems	Active countermeasures	• Choice of propagation and storage methods • Improvement of protocols (control maturation) • Optimise use of plant growth regulators, cryoprotectants • Morphological, cytogenetic, and molecular checks, then cull "off types" • Protocols to limit pathogen spread • Checks for cultivar decline
Imperfect evaluation	• Lack of genetic buffering against GE interaction • Clone x environment interactions	• Loss of clonal predictability • Under-performance • Loss of genetic gain • Sub-optimal management	Active countermeasures (risk spread)†	• Commitment to more thorough field testing, including good trial design and multiple test environments • Deploy clones only in site types where tested • Clonal certification process
Climatic Wind, snow†, frost†, drought†	• Siting • Propagule type • Establishment practice • Silvicultural regime • Climatic changes	• Under-performance‡ • Crop failure†	Active countermeasures (risk spread)†	• Choice of propagule type • Deployment tactics • Clonal diversity†

* In addition to requirements for seedlings or vegetative multiplication
† Of secondary importance for the case in question.
‡ Some beneficial effects of maturation can arise, creating an element of negative risk and associated under-performance

substandard propagation performance of a clone can completely deter propagators and forest growers from its operational use. If such failure is concentrated in specific families, which is often the case, the impact on genetic diversity of plantations can be particularly severe. Selection imposed by any propagation or storage system will have more impact if adversely correlated with traits of economic importance (Haines & Woolaston 1991). Some genotypes can be highly amenable to propagation and clonal storage, and can then become over-represented in a clonal programme; conversely, genotypes that are not amenable easily become under-represented or even lost. For instance, genotypes that branch profusely are likely to be favoured by propagators over ones that branch sparsely, but the forest industry generally prefers genotypes that allocate most of their biomass to stem wood (Sonesson *et al.* 2001).

Rates of failure, and incidence of failure among families, can both be highly dependent on the mode and degree of technical development of propagation or clonal storage technologies, viz juvenile stool-bed cuttings, field-collected cuttings, or various *in vitro* culture systems. Longer-term storage can often exacerbate uneven representation. High genotypic representation has been achieved with juvenile cuttings systems and some tissue-culture systems (Menzies & Aimers-Halliday 1997, in press). The rate of failure, complete or partial, and specificity to individual families and clones, progressively increase with increasing maturation in the starting material.

Some *in vitro* propagation systems, such as somatic embryogenesis, can favour certain families and not others. However, recent improvements in somatic embryogenesis technology with *P. radiata* have greatly increased the genotypic representation possible (L.J.Grace pers. comm.). Unfortunately, with control-pollinated seed, which includes the seed of highest genetic quality, the success rate of somatic embryogenesis is currently well below that with open-pollinated seed, and this poses a research challenge (C.L.Hargreaves pers. comm.). Despite these problems, the somatic embryogenesis system has great potential with its very high multiplication rates, amenability to genetic transformation, and suitability for cryopreservation which is a particularly efficient method of clonal storage (Menzies & Aimers-Halliday 1997, in press).

Quantitative Considerations

It is possible to infer the expected impact of propagation failure, whatever the form, in terms of potential genetic gain, by deterministic calculations based on:

- failure rate (outright propagation failure, or unacceptable costs)
- the broad-sense heritability of traits under selection
- number of candidate clones for desired selection intensity
- clones to be selected
- correlation between crop performance and rate of propagation failure (r).

Expectations were derived by Burdon (1989) assuming $r = 0$ and an undifferentiated population of candidate clones, and by Haines & Woolaston (1991) extending to cases of $r < 0$ and $r > 0$. Factors conducive to significant loss of potential gain, which can be very severe, are relatively few candidates per selection, high failure rates, and adverse r (i.e., $r \ll 0$).

Calculations of impacts of propagation failure on genetic diversity do not appear to have been published, and so the question of the extent of family variation in susceptibility to propagation failure could assume special significance.

Countermeasures

Whether propagation failure involves total loss of genotypes, or amounts to unacceptable cost per propagule for a clone, it can be addressed primarily by active countermeasures. These involve the development of more reliable and economic propagation and clonal storage systems, leaving risk spread as a secondary consideration.

Losses of genetic diversity can be mitigated if sufficiently large numbers with a good genetic spread are initially made available to counter potential losses. This includes production of a greater number of parental crosses to counter the poor propagation ability of some families. However, this will increase the costs of the clonal programme. Secondly, genotypic representation can be improved if protocols optimise the propagation and storage environments and the treatments applied. In particular, genotypes will progressively fail in systems where maturation is not contained.

Extra security can be incorporated into clonal storage systems to counter risks associated with losses of important clones. For example, nursery stool-beds (hedged donor plants) can be replicated in different locations for added security against accidental losses of individual clones. Another possibility is using more than one clonal propagation/storage system for important clones — a risk-spread option.

The problem of low numbers of genotypes successfully propagated with embryogenesis from control-pollinated seed could be reduced with more research. A greater understanding of seed development in the control-pollination system will lead to improved protocols to ensure a greater proportion of the seed contains vigorous embryos. Also, techniques could be developed either to screen for non-viable genotypes in unripe seed, or to utilise more mature (riper) seed in which non-viable genotypes are more apparent. Meanwhile, larger amounts of control-pollinated seed must be used and laboriously handled in the somatic embryogenesis system to safeguard broad genotypic representation.

RISKS ASSOCIATED WITH MISLABELLING AND CLONAL MISIDENTIFICATION

Maintenance of correct clonal identity and pure clonal lines is essential for successful clonal forestry. A good clonal testing programme contains thousands of candidate clones from various sources, and in different stages of evaluation. Thus, far more genetic entries are tracked than in breeding programmes based just on seedling families. Each ramet* must have a label encoding information useful to its management and giving unambiguous information about its identity.

Degradation of tags and vandalism can cause considerable errors in identity (W.J.Libby pers. comm.). There is good evidence of errors in identity or original pedigree in most tree

---

* A ramet is a vegetative propagule taken from the original mother plant, which will normally be the seedling ortet, and all ramets from an ortet will be genetically identical except for mutation.

improvement programmes, and these errors are particularly damaging in clonal programmes (Cheliak 1993). Errors will have a different impact depending on where they occur in the clonal programme. An error in the identity of a single foundation plant will affect a large portion of that clone, and all of it if there is only one foundation plant. An error in the identification of a single plant during serial propagation will affect all plants in that series, which is a drawback of serial propagation in clonal storage systems or clonal production systems, in contrast to systems with stable donor plants (e.g., hedged stool plants). Mistaken identity of a single test plant will simply put unwanted error into the clonal trial (Cheliak 1993).

Associated with somatic embryogenesis systems in conifers is the possibility of clonal misidentification due to archegonial polyembryony. In *P. radiata*, embryogenic tissue is initiated from unripe seed at the stage when multiple immature embryo genotypes can still be present. Therefore, it is possible that a particular cultured line may be composed of somatic embryos derived from different pollen genotypes rather than a single genotype (cf. Burdon & Zabkiewicz 1973). This has been reported in *Pinus taeda* L. (Becwar *et al.* 1991). In embryogenic systems where tissue can be initiated from mature or single isolated embryos, e.g., *Picea* spp., this is not a problem; unfortunately, with *Pinus radiata*, initiation of tissue from single mature embryos is still not possible on an operational scale (L.J.Grace, pers. comm.). Nevertheless, the problem of multiple genotypes being labelled as one is thought to be very rare, and there is more concern regarding mislabelling (from the seed orchard on) in somatic embryogenesis systems (L.J.Grace & B.C.S.Sutton pers. comm.).

