

Chapter 16

The Future of Forest Tree Improvement in New Zealand



16.1 Summary

Clonal testing for within-family ‘forwards’ selection has begun in the RPBC breeding programme and should also provide a powerful means of increasing gains in the breeding population. Within-family selection does not reduce breeding population genetic diversity, like selection among families.

NZ wood and wood products need innovation to move radiata pine wood from commodity to higher value uses. Economic breeding objectives are needed in which the aggregate genetic value improvement is described in dollar terms. However, as James pointed out, in the long term, a single breeding objective can be the most efficient.

Quicker selection is a key target since it can enable higher genetic gains per year in both the breeding and deployment operations (improving gain per year is a key objective for this program). OP breeding methods can shorten the breeding cycle by saving time in the progeny testing stage while achieving almost similar levels of genetic gain to those from a control-pollinated (CP)-based program. OP seed collected from plus trees and OP forwards selections can be used for progeny testing, obviating grafted archives for subsequent CP development.

At a recent review, the previously planned Elite, Main and Superline structures have been combined into a single breeding population, utilising mate selection by algorithms developed by Kinghorn and others. Clonal testing of candidate for forwards selections using rooted cuttings allows within-family selection without loss of genetic diversity in the BP. It also provides benefits of higher heritability, leading to higher genetic gains/year in PP and BP. Improved confidence in EBVs (estimated breeding values) for the cloned forwards selections has enabled seed providers to begin routinely grafting cloned forwards selections into clonal seed orchards.

Efficiency gains come from improved methods of breeding value estimation and improvements in progeny trial designs such as ‘multiple environment trial’ (MET)

analysis methods for radiata pine, using the BLUP reduced animal model, combined with factor analysis.

Recent work has demonstrated wood property screening of tree stem and core samples from trees as young as age 2, using new laboratory techniques. Traits screened with these tools include wood basic density, spiral grain, resin content and compression wood. Tree improvement programmes will face new challenges from pests and diseases. New Zealand's exotic forest plantations are losing their benefits of remoteness as new exotic pests and diseases continually arrive. The implementation of genomic selection (GS) is becoming a key issue for tree breeding programmes worldwide, as technical development challenges are being met.

Implementation of GS will mainly be for earlier selection than can be achieved using current screening methods. Such early selection is particularly valuable for economically important traits like cell-wall galactans and slow-to-develop heartwood. Earlier selection for low heritability traits like growth rate will also be valuable in shortening the progeny testing phase. The transfer of GS-based genetic gains into plantations will be most efficient and effective using somatic clones (due to their higher trait heritabilities), less so for CP seedlings and cuttings, and substantially less for OP seedlings. Cloned forwards selections cannot be multiplied for deployment as they are irreversibly matured during progeny testing.

Somatic cloning (through seed-based embryogenesis and cryogenic storage) is one method of cloning the breeding population, which could lead to a 'step-change' in delivery of genetic gains, in spite of only 10–15% of seeds and few families yielding somatic clones. Somatic clones, after field testing, can be immediately mass-propagated from cryogenic storage and deployed in plantations which would allow both higher genetic gain and increased gains/year from the breeding programme. Direct deployment of somatic clones is the best means of using genomic selection, as well as CRISPR-type gene editing and gene transfer methods.

16.2 Introduction

As for all major tree improvement programmes, the future of NZ tree improvement will be based on the population structures and levels of genetic gain achieved from previous generations of tree breeding (Shelbourne et al. 1986; Jayawickrama and Carson 2000; Dungey et al. 2009). The plantation estate is dominated by radiata pine, which will continue to provide the model for future tree improvement. The breeding programme for radiata pine is managed by the Radiata Pine Breeding Company (RPBC) which supplies treestocks from seed and somatic clones to the NZ pine estate of over 1.5 million hectares, as well as some 200–300,000 hectares of the Australian plantation estate.

