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# Design of Future Large Plot Genetic Gains Trials

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# EXECUTIVE SUMMARY

The investment in tree breeding hinges on the need to quantify genetic gain in terms of per hectare benefits in a number of metrics. Coupled to this is the need to modify growth and yield prediction models to forecast changes in product flows.
The Radiata Pine Breeding Company technical steering committee and the Future Forests Research/Radiata Pine Management Theme/Objective 1/technical steering committee agreed to collaborate in the development of a strategy for a new series of genetic gain (GG) trials.

Consideration was given to whether such a significant trial series can also break new ground in answering some of the big questions. To do this, we may have to establish a very wide range between the minimum and maximum genetic gains for any given trait, and to consider extended rotations to examine empirical data well beyond the rotation age.

The task team has developed a recommended design: being a replicated large-plot (8x8 tree plot) including an inner plot of at least 49 trees (7x7) in a single-tree-plot design, and where necessary including both control-pollinated and clonal material. Previously these trials have been established in the eight or nine *P. radiata* growing regions across NZ, and at least two regions in Australia. The RPBC breeding plan calls for fewer installations of big plot trials, with two trial sites to be planted in each of four NZ districts, presumably Northland, Kaingaroa, East Coast and either/or Golden Downs and Southland. In Australia, trials should be placed on both high and low elevation sites. At least one large trial will be established per region/site type, and additional small, unreplicated blocks spread across the estates of the member companies. Regions/site types are defined by climatic zones/site factors. Deciding on this classification may require further cooperative discussions.

# INTRODUCTION

The Radiata Pine Breeding Company technical steering committee and the Future Forests Research/Radiata Pine Management Theme/Objective 1/technical steering committee have agreed to collaborate in the development of a new series of genetic gain (GG) trials.

This paper forms the basis of discussion for the development of the next generation of genetic gains trials in New Zealand. The background to this discussion paper is contained the ***Technical Note*** [1] and ***Work Plan*** [2].

The interest in genetic gain hinges on the need to demonstrate gain in response to investment in genetic improvement, and in the need to modify growth and yield prediction models to forecast product flows caused by the adoption of the new genotypes. The needs range from snapshots of differences between genotypes, through to longitudinal forecasts of wood product flow and quality for strategic decisions at the company and national level (**Table 1**). There is a range of activities in the testing of genetic gain that is being undertaken by stakeholder entities. Most of these activities are complementary, and the broad range offers some flexibility in the design of new trials as some questions can be dealt with by existing trials. A major use of these trials is of course the need to include, and compare seedlots in wide existing use. Another need is for trials to be available to determine growth losses to diseases, for instance the series of Dothistroma trials established in the 1980s. These represent a subset of large block trials targeted at high hazard sites for the given disease.

**Table 1. Activity of entities interested in genetic gain of radiata pine**

| Entity | Objective | Activity |
|---|---|---|
| FFR Program 1 | Quantify perturbation of silviculture and genetics to GF7 based and 300I growth models | Establish large plot trials with a few seedlot treatments along with site, tending and thinning/stocking treatments. |
| RPBC | Demonstrate long term gain | Establish genetic gain trials with large blocks once per breeding cycle [3], or more frequently, once each breeding cycle (Carson pers comm.). Design to provide rotation length inference on the gain achieved, particularly for growth rate. Also, any breeding regions thought to express G X E should be covered. |
| | Demonstrate short term gains, analyse pre-canopy closure performance | Establish short term demonstration trials (e.g. International GFPlus trial series). Also good for wood property sampling |
| | Estimate variance components | Establish single tree plot genetic evaluation trials regularly during each breeding cycle. Not suited to examining yield due to inter- tree competition effects [4] |
| Companies | Demonstrate increase in stand value due to investment in genetic program | Establish control or improved seedlots within operational plantations. These are designed to determine long term changes in yield and wood quality.<br><br>Establish annual &/or periodic single site demonstration/trials of all seedlots used. |

**Forest Management Questions**

Forest establishment can be carried out with different types of planting stock: open pollinated seedlings, control pollinated seedlings or cuttings, and clones.