Countermeasures

Effective use of a clone depends on proper identification and careful labelling. The label must be coded in such a way that it refers back to a clonal database and links with information on the pedigree, time of introduction into the programme, and relevant management information. A standard format is needed for the coded label, preferably limited to between five and seven digits. Over-detailed labelling will have a higher risk of transcription errors, which argues for coding systems rather than explicit information, although elements of redundancy can afford protection against many errors. Labels must be prominent, easily read, durable, smudge-proof, difficult to tamper with, and preferably printed rather than handwritten. The back-up of a second labelling system, plus the mapping of the spatial arrangement of donor clonal hedges and clonal field trials, will also help minimise errors.

To help minimise identity errors, serial propagation should be kept to the minimum that is needed on technical grounds. Instead, reliance should be placed on semi-permanent donor plants — for example, hedged stool plants in a nursery-based cuttings system (Cheliak 1993).

The type of labelling needed depends on the type of clonal stock (W.J.Libby (pers. comm.):

Foundation stock is the plant material the clone originates from, such as clonal hedges, or stored tissue culture or cryo-stored embryogenic tissue. Multiple labelling (branch tags, pot labels, or indelible writing on the container) should be used. If there is any confusion in identity, the ramet or culture should be discarded. Foundation

stock is generally maintained as four or more ramets or cultures per clone, under the best conditions available.

Expansion stock comes from foundation stock and involves multiplication of a clone. The cuttings or cultures are also multiply labelled, and those with dubious labelling are discarded. In some clonal programmes there is also **production stock**, which comes from expansion stock. These plants or cultures are generally singly labelled, but clonal hedges can be mapped in clonal blocks. Again, any ramet or culture of dubious identity should be discarded.

Deployment stock, derived from production stock or expansion stock, represents the plants for production plantations. Except for ramets to be included in trials or other monitoring, the individual plants are not usually labelled.

A clonal database is essential for managing any significant clonal programme. The New Zealand Forest Research Institute Clonal Register records each genotype with a numeric code. This code is prefixed with the year of propagation and suffixed with an alphabetic code indicating the propagation event within that year.

For example:

The 2897th genotype to be recorded in the database, propagated for the second time in the 2001 season, has the label number "**2001 2897 b**"**.** Although this number exceeds the desirable "five to seven digits", the prefix-year digits can be omitted as the planting records will state the year the plant was propagated; thus "**2897-b**" would suffice for the field code, otherwise "**01 2897 b**" would be appropriate.

Entering the label number into the Clonal Register can retrieve the following data on any propagation of a clone:

- Breeding (female and male parents) which may be traced via the breeding database
- Propagation history through all serial propagations
- Year of propagation
- Year of introduction into the clonal programme
- Details of where the propagule is to be used and for whom the clone was propagated.

The database can be interrogated on Ancestry, Clone, Propagation History, and Customer. Also, this database can be expanded to include information on field performance. Maintaining an appropriate database, with protocols for inputting and editing data, will help minimise labelling errors. However, problems other than mislabelling can occur.

Somatic embryogenesis needs to be sufficiently researched and understood to develop protocols to minimise the risk of clonal misidentification, and also to identify and eliminate any off-types. Use of DNA markers may be necessary to confirm that all somatic embryos of a single recorded clone are indeed genetically identical — even if mixed genotypes arising from a single seed culture due to polyembryony are thought to be very rare.

Indeed, molecular markers are invaluable for verifying clonal identity in clonal propagation systems, especially for intensively selected material. This could become part of a clonal certification process (Cheliak 1993). Nevertheless, visual assessments by experienced individuals to identify off-types should be standard practice in all systems. Also, for any system, it would be prudent to check selected clones against representatives of the same clone in storage before large-scale commercial propagation.

RISKS ASSOCIATED WITH UNWANTED VARIATION WITHIN CLONES

The Problems

Crucial to successful clonal forestry is the delivery of a uniform and predictable product, i.e., the well-characterised clone (Aimers-Halliday *et al.* 1997). Therefore, significant variation in the performance of a clone negates these two key advantages. In theory, all ramets of a clone are genetically identical. For many agricultural crops, clonal propagation has been associated with high clonal fidelity, but variation within clones has sometimes been observed. In various clonal forestry systems, such variation can easily arise unless precautions are taken.

Kester (1983) identified four causes of variation within clones:

(1) **somaclonal variation** due to genetic mutations, spontaneous or induced by the propagation process;

(2) **chimeral** re-arrangements of pre-existing mutants;

(3) **systemic infection by pathogens** promoted by vegetative propagation; and

(4) **epigenetic variation** due to differential gene expression related to conditions in the original mother plant, and the propagation environment.

However, discriminating between these causes in individual examples of within-clone variability may be difficult.

Epigenetic effects are possibly the predominant source of intraclonal variability that is encountered in *P. radiata*. Whatever its exact causes, intraclonal variability is emerging as a problem with *P. radiata*, especially with *in vitro* propagation systems. A lack of published documentation of the troubles encountered does nothing to mitigate the problem. While a problem in itself, intraclonal variability also contributes to market risks (Burdon & Aimers-Halliday 2003).

Somaclonal variation

Somaclonal variation occurs when cells in tissue culture accumulate changes in both the number and structure of chromosomes (Griesbach 1987), or point mutations accumulate, sometimes at rates much higher than normal (Evans & Bravo 1986). These genetic alterations may occur spontaneously or be induced by the conditions *in vitro*, particularly with application of excess synthetic plant-growth regulators (PGRs) (Högberg *et al.* 2003). Somaclonal variation is much more prevalent in cultures from explants with no pre-organised meristem, or if cultures are maintained as unorganised callus prior to plantlet regeneration (Brown & Sommer 1982). However, current *in vitro* propagation systems for conifers largely avoid these risk factors, and conifer plantlets and somatic seedlings appear to be relatively stable genetically (Bornman 1984; Noh *et al.* 1988; Cyr 1999). “Off types” that are produced are often readily apparent (Högberg *et al.* 2003).

The potential for somaclonal variation in somatic embryogenesis systems in woody plants was discussed by Sutton & Polonenko (1999). They concluded that somaclonal variation is rarely observed in propagules that become successfully acclimated, and that it is significantly less of a problem than in organogenesis systems. Fourré *et al.* (1997) and Hanáček *et al.* (2002) failed to detect genetic instability in Norway spruce (*Picea abies* (L.)

Karst.) embryogenic cultures using random amplified polymorphic DNA (RAPD) markers. However, Fourré *et al.* (1997) did detect some morphological and cytogenetic intraclonal variations, including immature embryos with a diffuse organisation, complete or part albino mature embryos, acclimated somatic seedlings comparable to dwarf mutants, and acclimated somatic plants that were trisomic or chimeric (with trisomic buds and diploid roots). Similar "off types" have been observed in *Pinus radiata* somatic embryogenesis systems (L.J.Grace pers. comm.), including dwarf and albino variants, but very little has been published on quantifying somaclonal variation in *P. radiata* clonal propagation systems.