By comparison, plantation areas and the related tree improvement efforts in NZ for other species are small. NZ Douglas-fir plantations have peaked at around 100,000 hectares, all eucalypts total approximately 23,000 hectares, with cypresses and redwoods next at approx. 10,000 and 8000 hectares, respectively. Planting of

New Zealand's indigenous species has historically been negligible, although the recent emergence of NZ manuka (*Leptospermum scoparium*) as a producer of honey for both consumption and as a component of various health products has led to increased plantings. Similarly, a combination of renewed Maori interest and the current government's 'Billion Tree' policy may lead to a surge in planting of indigenous species.

The available genetic diversity in the radiata pine breeding programme is greatest in the 'Gene Resource Population', which is comprised of the landraces established in NZ since the 1860s, and stands of the five native provenances from California and Mexico. The 'breeding population' has been formed from plus-tree selections, originally from the landraces, plus limited infusions of native provenances (principally from Guadalupe Island).

The 'production population' comprises the progeny-tested parent clones established in open-pollinated and control-pollinated seed orchards and contains less genetic diversity but represents substantially greater gains from selection. Finally, the 'deployment population' represents the point of delivery for all genetic improvement efforts and is comprised of the highly selected and improved seed orchard seedlots plus individual improved somatic clones that are established in forest plantations (as yet, only 10–15% of seeds and few families yield somatic clones). In this population, genetic gain is maximised at the expense of reduced genetic diversity and potentially increased levels of inbreeding, and the trade-offs among these factors become important.

16.3 Population Structure and Development

The population structures described above are likely to be maintained in future tree improvement, as will the continuing use of classical breeding methods based on quantitative genetic theory, which will be increasingly informed by genomics tools. Until very recently, the RPBC breeding population has been further structured into a small Elite population and a larger Main population, with both populations divided into two superlines, in order to delay inbreeding by facilitating outcrossing in the deployment population (ex Dungey et al. 2009). However, a major recent review has resulted in the decision to combine the Elite, Main and Superline structures into a single breeding population and to utilise mate selection for achieving assortative mating, using efficient algorithms developed by Kinghorn and others (1999). These changes are expected to achieve the necessary optimisation of breeding gains and control of inbreeding versus any associated loss of genetic diversity from the breeding population. Clonal testing for within-family 'forwards' selection, which was not feasible in the past, has commenced in the RPBC breeding programme and should also provide a powerful means of increasing gains in the breeding population.

Tree breeders traditionally have defended the maintenance of large breeding populations, and, if costs are not an issue and everything else works as it should, few would argue against this.

Arguments for a large breeding population have included:

- Needed to ensure access to most of the species' available genetic diversity/ variance
- Enables higher selection intensities to be used in each breeding and selection cycle, therefore potentially achieving higher gain
- May protect against loss of rare alleles (e.g. for disease resistance)
- Delays the onset of relatedness and associated risks from inbreeding

However, recent studies based in part on population simulations support the use of much smaller breeding populations in line with practice in numerous other plant crops. For example, simulation work by Wu et al. (2016) indicates that maintenance of a per generation population size of around 200 individuals can be sufficient to balance the genetic gain versus diversity equation.

A rolling front approach, defined as '... a strategy in which all the main breeding activities are undertaken on a yearly basis' (Borralho and Dutkowski 1998), has become the standard approach used in advancing the Australian STBA tree improvement programme and has been recently adopted for the NZ programme. Each year parents are crossed, new progeny field trials are established, some existing trials are measured, new data are incorporated into the genetic evaluation system, and new parents are selected on the basis of the updated genetic values.

16.4 The Breeding Objective

The challenge of maintaining international markets for NZ wood and wood products will continue to require innovation as the forestry sector attempts to increase value-add by moving radiata pine wood from commodity to higher value uses. For the NZ radiata pine breeding programme, this necessitates use of a well-defined economic breeding objective, in which the aggregate genetic value of desired improvement is described in dollar terms (Alzamora 2010). The definition of genetic merit can vary along the production and processing chain. For example, Ivkovic et al. (2010) showed that the relative values of mean annual increment, sweep, branch size and modulus of elasticity vary between growers, sawmillers and integrated enterprises. However, James (1987) pointed out that, in the long term, a single breeding objective can be most efficient, and Apiolaza and Garrick (2001) have shown this to be appropriate for NZ radiata pine. A current NZ breeding objective will be likely to place most emphasis on improved growth rate (and/or associated reductions in rotation length), with secondary emphases on wood quality (strength and stiffness), tree form (stem straightness and reduced leader malformation) and resistance to *Dothistroma*.