1. Volume: what is the difference between (top ranked) open pollinated and (top ranked) control pollinated including clones?
2. Quality: control pollinated families and clones can be tailor made for traits of interest – but at what cost and benefit?
3. How applicable are the results of a few genetic gain trials put out on carefully selected sites to the whole of the estate?
4. What is the slippage between carefully tended replicated trials and operational performance on a large scale

**Research Questions and Modelling Needs**

1. Do different genotypes substantially affect slope or asymptote of projection functions? Extreme different treatments may affect the parameters (Apparently there is not much influence within a small 'Growth' rating range [(5)]). The wide range from GF7 to GF22 in the earlier 1978-1980 GG trials series would allow this assumption to be tested on any remaining trials in that series, and would provide results much earlier.
2. With new genotypes, what is the form the growth/density/biomass function beyond the current 25 year rotation? For instance, do we look at locking these trials in for extra long rotation and measurement? Note that there is a substantial opportunity cost to the grower.
3. Confirm if high site quality equates to greater magnitude of response in higher GFPlus rated seedlots. This has already been established by Sue Carson, but could be further analysed and publishing.
4. Track wood quality gains and variation to age 50;
5. Develop carbon models for stands aged up to 100 years;
6. Track compartment-sized blocks of a range of GFPlus seedlots and clones for changes in mortality under inter-tree competition.

Consideration was given to whether such a significant trial series can also break new ground in answering some of the big questions. To do this, we may have to establish a very wide range between the minimum and maximum genetic gains for a given trait, and consider extended rotations to examine empirical data well beyond the rotation age

Genetic multipliers were developed to modify the 'state-space' models developed by Oscar Garcia, which were largely based on data from GF7 material. Subsequent NZ growth Models including the 300 Index model (300I) have been developed and used in Forecaster. The original intention of 300I was to account for site and silviculture effects on growth rate; and it may also accommodate genetic gains, according to the input adjustments indicated from genetic gains trial analyses, although there is some indication that highest levels of gains did not fit the adjustment well [5].

## Review of Older Genetic Gain Trials

Formal testing of genetic gains includes six trial series established since 1978. The establishment and ownership of the trials has variously been through FRI/industry cooperatives, SCION and private companies (**Table 2**). They commenced with the 1978-Genetic Gains trials; based on seedlots on the GF system, rated up to GF23. The trials were simple with a common set of GF2, unimproved, GF7 climbing select, GF14 OP seed orchard and GF23 CP seed orchard material; these trials formed the basis of the genetic gain multipliers developed by Carson et al [6] to adjust the national radiata growth models, which were based largely on GF7 material. The period between establishment of the trials and the publication of this paper based on half rotation measurements indicates the likely period that information from the current proposed trials may become available. Some efficiency may be possible, in the rapid collation of appropriate seedlots, slightly shorter half rotation (12-13 years) and rapid inventory, evaluation and reporting, perhaps bringing this back to 17 years from the present.

A concern is that subsequent trial series have not been reported in such a sophisticated style. Despite a great deal of monitoring and analysis, it is only recently that a comprehensive publication detailing genetic gain in growth of the Silviculture Breed series has been completed; to draft report stage [5]. This trial series should be further evaluated for the GG of the highest GFPlus levels for growth, as well as wood quality, within those trials. While some stiffness measurements have been taken, the Special Purpose Breeds trial series has not been reported for mid-rotation wood property contrasts (inter-nodal clears were examined, as well as density) between the base level and high value seedlots included in the design. Analysis of this might inform decisions on whether to include wood and log property traits in yield plots in the current proposed trials. Some sites of the GFPlus international trial series have been measured at around age 7-8 years.

**Table 2. Previous NZ genetic gains trial series**

| Year established | Name | N sites | Reports | Description |
|---|---|---|---|---|
| 1978-80 (84) | 1978 Genetic gains trials | 10 | [6, 7] | First large plot GG radiata trials |
| | | | [6.5] GTI/RPBC | 10x10 block+ 6-tree row-plot trials |
| 1988 | Australasian Breeds series | | [6.6] RPBC reports, including Low | Demonstration trial with single or paired row plots |
| 1987-1991 | Silviculture Breed series. | 28 | [5, 5.5] | Second set of large plot radiata trials |
| 1992-94 | Special Purpose Breeds Series | 8 | [8] 92 SL meas. age 7.5 to 9; 94 S/L meas. age 6-6.5 | Developed for wood properties and other special purposes<br>Note: 92 seedlots OP, no rep, 94 S/L CP and 2 reps. |
| 2003 | GFPlus international trials | ~10 | RPBC tech reports on growth and form | Single or paired row plots. |
| 2001-2004 | Response surface | ~10 | [9] | Genotype × thin × prune factorial, large plots Dean (2005). |

The design of these trials appears to have in general, been reasonably suited to their designed purpose (M. O. Kimberley pers. comm.). However management of trials across the various companies has not been consistent; which at times leaves temporal gaps in the datasets. The utility of the response surface trials remains to be evaluated. Note that at the level of mixed-

genotype seedlots, the Silviculture/breed trials have established/confirmed that growth rate, wood density and stiffness gains do not interact with differing thinning and pruning treatments (D. Stackpole, pers. comm.), and that future trials can omit differing silvicultural treatments.