However, Maddocks *et al.* (1995) conducted an early screening of *P. radiata* cell lines using two methods: double-stain microscopy and flow cytometry. While the majority of cell lines were diploid, some tetraploid lines were detected, suggesting that the loss of embryo-forming capacity may be related to onset of tetraploidy, which typically has catastrophic effects on vegetative vigour of conifers. Variation in ploidy level appears to be associated with fluctuation and eventual decline in culture productivity in other conifer somatic embryogenesis systems (Fourré *et al.* 1997; Cyr 1999; Sutton & Polonenko 1999).

Somatic embryogenesis coupled with cryopreservation has been viewed as an ideal method of clonal propagation and storage with long-term genetic (and epigenetic) stability (Cyr 1999). Unfortunately, few studies have been published on the effects of long-term storage on the genetic fidelity of woody material (Häggman *et al.* 2000). Park *et al.* (1998) demonstrated general stability of cryopreserved clones from a somatic embryogenesis system with white spruce (*Picea glauca* Moench), assessing morphological characters during *in vitro* development and *in vivo* survival and growth. Some concern exists over the use of cryoprotecting agents and their mutagenic potential (Häggman *et al.* 2000). Aronen *et al.* (1999) stated that the use of DMSO (dimethylsulfoxide) as a cryoprotectant may cause genetic aberrations in embryogenic cultures.

Genetic transformation, which is likely to depend on embryogenic culture, has obvious potential to introduce unwanted somaclonal variation, in both the gene-insertion process and the associated *in vitro* culture.

Chimeral phenomena, which represent special cases of somaclonal variation, are not seen as causing serious clonal fidelity problems with conifers. A chimera is a plant that is composed of tissue of more than one genotype. It usually results from mutated cells in a meristem dividing and the resulting shoot developing with layers of mutated and non-mutated tissue, which is then propagated (Hartmann *et al.* 1990). Chimeras have far more important applications in horticulture than in forestry — for example, the origin of varieties with leaf variegation. Because of differences in angiosperm and gymnosperm shoot anatomy and development, stable chimeras are rarer in gymnosperms, although they have been reported with embryogenic cultures (Fourré *et al.* 1997). However, chimeras may be a problem with genetically transformed plants.

Systemic infections

Systemic infections do not appear to cause serious problems for clonal fidelity in conifers, in contrast to experience with some horticultural crops. Such infections are typically viral in nature, and viral infections have not been reported for conifers. However,

caution is warranted, particularly when collecting and bringing together many different types of plants in one location (nurseries, breeding centres), widely disseminating clones from one environment to another, and combining different genotypes by budding or grafting (Hartmann *et al.* 1990).

Epigenetic effects

Epigenetic effects are likely to cause many if not most of the problems with clones performing "off type" in *Pinus radiata*, particularly when maturation is involved. Epigenetic variation involves phenotypic changes rather than genotypic changes (Evans & Bravo 1986). In other words, epigenetic variation results from variation in gene expression, but does not involve changes in the genes themselves since it can be erased in the course of sexual reproduction. Changes in gene expression, which cause the phenotypic instability, can reflect the position, developmental stage, and environment of the original explant (Olesen 1978; Meins & Binns 1979; Kester 1983; Gupta & Durzan 1987).

The process of ontogenetic change, from embryonic through juvenile, adolescent, and mature states, has been termed either maturation (Wareing 1959, 1987) or physiological ageing (Robbins 1957; Sweet 1964; Borchert 1976; Menzies *et al.* 2000), and is difficult to reverse. (There is some confusion in the literature regarding the use of the term physiological ageing; these terminology differences have been discussed by Menzies *et al.* 2000.) In this paper, we use the term maturation for the ontogenetic progression from embryonic through to the fully mature state. "Physiological age" (Menzies *et al.* 2000) is defined as the apparent maturation state of a tree; this is the result of the combination of ontogenetic processes and the loss of vigour associated with increasing tree age *per se*. The ontogenetic processes are still largely irreversible in *P. radiata*, except through sexual reproduction. By contrast, the loss of vigour that typically occurs in minor branches or the tops of old trees is essentially reversible in vegetative propagules that are successfully produced. With current *P. radiata* clonal programmes, it is generally accepted that the apparent physiological age of a tree is essentially due to ontogenetic ageing, rather than loss of vigour and, as such, is very difficult to reverse.

Since maturation state appears to be dominated by cumulative distance along stem axis from the position of the seedling's cotyledons, it is implicitly the maturation state of the upper shoots of a tree, of typical genotype, that has grown at a typical rate. For quantifying maturation state in terms of physiological age, morphological indicators have been described by Menzies *et al*. (2000).

Significant maturation results in plants that are difficult and expensive to propagate, with low multiplication rates and poor field performance, compared with juvenile plants. However, the successful deployment of clones after clonal testing is crucial for clonal forestry. Thus, either the clonal material must be rejuvenated after testing, or maturation must be arrested, or at least minimised, in a clonal storage system (Menzies & Aimers-Halliday 1997). Changes in the physiological age (largely due to ontogenetic ageing) during clonal storage can make some *P. radiata* clones very difficult to propagate as cuttings (Wilcox *et al.* 1976), and are likely to cause changes in clonal rankings, particularly for growth and form traits. Such rank changes would effectively amount to genotype × physiological age interaction. Several fairly successful clonal storage systems, both

nursery-based (e.g., hedging) and *in vitro* (e.g., cold storage of tissue culture or cryopreservation), are currently used to contain maturation, although they still have problems (Hargreaves & Smith 1992; Horgan *et al.* 1997; Menzies & Aimers-Halliday 1997, in press). Cryopreservation has promise for completely halting maturation. Although cryopreservation of embryogenic *P. radiata* tissue (including cotyledons) is now routinely achievable, cryopreservation of axillary meristems is still under development (Hargreaves *et al.* 1997, 1999, 2002; Menzies & Aimers-Halliday in press).

While unrestricted rejuvenation is the ideal, it is still technically uncertain and far from operational with *P. radiata*. Horgan (1987) successfully micropropagated explants from apical meristems of mature trees. Smith (1999) reported successful rejuvenation of five *P. radiata* clones from a 20-year-old stand by initiating embryogenic cultures from apical meristems, but no application appears to have been reported. If these protocols can be reliably replicated at an operational scale, then the inefficiencies resulting from needing to store thousands of clones during clonal testing, before selecting only a few clones for plantations, would be removed.

However, some controlled maturation can be beneficial. With *P. radiata*, there is an optimum physiological age of 3–4 years when there are advantages of improved stem form without the early loss of diameter growth that is associated with greater physiological ages (Menzies *et al*. 1988, 1991; Forest Research Institute 1991). Such maturation may give valuable improvement in mechanical stability for topple-hazard sites.

Current clonal forestry programmes with *P. radiata* in nursery-based systems would likely involve many propagation events from multiple stool-beds per clone. In other words, multiple stool-beds from a single clone would probably be planted in separate locations within a nursery (for clonal security), and possibly in separate nurseries (distribution of clonal stool-beds to clients). With *in vitro* propagation systems, tissue in culture can be (and is!) widely distributed for producing planting stock in clients' nurseries. With current clone maintenance systems, collection of clonal material from stool-beds or tissue-culture storage systems would occur over indefinite periods. With propagation events thus separated in space and time, epigenetic variation is likely to be generated. Non-containment of maturation is widely thought to be the most critical risk factor for unwanted and intraclonal variation in clonal forestry with *P. radiata*.

Countermeasures

The risk of significant variation in the performance of a clone can be minimised with careful management of clonal propagation and storage systems, coupled with sufficient field and molecular testing to verify clonal fidelity.