Today's economic values may not adequately reflect those prevailing when wood harvest will occur several decades into the future. To combat this risk, Evison and Apiolaza (2014) have outlined a method (termed 'robust selection') for dealing with

market uncertainty through selection for individuals expressing less variability in their expected economic value, as estimated from a multi-trait selection index.

16.5 Improved Breeding Efficiency

Other proposed changes to the breeding strategy are aimed at achieving improvements in overall efficiency, plus increasing gains per year through earlier selection (including genomic selection) and more sophisticated methods of seed and plant delivery.

Earlier (and often, indirect) selection is a key target for all tree improvement programmes, since it can enable achievement of higher genetic gains per year in both the breeding and deployment operations (King and Burdon 1991; Kumar and Lee 2002; Li and Wu 2005). For the NZ radiata pine programme, the choice of the minimum selection age in progeny trials has been largely set as the earliest age at which the breast height diameter (DBH) can be considered to be a reliable estimator of rotation-end tree volume. In recent years, the practice has been to select at age 8 from trial establishment, by which time both the expressions of genetic variance and heritability for DBH are close to their maximum and the trial trees have not yet been strongly affected by inter-tree competition effects due to canopy closure. By age 8 also, the key form traits of stem straightness and branch cluster frequency (and associated internode length) can be easily assessed (Carson and Inglis 1988) and also the key wood property traits of wood density and corewood stiffness (Apiolaza et al. 2008; Apiolaza 2009; M. Lausberg, personal communication) and resistance to the *Dothistroma* needle blight (Carson 1989).

More efficient and often earlier selection will be achieved in a number of ways, including through use of:

- Open-pollinated (OP) breeding methods (Shelbourne et al. 2007)
- Clonal testing, combined with forwards selection
- Improved breeding value estimation and progeny trial designs (Cullis et al. 2014)
- New wood property screening methods (Apiolaza et al. 2008)
- Remote sensing applications
- Databases and decision support tools
- Screening for disease resistance
- Applications of genomics methods

16.6 Open-Pollinated (OP) Breeding Methods

OP breeding methods can shorten the breeding cycle by saving time in the progeny testing stage while achieving almost similar levels of genetic gain to those for a control-pollinated (CP)-based programme. OP seed collected from plus-tree and OP

forwards selections (assumed half-sib in both cases) can be used for progeny testing, thereby saving the time taken to establish and achieve flowering in grafted archives for subsequent development of full-sib crosses using controlled pollination. While the associated loss of pedigree information on the male line has been of concern in the past, genotyping tools are now available to identify relatedness among OP trees in most situations. For the present, however, the NZ radiata pine breeding programme is continuing to rely on fully pedigreed CP crossing for breeding population development. The additional time needed for pursuing a CP breeding strategy can be reduced if selection and CP crossing take place in the year of final progeny trial assessment; achieving this is now a practical goal for the NZ breeding programme.

16.7 Clonal Testing with Forwards Selection Within Families

Clonal testing of candidate for forwards selections using rooted cuttings is now an accepted practice in the NZ RPBC programme, providing benefits of higher heritability, increased genetic diversity in the BP and associated greater confidence in estimated breeding values (EBVs), leading to higher genetic gains/year. Cloning of radiata pine can be achieved through any of the following:

- Grafting of scions from both juvenile and mature trees, for establishment of clonal seed orchards and genetic archives
- Rooting cuttings from juvenile trees, usually combined with hedging to delay the effects of physiological aging and used routinely for multiplication of CP cross treestocks for deployment.
- Tissue culture, usually shoot cultures of axillary bud tissue from juvenile plants, used currently for an intermediate bulking of somatic cell lines prior to their establishment in cuttings stoolbeds.
- Somatic clonal lines developed from immature seed and cryostored in liquid nitrogen in order to delay the negative effects of physiological aging (as yet, only 10–15% of seeds and few families yield somatic clones). Somatic clones being deployed in NZ represent approximately 8% of the annual planting programme.