Somaclonal variation

Somaclonal variation in tissue-culture systems can be largely avoided by minimising the use of plant growth regulators, limiting unorganised callus growth, and limiting subcultures (Hartmann *et al.* 1990). Systems relying on pre-formed (e.g., axillary) meristems are genetically more stable than systems where adventitious buds are induced *de novo* (Brown & Sommer 1982). Since "off types" that are produced are often readily apparent (Högberg *et al.* 2003) they can often be eliminated quickly.

Sutton & Polonenko (1999) suggested methods of reducing the risk of somaclonal variation in somatic embryogenesis systems. These included minimising time in culture before cryopreservation, discarding production cultures after 12 months of active culture (and reconstituting a new starter culture from cryopreservation), and rigorously field testing somatic seedlings alongside genetically related zygotic seedlings. Also, on the basis of results obtained with embryogenic cultures of *Pinus sylvestris* L. (Häggman *et al.* 1998) and *Abies cephalonica* Loudon (Aronen *et al.* 1999), the use of dimethylsulfoxide (DMSO) as the sole cryoprotectant for conifer somatic embryogenesis systems should be reviewed because of its potential mutagenic properties. The use of a mixture of polyethylene glycol, glucose, and DMSO is recommended by some researchers (Häggman *et al.* 2000).

More research is needed on intra-clonal variation and genetic fidelity in current *in vitro* propagation and clonal storage systems for *P. radiata*. Risks of increased somaclonal and epigenetic variation could then be quantified and means of risk mitigation identified.

It has been recommended that morphological and cytogenetical approaches should be used as complementary tools to molecular markers to detect somaclonal variation (Fourré *et al.* 1997; Häggman *et al.* 2000). Molecular markers seem inefficient for detecting rare point mutations or variations in ploidy, but may help detect genetic changes that are not readily visible as morphological or physiological variations in young plants (Häggman *et al.* 2000).

Care must be also taken to cull deleterious "off types" in nursery propagation systems. Although mutations may be very rare, limiting apical growth and forcing lateral meristems for propagation increases the "capture" of mutations (Hartmann *et al.* 1990). In general, clonal propagation is likely to be efficient at capturing even rare mutations, whether useful or not.

Systemic infections

Although systemic infections are not thought to cause serious problems with clonal fidelity in conifer species, propagators should be wary of any practices that favour cross-infection, as discussed above (Hartmann *et al.* 1990).

Containment of maturation

This factor, which can generate much unwanted variability, is defined as the failure to control maturation at the desired level. This is probably the most critical risk associated with unwanted clonal variation in clonal forestry with *P. radiata*. Care must be taken that all material is juvenile (or embryogenic) when placed in clonal storage. Also, each propagation line needs to be maintained in the same desired maturation state. Embryonic or early-juvenile maturation states will generally be preferred for most forestry purposes, as they provide the greatest flexibility with today's technology. By allowing the expansion and production stock of a line to mature to varying degrees, some stock can be deployed with older physiological ages, such as late-juvenile or mid-adolescent.

Propagators should visually screen for maturation state and general vigour on a regular basis, and avoid conditions in propagation and clonal storage that are likely to accelerate rather than contain maturation. There is some evidence that minimising the number of

propagation cycles in nursery-based storage systems will help limit maturation (Aimers-Halliday *et al.* 2003).

Clonal-fidelity field tests are recommended 2–3 years prior to the final assessment of the main clonal trials, using a subset of clones. This subset could be clones identified in early assessments as likely to be in the final selection. If the trials are established on fast-growth sites, the effectiveness of the clonal storage system(s) in containing maturation could be assessed within 2 years. The clones can be visually assessed for physiological age using morphological indicators (Menzies *et al.* 2000). These clonal-fidelity trials would be most useful when new clonal-storage technology is implemented. After the technology is proved to be reliable for containing maturation and minimising variation within clones, clonal-fidelity trials may become unnecessary. Assessment of physiological age in the nursery, before deployment, would probably suffice and would be an integral part of clonal certification.

However, as stated above, some degree of maturation can be useful.

Maintaining quality of planting stock

Particular attention must be given to the quality of clonal planting stock, as described by Menzies & Aimers-Halliday (in press). Not only is it technically desirable, but it also addresses a major short-term market risk (Burdon & Aimers-Halliday 2003). As stated above, vegetative propagules must both look good and perform at least as well as seedlings of similar genetic quality after planting, to be fully accepted by industry and other clients. Minimal standards of acceptance for planting stocks have been developed for plantations to ensure good field performance, and these standards should not be relaxed for clonal material (q.v. Grossnickle 1999; Sutton & Polonenko 1999). Much research has been done on the field performance of *P. radiata* rooted cuttings, and research is continuing into the field performance of tissue-culture plantlets and embryogenic propagules (Menzies & Klomp 1988; Menzies & Aimers-Halliday 1997, in press; Menzies *et al.* 1988).

A hybrid production system may be best. For example, organogenesis or embryogenesis could be used initially to capture and cryopreserve genotypes and to produce sufficient plants for clonal testing. Once clones have been selected for clonal production, sufficient individuals could be produced by tissue culture, to be planted as stock plants in clonal hedges for the mass-propagation of cuttings, producing more robust and cheaper plants for field deployment (Menzies & Aimers-Halliday 1997).

Similarly, a hybrid storage system may be optimal. Cool-storage and cryopreservation are very expensive for large numbers of clones, and the cost is high for producing only a few plants per clone, via micropropagation, for clonal testing (10–18 plants). An alternative might be to use cuttings technology to establish the clonal tests and for stool-bed storage. Following clonal-test assessment at about age 4–5 years, the best-performing clones could be initiated into tissue culture by organogenesis (or embryogenesis, when the technology becomes available) for cool or cryogenic storage. With a favourable environment and management, nursery stool-beds should undergo minimal maturation over the first 5 years. The high tissue-culture costs would then be applicable only to clones with the best early performance (10–50% of the initial candidates, depending on the strategy adopted) (Menzies & Aimers-Halliday 1997). However, the costs of *in vitro* culture, particularly embryogenesis, may decrease significantly through automation, particularly if artificial

seeds become available (Sutton 2002), making a hybrid system unnecessary. Such artificial seeds should serve well for short-term storage (≤20 years), with cryogenic storage of embryogenic foundation stock for longer periods (W.J.Libby pers. comm.).

Early culling during the propagation period is useful for eliminating, before further waste of time and resources, clonal propagules that are highly unlikely to meet minimum standards of acceptance as planting stock. Högberg *et al.* (2003) developed early selection criteria for embryogenic propagules of *Picea abies*, at *ex vitro* transfer, which resulted in better performance and less intraclonal variation.

CLIMATIC RISKS

Climatic damage can be more predictable than certain biotic risks, at least in terms of being able to identify hazards and quantify probability of occurrence within a given period in a given place, although climatic change can shift the probabilities. These hazards can be addressed by deployment of material, especially in terms of siting, on the basis of the typical trade-off between growth potential and resistance to damage. A classic case is siting of species in relation to the snow hazard at moderate altitudes in the eastern South Island. While there are some prospects of genetic gains from selection for resistance to direct climatic damage in *Pinus radiata* in New Zealand (Burdon *et al.* unpubl. data; Menzies *et al.* 1987) such gains have not been actively pursued.

The behaviour of climatic risk factors in plantations can be very complex. Resistance or susceptibility to wind damage, for instance, can probably be expressed best in monoclonal plantations. The exact impact of growing clones in mixtures may be hard to predict. Having a susceptible clone in a mixed stand may allow wind damage to spread through the stand and possibly affect inherently resistant components. On the other hand, the presence of resistant clones may protect the susceptible ones from the damage that they would suffer if grown in pure stands.

Among specific risks, tree toppling can be very serious in young *P. radiata* plantations on exposed farm sites of moderate to high fertility (Mason 1985; Forest Research Institute 1987). Toppling occurs before canopy closure, particularly 1–3 years after planting, and is associated with damage to the root system after wind-induced tree sway. Grossly toppled trees have poor survival, but trees with slight to moderate topple usually continue to grow and correct to an upright form. Unfortunately, this often results in a sinuous stem, butt-sweep, and compression wood, with subsequent down-grading of the butt log and financial loss (Forest Research Institute 1987; Mason 1988; Somerville1988; Maclaren 1993; Holden *et al.* 1995).

It is likely that some genotypes will be more susceptible to toppling, particularly fast-growing genotypes with dense crowns and shallow rooting. Also, there is good evidence that rooted cuttings taken from early adolescent donors (physiologically aged material) are more stable and less likely to topple than seedlings and rooted cuttings taken from very juvenile material (Forest Research Institute 1999).

Countermeasures

Clonal ideotypes that are known to be prone to climatic damage should be deployed judiciously. For example, long-internode clones are not recommended for sites that are

highly fertile and exposed. Such trees can be very badly affected by leader damage and, with their propensity to develop very large branches, they can have unacceptable tree form on fertile sites. Appropriate deployment of well-characterised clones would exclude all high-risk genotypes from fertile and exposed sites, as compared to the variable numbers of genotypes susceptible to damage that can occur in seedling planting stocks.

Similarly, *P. radiata* planting stock should not be deployed on sites with a high risk of topple unless they are clones that have been exposed to high-risk conditions in clonal tests and have proved to be mechanically stable. Care must be taken on such sites to ensure that all planting stock have well-balanced root systems before deployment and that appropriate planting techniques are used. The deployment of post-juvenile clones is recommended. Stock derived from field-collected (early adolescent physiological age) cuttings have proved to be more stable, probably due to more open permeable crowns (resulting in less wind resistance), although sturdier root systems may also be a factor. The greater mechanical stability and better returns from planting physiologically mature (early adolescent) clonal material on high-risk sites should more than compensate for the greater initial costs and slightly slower growth rates (Forest Research Institute 1999). Early crown pruning can reduce wind resistance, although it does not negate the advantages of topple-resistant planting stock.

However, because the conditions leading to toppling are so complex, variable, and to a certain extent unpredictable, it will be very difficult to develop clones that are entirely topple-resistant. With severe (but fortunately very rare) storms such as Cyclone Bola, all trees of *P. radiata* are vulnerable to topple, regardless of their origin. The only sure risk management on highly topple-prone sites may be to not plant the species at all!

RISKS OF INADEQUATE EVALUATION

General Evaluation Issues

Risks relating to field performance that we are addressing here involve imperfect yet inherently accessible knowledge about the clones. Clonal testing is an essential part of clonal forestry and provides critical information for clonal deployment and risk management. Despite their cost and need for long-term commitment, clonal field trials are indispensable and cannot be replaced with short-cuts. Unfortunately, clonal testing will never be perfect, as firm conclusions on safe clonal deployment require unrealistically large and extensive trials (Lindgren 1993) possibly carried through to several rotations. However, this already applies to some extent with testing seedling progeny in breeding programmes.

Forestry trials are particularly vulnerable because of their longer lifespan, due to a much longer rotation time than annual crop plants. The commitment to clonal field trials and associated research projects can be eroded by changes in priorities and policy, particularly where industry partnerships are involved. It is critical that the clonal testing period is not foreshortened. Selection of clones not before one-third of the rotation age is generally accepted practice with *P. radiata* clonal trials in New Zealand (M.I.Menzies pers. comm.). It is acknowledged that final selection of clones for operational use, from very early in clonal testing, could result in serious loss.

Zobel (1993, p. 142) described the situation in a Brazilian clonal forestry programme for short-rotation tropical eucalypts. One clone "produced a very superior clonal planting

for the first 1.5 years of its test. A lot of wind and rain then occurred and many of the trees (about 9 m tall) fell over … we found that every member of this particular clone had only two major roots that were approximately 180° apart. In the wet soil the wind just pivoted the trees out of the ground. Without testing long enough, operational planting of this clone would have been a disaster."

The impact of inadequate evaluation can be addressed quantitatively in terms of expected genetic gain (ΔG) which is proportional to the product:

$H_C \times r_g$,

where H_C = the square root of the heritability (or repeatability) ($H^2{}_C$)* of clonal values (usually means) at assessment date, in this context varying according to the precision of evaluation,

and r_g = genotypic correlation between performance at assessment date and harvest date.

With evaluation on a random sample of *k* sites with *m* ramets per site, we have

$$H^2{}_C = \sigma^2{}_g/(\sigma^2{}_g + \sigma^2{}_{gE}/k + \sigma^2{}_e/km) = \sigma^2{}_g/(\sigma^2{}_g + \sigma^2{}_e) \quad (1)$$

where $\sigma^2{}_g$, $\sigma^2{}_{gE}$, and $\sigma^2{}_e$ denote variances attributable to clones, random clone × environment interaction†, and ramets within clone/environment subclasses, while $\sigma^2{}_e$ represents the error variance of estimates of clonal means. The roles of the various parameters in governing H^2 are basically self-evident. The behaviour of r_g is known in general terms, showing roughly a linear relationship with the logarithm of the ratio of assessment age and rotation age (e.g., Apiolaza *et al.* 2000), such that it typically rises rapidly from a low value during the early part of the rotation to make a gradual approach to +1 at the end. Note that within a single environment $H^2 = (\sigma^2{}_g + \sigma^2{}_{gE})/(\sigma^2{}_g + \sigma^2{}_{gE} + \sigma^2{}_e/m)$, which illustrates both how genotype × environment interaction can erode across-environment heritability and how the replication among the *k* sites can tend to boost it.

Clonal selection can be revised right up to rotation age in clonal tests, with r_g (and possibly $H^2{}_C$) rising with time, provided the clones can be stored in the meantime for satisfactory repropagation. This is in contrast to the early commitment of genotypes to mating within a breeding population, which is needed for pursuit of gain per unit of time.

Some issues of clonal testing in *P. radiata* are still not fully resolved. These include the range and number of sites needed for testing, and optimal clonal field-trial design. Testing very large numbers of genetic entries, with few replicates per site, poses particular challenges for experimental design. These issues have been discussed by Aimers-Halliday *et al.* (1997).

Risks Associated with Complex Interactions with Growing Environments

Adverse effects of genotype-environment interaction (G×E) represent one possible category that is likely to involve under-performance of clones. They can arise through it

---

* This value is not to be confused with broad-sense heritability (H^2) with the composition of $\sigma^2{}_g/(\sigma^2{}_g + \sigma^2{}_{gE} + \sigma^2{}_e)$.