Cloned forwards selections from progeny trials are sexually mature by around age 4–5 from planting, and this currently sets a lower limit on the time required for completion of a breeding cycle. When applied appropriately, top grafting of material from juvenile selections onto mature plants offers opportunities to reduce this age by 1–2 years (Li and Dungey 2018). Either way, the more accurate EBVs estimated for cloned selections, replicated as they are both within and across trial sites, provide added confidence that can be translated into a reduction of 2 or more years of the current 8 years required for progeny testing.

The same improved confidence in EBVs for the cloned forwards selections has enabled seed providers to begin routinely grafting them into clonal seed orchards

earlier to produce commercial seed for treestock deployment. Both OP and CP seed are produced in these orchards, with the CP component usually being deployed in the form of rooted cuttings from hedged stoolbeds of CP seedlings. Somatic clones are similarly deployed as rooted cuttings, following a tissue culture bulking up phase, and continued supply of these is made possible through replacement of aging stoolbeds with new juvenile stocks extracted from cryostorage.

16.8 Improved Breeding Value Estimation and Trial Designs

Efficiency gains are coming from improved methods of breeding value estimation and improvements in progeny trial designs. University of Wollongong researchers have developed ‘multiple environment trial’ (MET) analysis methods for radiata pine, using the BLUP reduced animal model, combined with factor analysis (Cullis et al. 2014). This work has provided new selection tools for separately characterising and selecting candidates for both their overall performance and stability of performance across sites, thereby offering opportunities to better manage G X E and to achieve improved genotype/site matching (or ‘G + E’) (Smith and Cullis *In Press*). New ‘sparse phenotyping’ progeny trial designs promise to further reduce costs of investment in trials while also improving trial coverage (B. Cullis, personal communication).

16.9 New Wood Property Screening Methods

Recent work at the University of Canterbury, School of Forestry, has demonstrated the potential for wood property screening of tree stem and core samples from trees as young as age 2, using new laboratory techniques (Apiolaza et al. 2008). Traits screened with these tools include wood basic density, spiral grain, resin content and compression wood. These methods should enable earlier and more intensive screening of selection candidates.

Trial assessment of corewood stiffness and wood density with new tools like the Resistograph may prove cost-effective and can be used in combination with the existing ‘time-of-flight’ Treetap and Hitman tools (M. Lausberg and G. Downes, personal communication).

16.10 Remote Sensing Applications

Remote sensing applications of tools like LIDAR (‘Light Detection And Ranging’), and UAVs (‘Unmanned Aerial Vehicles’) are enabling more cost-efficient phenotyping of genotypes to be achieved in both trials and forest stands. LIDAR can already

provide cost-effective data on trial plant survival and trial heights, as well as disease traits and nutritional status. Similar data collected on forest stands will enable tracking of the plantation performance of improved family and clonal treestocks, providing early feedback for guiding decision-making in both the NZ RPBC breeding and deployment populations. In addition, these tools will provide early screening opportunities against new pest and disease incursions.

16.11 Databases and Decision Support Tools

Tree breeding programmes have increased in their complexity with increasing use of sophisticated trial designs and both multisite and multi-trait analysis methods and the extended pedigrees resulting from progressive breeding cycles. Various computer-based tools are now evolving to enable the NZ breeding programme to be managed more efficiently, including the Gemview (www.gemnetics.com) and Katmandoo relational databases. In addition, forest growers are being provided with decision support tools for optimising deployment of improved genotypes, with current examples including the RPBC's GenSelector software and a Univ. Wollongong application for use with mobile devices.

16.12 Screening for Disease Resistance

While the implementation of these new technologies will bring welcome increases in forest productivity, tree improvement programmes will also face new challenges from pests and diseases. New Zealand's exotic forest plantations are progressively losing the benefits of remoteness that they previously enjoyed, as new exotic pests and diseases continually arrive and threaten plantation health and productivity.

Such incursions may further increase as a result of climate change. While conventional field screening has been very effective for selection of *Dothistroma*-resistant trees in NZ (Carson and Carson 1989), research is continuing into development of both field and indirect screening methods for other foliar diseases, for example, the recently identified red needle cast (RNC) disease (Dungey et al. 2014). Pre-emptive resistance screening is often prescribed for protection against diseases that may not have yet arrived, but have proven damaging in other countries. For example, tree breeders in the USA, Chile, Australia and NZ combined to complete a pre-emptive screening project that has provided preliminary resistance scores to the pine pitch canker disease in *radiata* pine, which is endemic in the US Pacific NW (Matheson et al. 2006).

16.13 Applications of Genomics Methods to Enhance Breeding

The implementation of genomic selection (GS) is becoming a key issue for tree breeding programmes worldwide as technical development challenges are being met (Resende et al. 2012; Isik et al. 2016; Grattapaglia 2017). NZ R&D providers are close to completing a major project that will deliver a single nucleotide polymorphism (SNP) panel, capable of being used for GS in both the radiata pine breeding and deployment programmes. Current costs are estimated at approx. NZ\$37 and potentially as low as NZ\$25 per genotype for the SNP-based GS test, depending largely on scale (J. McEwan, personal communication).

Although there are challenges in terms of both biological and cost constraints to GS implementation, reports of successful progress with GS in relatively small breeding populations in other crops have been encouraging, and statistical methods for combining GS and field measurement data are continually being improved. Implementation of GS will principally be for earlier selection than can be achieved using current field and greenhouse screening methods. Such early selection is particularly valuable for economically important traits that either are very expensive to measure using conventional methods (e.g. cell-wall galactans) or take a long time to develop in field trials (e.g. heartwood development). However, even earlier selection for more conventional, low heritability traits like growth rate will also be valuable in shortening the progeny testing phase.

In a radiata pine breeding programme, a new selection cycle/breeding generation might be accomplished using GS within a 7–10-year timeframe, instead of the current 12–15-year breeding cycle using conventional field testing. GS may also be used for rapid improvement of specific key traits and/or for a potential emergency response to a new pest or disease threat, enabling repeated generation cycles to be implemented for single traits, leading to accelerated breeding gains/year for that trait.

16.14 Applications of GS to Enhance Deployment

The implementation of GS for improving commercial treestocks which are directly deployed by growers of radiata pine represents a much greater challenge than that for breeding. The transfer of GS-based genetic gains into plantations will be most efficient and effective using somatic clones (due to their higher trait heritabilities), less so for CP seedlings and cuttings, and substantially less for OP seedlings.

The cloned forwards selections selected in the breeding programme cannot currently be multiplied for deployment since they will have undergone irreversible maturation during the progeny testing and reproductive development phases. New CP cross seed derived from breeding population parents can be screened using GS, but then there will be a requirement for the selected set of individual genotypes to be massively

multiplied (using some combination of tissue culture bulking and stoolbed cutting production) in order to create a sufficient number of plants for establishment of meaningful areas of commercial plantations. This will inevitably involve a trade-off between achieving worthwhile selection intensity and incurring costs for a large number of SNP reactions, and these costs will be further increased by the requirement to turn cuttings stoolbeds over every 4 years, in order to control maturation.

NZ clonal providers have a resource of several thousand improved clones that have been captured into somatic embryogenesis and cryostorage. These represent an efficient and effective opportunity for applying GS to realise higher genetic gains for both breeding and commercial deployment. Plant production costs will, of course, continue to be higher for somatic clones and CP treestocks versus OP seedlings, with today's costs being in the order of \$700/thousand for somatic clones, \$450/thousand for CP cuttings, \$350/thousand for CP seedlings and around \$250/thousand for OP seedlings. However, the gains from clonal deployment plus genomic selection will outweigh the additional costs of production.

There are additional potential benefits from the use of genomic methods beyond implementing GS selection, including:

Breeding values can be estimated more accurately, through reductions in identification errors, plus better estimates of relatedness from the additional genomic information.

Improved accuracy in genotyping should assist in improved quality control in breeding operations.

Genomic data can play an essential role in augmenting any future applications of genetic editing and manipulation techniques.

SNP associations with key traits can be used to identify higher gain candidate selections for breeding crosses, through improved detection of both favourable gene clusters and potential inbreeding.