† Clone × environment interaction represents difference among sites in the relative performances of various clones, which usually results in differences in clonal rankings among environments.

being impossible to evaluate selection candidates in the full range of environments that might generate G×E at the clonal level. Moreover, clone × environment interaction may comprise much more than the G×E that is expressed in seedling material. Such clonal interaction can also involve environmental and epigenetic effects. Thus, variation arising among clones in maturation state would not only generate main effects on clonal performance, but it could also create a set of interactions involving both clones and environments. However, while G×E of *P. radiata* appears to be fairly well understood for seedling material (Johnson & Burdon 1990; Burdon, Firth, Low & Miller 1997; Burdon, Hong, Shelbourne, Johnson, Butcher, Verryn, Cameron & Appleton 1997), almost nothing is known of either the magnitude or the pattern of the interactions involving epigenetic effects.

It can be argued that, because clones are genetically uniform, they are likely to have less stability in their performance across different sites than full-sib and half-sib families. Families have considerable genetic heterogeneity. Such heterogeneity should provide buffering against G×E and other interactions, since the better-adapted segregants should be able to take advantage of reduced competition from ill-adapted ones. However, it can also be argued that interactive clones can be eliminated and, consequently, a set of non-interactive clones would likely have much greater stability than seedlings over a range of sites.

It is likely that some clones will be highly unstable in their performance across sites, i.e., varying widely among sites in their rankings, while other clones will be stable with little or no change in rank across sites. However, there is some evidence that rankings for individual genotypes remain relatively constant, even though the expression of genetic and phenotypic variation may differ dramatically among environments (Burdon 2000), unless phosphorus deficiency is involved (Burdon, Firth, Low & Miller 1997).

Clearly there is a need for further research on G×E at the clonal level for *P. radiata* and this is being addressed by both industry and the New Zealand Forest Research Institute. In the winter of 2001, 72 clones from 35 families were planted by the Forest Research Institute in nine field trials in widely varying environments throughout New Zealand. Similar trials were planted by industry via the New Zealand Radiata Pine Breeding Company (RPBC). The Forest Research Institute has also planted clonal field trials investigating epigenetic effects and associated interactions.

Addressing Risks of Inadequate Evaluation, and Reduction of Clonal G×E

Forest managers should ensure that clones deployed in their forests are adequately tested and deployed only in environments very similar to those they have been tested in. Care must be taken to ensure that deployed clones are either highly stable in their performance or, if they are unstable, that they are closely matched to the environments they perform well in. Those marketing tested clones should provide documentation that the clones have been tested to at least one-third of rotation, with accurate (not inflated) extrapolation to full-rotation, and that the field-test environments are quantified as well as possible. This could become another part of a clonal certification system (*see earlier*).

Selection of clones for mass-propagation is inherently a process of progressive culling. Four steps have been suggested by Libby (1987):

initial screening of very large numbers of single-copy seedling genotypes (not clonal testing as such);

candidacy testing of large numbers of genotypes, each cloned a limited number of times;

clonal performance testing of moderately small numbers of genotypes, each cloned many times; and

compatibility trials of very small numbers of successful and well-known genotypes, which are then tested in various neighbour combinations. Not all these steps, however, need feature in any particular programme.

In clonal trials, testing very large numbers of genetic entries with few replications per site poses particular challenges for experimental design (Aimers-Halliday *et al.* 1997). More complex incomplete-block designs may yield better genetic information than the sets-in-reps design (cf. Schutz & Cockerham 1966) that is widely used in New Zealand, but there are operational disadvantages in using complicated designs in large-scale testing programmes. They will require careful planning and more logistical effort, otherwise clonal trial establishment will be compromised, to the detriment of the entire clonal forestry programme.

According to W.J.Libby (pers. comm.) utilising better-designed clonal-evaluation programmes, with larger tests that are maintained and evaluated over longer periods of time, allows for better evaluation and characterisation of clones, which subsequently reduces the risk in deploying those clones. While larger experiments provide better information, they also do so with diminishing returns, and thus optimum sizes should be considered.

The general criteria for adequate evaluation in terms of numbers of test sites, numbers of ramets, and age of evaluation are evident from Eq. 1. However, there will be trade-offs between selection intensity, governed by the pool of candidate clones, and the quality of the information on each clone. In many cases (cf. Cotterill & James 1984) selection intensity may be more critical, unless there are high fixed costs of initial production and propagation of a clone. Ideally, choice of test sites should be based strongly on the roles of different site categories in generating G×E interaction (Burdon 1977). While clones are a particularly promising tool for obtaining such information, we are only beginning to use their potential for that purpose.

Planting clones in an intimate mixture can afford some protection against the effects of imperfect information on individual clones. They should effectively give some buffering against G×E and other sources of under-performance, since the under-performance of some clones can be compensated by better-performing clones, taking advantage of the reduced competition.

If clones are well tested and characterised, interactive clones can be eliminated, although the testing will need to include important site categories that are interactive with respect to other site categories (cf. Johnson & Burdon 1990). A mixture of non-interactive clones is likely to have greater stability than seedlings over a range of sites. This is because the proportion of seedlings that are interactive cannot be eliminated, or accurately deployed to the subset of sites on which each will do better than the predicted average. Therefore, the

risks associated with G×E in clones can be reduced by identifying and deploying above-average clones with above-average stability.

Evaluating clones for crop performance in a species such as *P. radiata*, either as monoclonal blocks or for compatibility of combinations of clones, is a long-term goal and can address attributes that cannot be evaluated in conventional tests (Burdon 1989, 1991). However, it is an enormous challenge. It would be feasible for only a small fraction of candidate clones, and would entail much anecdotal experience, but the material may be genetically obsolete by the time it is proven. Definition of clonal ideotypes that can be predicted to perform well as crops rather than competitors remains a major part of the challenge (Burdon 1989, 1991).

CONCLUDING REMARKS

Clonal forestry with a species such as *P. radiata* faces a number of known difficulties, which pose ever-present problems, in addition to the uncertainties addressed by Burdon & Aimers-Halliday (2003) which call for risk spread. These known problems must be addressed on a broad front if clonal forestry is to fulfill its promise. But, while the problems are well recognised and there are already many available countermeasures, much research remains to be done in order to provide dependable solutions.

ACKNOWLEDGMENTS

We are grateful for major critiques by W. J.Libby and C.J.A.Shelbourne, and valuable input from L.J.Grace, C.L.Hargreaves, L.D.Gea, M.I.Menzies, J.Find, and M.J.Dibley.

REFERENCES

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

AIMERS-HALLIDAY, J.; MENZIES, M.I.; FAULDS, T.; HOLDEN, D.G.; LOW, C.B.; DIBLEY, M.J. 2003: Nursery systems to control maturation in *Pinus radiata* cuttings, comparing hedging and serial propagation. *New Zealand Journal of Forestry Science 33(2)*: 135–155.

APIOLAZA, L.A.; GARRICK, D.J.; BURDON, R.D. 2000: Optimising early selection using longitudinal data. *Silvae Genetica 49*: 196–200.

ARONEN, T.S; KRAJNAKOVA, J.; HÄGGMAN, H.M.; RYYNÄNEN, L.A. 1999: Genetic fidelity of cryopreserved embryogenic cultures of open-pollinated *Abies cephalonica. Plant Science 142*: 163–172.

BECWAR, M.R.; BLUSH, T.D.; BROWN, D.W.; CHESICK, E.E. 1991: Multiple paternal genotypes in embryogenic tissue derived from individual immature loblolly pine seeds. *Plant Cell Tissue and Organ Culture 26*: 37–44.