16.15 Realised Gains and Tree Crop Modelling

Proof of profit is an essential prerequisite to market demand for improved treestocks, and most forest growers in NZ believe that the continued quantification of genetic gain is essential. Forest growers use genetic gain information in new crop investment decisions as to what treestocks to plant, in yield tables and in determining woodflow and harvest levels.

Tree breeders have sometimes relied on predicted genetic gains from breeding values developed from progeny trial data to be realistic estimates of realised genetic gain. For example, the STBA uses this method to estimate annual gains in their programme (Powell et al. 2004). However, the main limitation of this approach is that it does not take adequate account of the relatively major (compared to genetics) effects of site and silviculture (Carson et al. 1999a).

The much greater impacts of site and silviculture (especially on tree growth) mean that these can have a large influence on the expression of genetic gain, which

is not reflected in breeding value estimates. Instead, the use of well-designed and forward-looking long-term large-block trials combined with tree crop modelling is considered to be a more reliable approach (Carson et al. 1999b).

Tree crop models developed in NZ for growth rate, wood density and corewood stiffness are based on thousands of empirical measurements over a comprehensive range of forest sites. Early-age breeding values ‘grown on’ in these models are far more likely to yield reliable estimates of rotation-end values that can be used in genetic gain estimation, since they will take due account of site and silviculture effects. In addition, age/age genetic correlation estimates derived from progeny trial data are used in breeding value estimation and selection indices for key traits like growth rate. While conventional single-tree-plot progeny trials are very efficient at ranking genotypes for selection, they are as poor for providing reliable age/age correlation estimates as they are for estimating realised rotation-end gains. Instead, breeders may better accept the age 8 breeding values from progeny trials as providing the best- available information on genotype ranking for selection and use the growth (or other target trait) models to provide the necessary selection trait correlation with the requisite (and often quite different) rotation-end target trait (Carson et al. 1999b). The theoretical counter to this would be to challenge the implicit assumption that this approach makes, that is, that there is no crossover of genotype rankings between age 8 and age 25–30. However, we would argue that this is a safer option than the use of very unreliable age/age correlation estimates derived from progeny trial data.

Long-term ‘genetic gain trials’ comparing seedlots with various levels of improvement were first established in NZ in 1978 (Shelbourne et al. 1986). These trials were subsequently enhanced with the establishment of a comprehensive series of ‘silviculture/breed trials’ between 1987 and 1992 (Carson et al. 1999a), aimed at providing data on genetic performance of a mensurational standard over a range of silvicultural (largely tree spacing and thinning) options and across all NZ growing regions. The 1978 genetic gain trial series yielded valuable and convincing rotation-age evidence of realised gain from radiata pine improvement efforts, which has contributed to the forestry sector’s continuing strong support for the breeding programme.

Table 16.1 summarises gains realised at a high-productivity site of the 1978 genetic gain trial series. Although this result is exceptional, there is substantial

Table 16.1 Comparison for timber volume, percentage high-quality sawlogs, gross revenue per hectare and gain in \$NPV/ha of unimproved radiata pine seedlots with both an OP seed orchard seedlot and a good CP cross seedlot, from age 30 measurements in a replicated large-block genetic gain trial established at Mohaka Forest in 1978

Genetic seed source	Timber volume (m ³ /ha)	High value log grades (%)	Revenue at age 30 (\$)	Value gain per hectare of improved seedlots (\$NPV/ha @ 8% interest rate)
Unimproved seedlot	754	61	67,300	–
OP seed orchard seedlot	905	68	82,900	1550
Good CP cross	1165	69	108,800	4130

evidence from the subsequent silviculture/breed trials indicating that national tree volume gains/year from the NZ breeding programme are averaging in excess of 1% per year of breeding effort, which is consistent with gains being achieved in a number of other tree and plant crop species (Kimberley et al. 2015).

A number of recent publications have addressed the goal of estimating realised gains based on measurements of these long-term genetic gain trials and the inclusion of these estimates in NZ tree crop models. Genetic multipliers were first developed in the 1990s for modelling improvements in growth rate for individual genotypes (Carson et al. 1999b). Using a similar approach and based on similar evidence for realised gains in wood density and corewood stiffness (Carson et al. 2014), Scion researchers have now developed genetic multipliers for improvements in these traits (Kimberley et al. *In Press*).