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

BORNMAN, C.H. 1984: Application of *in vitro* culture technology in clonal forestry. Pp. 178–194 *in* Proceedings of the International Symposium on "Recent Advances in Forest Biotechnology", 10–13 June, Grand Traverse Resort Village, Traverse City, Michigan.

BROWN, C.L.; SOMMER, H.E. 1982: Vegetative propagation of dicotyledonous trees. Pp. 109–149 *in* Bonga, J.M.; Durzan, D.J. (Ed.) "Tissue Culture in Forestry". Martinus Nijhoff/Dr. W. Junk Publishers, The Hague.

BURDON, R.D. 1977: Genetic correlation as a concept for studying genotype-environment interaction in forest tree breeding. *Silvae Genetica 26*: 168–175.

———1989: When is cloning on an operational scale appropriate? Pp. 9–27 *in* "Breeding Tropical Trees: Population Structure, and Genetic Improvement Strategies in Clonal and Seedling Forestry". Proceedings of IUFRO Meeting, Pattaya, Thailand, Nov. 1988. Oxford Forestry Institute, United Kingdom, and Winrock International, Arlington, VA, USA.

———1991: Position statement – clonal forestry with *Pinus radiata*. Pp. 187–198 *in* Miller, J.T. (Ed.) Proceedings of FRI/NZFP Forests Ltd Clonal Forestry Workshop. *New Zealand Forest Research Institute, FRI Bulletin No. 160*.

———2000: *Pinus radiata*. Pp. 99–161 (Chapter 5) *in* Last, F.T. (Ed.) "Ecosystems of the World, Vol. 19. Tree Crop Ecosystems". Elsevier, Amsterdam, The Netherlands.

BURDON R.D.; AIMERS-HALLIDAY, J. 2003: Risk management for clonal forestry with *Pinus radiata* — Analysis and review. 1: Strategic issues and risk spread. *New Zealand Journal of Forestry Science 33(2)*: 156–180.

BURDON R.D.; ZABKIEWICZ, J.A. 1973: Identical and non-identical seedling twins in *Pinus radiata*. *Canadian Journal of Botany 51*: 2001–2004.

BURDON, R.D.; FIRTH, A.; LOW, C.B.; MILLER, M.A. 1997: Native provenances of *Pinus radiata* in New Zealand. *New Zealand Journal of Forestry 41(4)*: 32–36.

BURDON, R.D.; HONG, S.O.; SHELBOURNE, C.J.A.; JOHNSON, I.G.; BUTCHER, T.B; VERRYN, S.D.; CAMERON, J.N.; APPLETON, R. 1997: International gene pool experiments in *Pinus radiata*: Patterns of genotype-site interaction. *New Zealand Journal of Forestry Science 27*: 101–125.

CHELIAK, W.M. 1993: Clone identification. Pp. 101–109 *in* Ahuja, M.R.; Libby, W.J. (Ed.) "Clonal Forestry I, Genetics and Biotechnology". Springer-Verlag, Berlin/Heidelberg.

COTTERILL, P.P.; JAMES, J.W. 1984: Number of offspring and plot sizes required for progeny testing. *Silvae Genetica 33*: 203–209.

CYR, D.R. 1999: Cryopreservation of embryogenic cultures of conifers and its application to clonal forestry. Pp. 239–261 *in* Jain, S.M.; Gupta; P.K.; Newton, R.J. (Ed.) "Somatic Embryogenesis in Woody Plants, Vol. 4". Kluwer Academic Publishers, Dordrecht, The Netherlands.

EVANS, D.A.; BRAVO, J.E. 1986: Phenotypic and genotypic stability of tissue-cultured plants. Pp. 73–96 *in* Zimmerman; R.H.; Griesbach, R.J.; Hammerschlag, F.A.; Lawson, R.H. (Ed.) "Tissue Culture as a Plant Production System for Horticultural Crops". Martinus Nijhoff Publishers, Dordrecht, The Netherlands.

FOREST RESEARCH INSTITUTE 1987: Toppling of radiata pine. *New Zealand Forest Research Institute, What's New in Forest Research No. 147*.

———199l: 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 topple-prone sites. *New Zealand Forest Research Institute, What's New in Forest Research No. 248.*

FOURRÉ, J.-L.; BERGER, P.; NIQUET, L.; ANDRÉ, P. 1997: Somatic embryogenesis and somaclonal variation in Norway spruce: morphogenetic, cytogenetic and molecular approaches. *Theoretical and Applied Genetics 94*: 159–169.

GRIESBACH, R.J. 1987: Selected topic on induced chromosome changes in tissue-cultured cells. *Horticultural Science 22(6)*: 1204–1206.

GROSSNICKLE, S.C. 1999: Performance of conifer stock produced through somatic embryogenesis. Pp. 97–123 *in* Jain, S.M.; Gupta, P.K.; Newton, R.J. (Ed.) "Somatic Embryogenesis in Woody Plants, Vol. 4". Kluwer Academic Publishers, Dordrecht, The Netherlands.

GUPTA, P.K.; DURZAN, D.J. 1987: *In vitro* establishment and multiplication of juvenile and mature Douglas-fir and sugar pine. *Acta Horticultura 212*: 483–487.

HÄGGMAN, H.M.; ARONEN, T.S.; RYYNÄNEN, L.A. 2000: Cryopreservation of embryogenic cultures of conifers. Pp. 707–728 *in* Jain, S.M.; Gupta, P.K.; Newton, R.J. (Ed.) "Somatic Embryogenesis in Woody Plants, Vol. 6". Kluwer Academic Publishers, Dordrecht, The Netherlands.

HÄGGMAN, H.M.; RYYNÄNEN, L.A.; ARONEN, T.S.; KRAJNAKOVA, J. 1998: Cryo-preservation of embryogenic cultures of Scots pine. *Plant Cell Tissue and Organ Cult*ure 54: 45–53.

HAINES, R.J.; WOOLASTON, R.R. 1991: The influence of reproductive traits on the capture of genetic gain. *Canadian Journal of Forest Research 21*: 273–275.

HANÁČEK, P.; HAVEL, L.; TRUKSA, M. 2002: Characterisation and determination of genetic stability of early somatic embryo culture clones of Norway spruce using RAPD markers. *Biologia, Bratislava 57(4)*: 517–521.

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.

HARGREAVES, C.L.; FOGGO, M.N.; SMITH, D.R.; GORDON, M.E. 1999: Development of protocols for the cryopreservation of zygotic embryos of *Pinus radiata* and subsequent plant regeneration. *New Zealand Journal of Forestry Science 29(1)*: 54–63.

HARGREAVES, C.; SMITH, D.; FOGGO, M.; GORDON, M. 1997: Cryopreservation of zygotic embryos of *Pinus radiata* and subsequent plant regeneration. Pp. 281–284 *in* Burdon, R.D.; Moore, J.M. (Ed.) "IUFRO '97 Genetics of Radiata Pine". Proceedings of NZ FRI-IUFRO Conference 1–4 December and Workshop 5 December, Rotorua, New Zealand. *FRI Bulletin No. 203*.

HARTMANN, H.T.; KESTER, D.E.; DAVIES, F.T. Jr 1990: "Plant Propagation Principles and Practices". Prentice Hall, New Jersey. 5th edition. 647 p.

HOLDEN, D.G.; KLOMP, B.K.; HONG, S.O.; MENZIES, M.I. 1995: Growth and predicted timber value of *Pinus radiata* cuttings and seedlings on a fertile farm site. *New Zealand Journal of Forestry Science 25(3)*: 283–300.

HÖGBERG, K.-A.; BOZHKOV, P.V.; VON ARNOLD, SARA 2003: Early selection improves performance and reduces intraclonal variation of Norway spruce plants propagated by somatic embryogenesis. *Tree Physiology 23*: 211–216.

HORGAN, K. 1987: *Pinus radiata*. Pp. 128–154 *in* Bonga, J.M.; Durzan, D.J. (Ed.) "Tissue Culture in Forestry, Vol. 3". Martinus Nijhoff Publishers, Dordrecht, The Netherlands.

HORGAN, K.; SKUDDER, D.; HOLDEN, 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 FRI-IUFRO Conference 1–4 December and Workshop 5 December, Rotorua, New Zealand. *FRI Bulletin No. 203*.

JOHNSON, G.R.; BURDON, R.D. 1990: Family-site interaction in *Pinus radiata*: Implications for progeny testing strategy and regionalised breeding in New Zealand. *Silvae Genetica 39*: 55–62.

KESTER, D.E. 1983: The clone in horticulture. *Horticultural Science 18*: 831–837.

LIBBY, W.J. 1987: Testing for clonal forestry. *Annales Forestales 13*: 69–75.

LIBBY, W.J.; AHUJA, M.R. 1993: The genetics of clones. Pp. 5–13 *in* Ahuja, M.R.; Libby, W.J. (Ed.) "Clonal Forestry I, Genetics and Biotechnology". Springer-Verlag, Berlin/Heidelberg.

LINDGREN, D. 1993: The population biology of clonal deployment. Pp. 34–49 *in* Ahuja, M.R.; Libby, W.J. (Ed.) "Clonal Forestry I, Genetics and Biotechnology". Springer-Verlag, Berlin/Heidelberg.

MACLAREN, J.P. 1993: Radiata Pine Growers' Manual. *New Zealand Forest Research Institute, FRI Bulletin No. 184.*

MADDOCKS, D.; GOUGH, K.; HOPPING, M.; AITKEN-CHRISTIE, J. 1995: Early screening of radiata-pine embryogenic cells and occurrence of tetraploidy. Abstract. P.29 *in* Proceedings of Conifer Biotechnology Working Group 7th International Conference 26–30 June, Surfers Paradise, Queensland, Australia.

MASON, E.G. 1985: Causes of juvenile instability of *Pinus radiata* in New Zealand. *New Zealand Journal of Forestry Science 15(3)*: 263–280.

———1988: Toppling in New Zealand – Where do we go from here? Pp 23–25 *in* "Wind Damage in New Zealand's Exotic Forests", Proceedings from Workshop, Rotorua, New Zealand. *New Zealand Forest Research Institute, FRI Bulletin No. 146*.

MEINS, F. Jr; BINNS, A.N. 1979: Cell determination in plant development. *BioScience 29(4)*: 221–225.

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 FRI-IUFRO Conference 1–4 December and Workshop 5 December, Rotorua, New Zealand. *FRI Bulletin No. 203*.

———: Propagation options for clonal forestry with conifers. *In* Walter, C.; Carson, M.J. (Ed.) "Plantation Forest Biotechnology for the 21st Century". Research Signpost Publishing, 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.) Proceedings of Workshop on Growing Radiata Pine from Cuttings, Rotorua, 5–7 May 1986. *New Zealand Ministry of Forestry, FRI Bulletin No. 135*.

MENZIES, M.I.; AIMERS, J.P.; WHITEHOUSE, L.J. (Ed.) 1988: Proceedings of Workshop on Growing Radiata Pine from Cuttings, Rotorua, 5–7 May 1986. *New Zealand Ministry of Forestry, FRI Bulletin No. 135*.

MENZIES, M.I.; KLOMP, B.K.; HOLDEN, H.G. 1991: Optimal physiological age of propagules for use in clonal forestry. Pp. 142–145 *in* Miller, J.T. (Ed.) Proceedings: 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.; BURDON, R.D.; HOLDEN, D.G.; WARRINGTON, I.J. 1987: Family variation in frost tolerance of *Pinus radiata*. *New Forests 1*: 171–186.

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

NOH, E.W.; MINOCHA, S.C.; RIEMENSCHNEIDER, D.E. 1988: Adventitious shoot formation from embryonic explants of red pine (*Pinus resinosa*). *Physiologia Plantarum 74*: 119–124.

OLESEN, P.O. 1978: On cyclophysis and topophysis. *Silvae Genetica 27(5)*: 173–178.

PARK, Y.S.; BARRETT, J.D.; BONGA, J.M. 1998: Application of somatic embryogenesis in high-value clonal forestry: deployment, genetic control, and stability of cryopreserved clones. *In Vitro Cell. Dev. Biol.: Plant 34(3)*: 231–239.

RITCHIE, G.A. 1994: Commercial application of adventitious rooting to forestry. Pp. 37–52 *in* Davis, T.D.: Haissig, B.E. (Ed.) "Biology of Adventitious Root Formation". Plenum Press, New York.

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

SCHUTZ, W.M.; COCKERHAM, C.C. 1966: The effect of field blocking on gain from selection. *Biometrics 22*: 843–863.

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

SMITH, D.R. 1999: Successful rejuvenation of radiata pine. Pp.158–167 *in* Proceedings of 25th Biennial Southern Forest Tree Improvement Conference (SFTIC), New Orleans, Louisiana, USA, 11–14 July.

SOMERVILLE, A.R. 1988: Tree wind stability and forest management practices. Pp. 38–58 *in* "Wind Damage in New Zealand's Exotic Forests", Proceedings from Workshop, Rotorua, New Zealand. *FRI Bulletin No. 146*.

SONESSON, J.; BRADSHAW, R.; LINDGREN, D.; STÅHL, P. 2001: Ecological evaluation of clonal forestry with cutting-propagated Norway spruce. *SkogForsk (The Forestry Research Institute of Sweden) Report No. 1*.

SUTTON, B. 2002: Commercial delivery of genetic improvement to conifer plantations using somatic embryogenesis. *Annals of Forestry Science 59*: 657–661.

SUTTON, B.C.S.; POLONENKO, D.R. 1999: Commercialization of plant somatic embryogenesis. Pp. 263–291 *in* Jain, S.M.; Gupta, P.K.; Newton, R.J. (Ed.) "Somatic Embryogenesis in Woody Plants, Vol. 4". Kluwer Academic Publishers, Dordrecht, The Netherlands.

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*. 8 p.

THOMPSON, D.G. 1984: 1. Clonal reforestation: Forests of the future? Pp. 3–28 *in* Duryea, M.L.; Brown, G.N. (Ed.) "Seedling Physiology and Reforestation Success". Martinus Nijoff/ Dr W. Junk Publishers, Dordrecht/Boston/London.

WAREING, P.F. 1959: Problems of juvenility and flowering in trees. *Journal of the Linnaean Society of 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.

WILCOX, M.D.; THULIN, I.J.; VINCENT, T.G. 1976: Selection of *Pinus radiata* clones in New Zealand for planting from cuttings. *New Zealand Journal of Forestry 21*: 239–247.

ZOBEL, B.J. 1993: Clonal forestry in the eucalypts. Pp. 139–148 *in* Ahuja, M.R.; Libby, W.J. (Ed.) "Clonal Forestry II, Conservation and Application". Springer-Verlag, Berlin/Heidelberg.