An ideal future approach to realised gain prediction and genetic gain modelling would be to (1) plant the range of genetic stock available for planting in a production forest in progeny trial-like experimental designs across the forest and (2) systematically allocate permanent sample plots of the type used by mensurationists for growth model development and validation to sites, representing the range of available genetic stock. Elements of this approach are already being tried in NZ plantations, in which ‘paired plots’ of unimproved and improved seedlots are being established within plantation stands and inventory plots are, in turn, being embedded in progeny trials. In the future, although physiological models developed for growth prediction have disappointed in the past, a new hybrid model combining physiological data with empirical measurements is showing promise and may assist gain prediction through an improved understanding of the underlying causes of $G \times E$ (Mason et al. 2018).

Another promising approach for improved prediction of genetic gain in plantation forests would be to substitute the age/age correlations used in the best linear unbiased estimation (BLUP) approach commonly used by tree breeders with estimates derived from crop model predictions. More accurate predictions could then be made for a range of silvicultural prescriptions, rather than assuming that realised gain will be the same under all planting conditions.

16.16 Major Challenges and Opportunities

Tree breeding programmes are subject to constant challenge, review and revision as new technologies and new thinking are brought to bear. The overall challenge for the NZ radiata pine breeding programme is to increasingly deploy to achieve substantial additional genetic gains/year in key traits, to provide protection against major risks posed by pests and diseases and to retain sufficient genetic diversity to maintain future gains while managing any effects of inbreeding. Genomic selection represents a technology that is close to implementation. Other attractive near-term opportunities are available, including:

Cloning of the breeding population and adoption of clonal deployment as the approach which delivers the maximum genetic gain in a cost-effective way.

New accessions sourced both from other breeding and clonal programmes and from ‘mining’ of existing plantations and gene editing and gene transfer.

16.17 Cloning the Breeding Population and Adoption of Clonal Deployment

Somatic cloning methods offer the potential to clone the breeding population, which could lead to a ‘step-change’ improvement in delivery of genetic gains. The potential benefits include:

Somatic clones that are tested in the breeding programme as potential forwards selections can be immediately mass-propagated and deployed in plantations through clonal deployment. This substantially reduces the time required for conventional seed propagation and deployment and delivers both higher genetic gain and increased gains/year from the breeding programme (but as yet, only 10–15% of seeds and few families yield somatic clones).

The capability to combat a new disease or other threat would be enhanced. Resistant stocks could be deployed more rapidly because somatic clones in cryostorage can be deployed as bulked or single clones for deployment within 3–5 years of recognition of a crisis. In addition, resistant genotypes can be more quickly established in breeding archives and deployed as seed from seed orchards and/or as cuttings from control-pollinated stoolbeds.

Direct deployment of somatic clones represents the best platform for deploying new genomic technologies. This includes genomic selection, as well as CRISPR-type gene editing and gene transfer methods. Whether in response to a threat or for making conventional breeding gains, improved stock could be delivered to plantations via the above propagation options within a 3–5-year timeframe.

Somatic clones can also be transferred *in vitro* from NZ to Australia, for testing and deployment in both countries, offering key opportunities for improving both gains and connectivity.

The main technical challenges revolve around the current costs of capturing genotypes into somatic embryogenesis and the low efficiency of the capture process. Only 10–15% of immature seeds cultured will become propagable somatic clonal lines. However, there is no evidence to date for any reduction in genetic diversity as a result of the low efficiency of the capture process, and the costs of clone line capture can be considered manageable when compared to the potential benefits. One of the largest forest owners in NZ has embraced this technology for deployment, and a pilot programme comprising over 1500 cryostored NZ breeding clones has been recently initiated.

16.18 New Accessions

Several of the recent changes in breeding strategy have arisen from the realisation that the NZ breeding population is now almost ‘closed’, in the sense that there are relatively few options for expanding genetic diversity without reverting to making selections in the relatively unimproved native provenances. This, however, represents only one of several unexercised opportunities to access and utilise potentially available genotypes.

New accessions could come from at least four different sources, that is:

Other breeding programmes with radiata pine, including progeny-tested selections from the Forest New South Wales, Southern Tree Breeding Association and Chilean Tree Breeding Association programmes. In each case, there are potential biosecurity barriers to overcome, but these may be resolvable with some creative planning and associated R&D investment.

Accessions from these programmes are likely to provide substantial additional genetic diversity, since they will be largely unrelated to material already in the NZ breeding population.

As mentioned already (under ‘Genomic Selection’), two NZ providers of somatic clones currently hold several thousand clonally tested genotypes in their cryostorage facilities, archives and field trials. Although these clones represent progeny and siblings of breeding parents already in the NZ breeding population, their introduction into the breeding population and provider seed orchards would undoubtedly create options for increasing gains/year in both the breeding and deployment populations.

A similar potential exists within the NZ plantation resource, even although this resource is largely comprised of seed orchard progenies derived from the NZ breeding programme. The NZ radiata pine estate covers 1.5 million hectares and has been planted within excess of 1.2 billion individual genotypes, of which in the order of 400 million trees are expected to be present at harvest age. In terms of population genetics, this vast genetic resource must be assumed to contain a very large number of genotypes possessing new gene combinations and mutations, when compared to the relatively tiny sample of genotypes being progressed in the breeding programme. New plus trees identified and sampled from this resource could be screened for their pedigree and performance, for example, using the new GS SNP panel, thereby creating selection options for achieving additional gains and genetic diversity in both breeding and deployment.

As mentioned above, new selections can be made in various existing NZ and Australian trials and stands containing material from the five native provenances of radiata pine. To date this has been an unattractive option, since (with the possible exception of some genotypes from the Guadalupe provenance) these native populations have progressively fallen further behind the NZ breeding population for their performance for the key target traits. Selections within them would likely need to be backcrossed with better parents for several breeding cycles before they could contribute to gains in deployment. However, these native populations offer potential contributions of valuable alleles not present in the breeding population, and such

genetic variation could become critically important in combating any new serious threats from pests and diseases. Also, the new genomics tools should make it possible to make the backcrossing process more efficient, such that these new alleles could be brought into play much more rapidly than otherwise (J. McEwan, personal communication).

16.19 Genetic Manipulation

There has been a plethora of academic and public debate about the pros and cons of genetic modification (GM), and this is likely to be ongoing. In NZ, the Royal Commission's recommendations in 2001 were favourable to cautious commercial deployment of GM plant stocks. However, since then there has been little practical progress towards achieving commercial applications of GM for radiata pine, despite years of subsequent research that has indicated both the high potential and the low risks associated with this option (Walter et al. 2005).

As was the case in 2001, the threat of negative public perceptions has continued to be the main barrier to implementation of GM methods, combined with an understandable reluctance among the large forest growers to be the first to challenge the *status quo*. Whether perceptions and regulations associated with the new gene editing methods will be any different remains to be tested. It appears clear, however, that these new techniques offer much more accurate manipulation of existing genes, without necessarily incurring the risk and/or fears currently associated with foreign gene transfers.

16.20 Conclusions

NZ radiata pine breeders have been highly successful in increasing the productivity of the large forest estate of radiata pine in NZ and parts of Australia. Improved statistical methods, trial designs and decision support tools are making breeding more efficient, while new indirect and remote screening methods show promise of increasing gains/year, particularly in deployed treestocks. New information has enabled simplification of the breeding population structure, and assortative mating will assist achievement of a balance among genetic gain, diversity and inbreeding effects. Opportunities are available for accessing other sources of improved genetic material, thereby enabling an increased emphasis to be placed on selection intensity, leading to increased genetic gains. Cryostorage and retrieval of somatic clones in juvenile form provide exciting options for immediately increasing realised genetic gain through deployment and breeding, as well as being the preferred platform for successful implementation of genomic selection and for potential deployment of improved, gene-edited treestocks. These biotechnology tools, when combined with sound conventional tree breeding practice, may well bring about a step-change improvement in future cycles of NZ tree breeding.

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