Information of genetic gain is generally tested through the growing of trees in field trials, since indirect measures of estimating genetic gain have invariably proven unreliable. The space occupied by a tree is considerable; hence trials tend to occupy large land areas. Competition between trees means that in small plot or single tree plot designs, the 'true' differences between seedlots of contrasting growth potential are exaggerated sooner than in large plots. Growth is particularly affected in this way, while wood properties appear to be less so. Hence, empirical measures of rotation length yield that accurately reflect the yield potential of the seedlots under test are best obtained with large plot designs. When appropriate replication is included, and buffers allowed for, the large areas of these trials means that few seedlots (typically up to 6) can be tested, hence considerable care must be taken in the selection and formulation of those seedlots. For investigating genetic gains for growth and (to a lesser extent) wood properties, we are mainly interested in Large and 'Medium' plot size (**Table 3**).

Assuming that log quality and wood physical and chemical properties are less prone to competition (Carson et al 1999) smaller plots can be used. Paired or single row plot designs, which can also be used for early age yield as well as for demonstration purposes, appear to be appropriate for wood property demonstration. The other benefit is that it may be possible to get estimates of genetic gain for wood and chemical properties for many more seedlots than is possible for growth. This assumption could be tested by comparing the response of wood properties in the Special Purpose Breeds Series (**Table 2**), with any extant smaller plot plantings containing the same seedlots.

**Table 3. Trial plot sizes and typical application in testing genetic gain.**

| Plot size | Purpose | n treatments + base/controls |
|---|---|---|
| Large | Growth modelling | 6 max |
| | Demonstration of sound long term response | |
| Medium | Demonstration of short term response | Dozens |
| | Short term statistical comparison between seedlots | |
| Small | Estimation of variance components by breeders | 100s |
| | Provide high selection intensity for recombined germplasm | 1000s |

Where large = 100+, medium =64, and Small = 6-10 i.e. row plots

**Trial Design**

The recommended design is a replicated large-plot (10x10 or 8x8 tree plot) including an inner plot of at least 49 trees (7x7) in a single-tree-plot design, including, where necessary both control-pollinated and clonal material.

At least one large trial will be established per region/site type, and additional small, unreplicated blocks (satellite trials) spread across the estates of the member companies.

The trial regions/site types are defined by climatic zones/site factors. Deciding on this classification may require further cooperative discussions.

## Identifying the Populations of Germplasm to be Tested

### Control or Base Genotypes

Three seedlots have previously been considered 'automatic inclusions' in almost any genetic gain or seedlot trial. This is because these were considered the likely standard in genetic trials, and served to link all these trials together. The seedlots were climbing select GF7, GF14 (seed orchard open pollinated) and GFPlus23. More recently, the GF7 seedlot has been omitted and the GF14 seedlot replaced by a bulked sample of GF19 seed. In addition, higher-rated CP seedlots have been introduced as they have become available, in a bulk of up to five specific clone-clone crosses. We need to address the appropriate number and composition of experimental controls to include in future gain trials. The increasing availability and planting of individual clones in pure stands underscores both the need for such genotypes to be represented in future gain trials, as well as consideration of their use as standard controls.

### GF or GFPlus Range of Genotypes Under Test

Recent analyses [5, 10] of 1978 and 1988 genetic gains trials indicate the difficulty in separating close ranked seedlots at the higher GF/GFPlus range. This indicates the desirability of using GFPlus ratings for diameter of at least 4 points separation in order to convincingly display differences. How this relates to density or stiffness traits is less well known, and although density was used as a selection trait in the 268 series, density has not been often used to help identify seedlots for genetic gain. More recently, the Special Purpose Breeds trials were designed specifically to confirm gain differences in density, and the recent GF Plus trials have also addressed this with specific mixtures of crosses.

### Clones

A committee of the then SGMC agreed that the next generation of modelling trials should include clonal treatments [4] as they represent the top end of the market and are being deployed on an operational scale. Clones must be considered not only as controls in gain trials, but also as important plantation candidates/'seedlots' in their own right.

### General

Previous Genetic Gain trial series have included the most advanced seedlots that could be formulated at the time of establishment. The results have usually been good, in that the gains forecast have been evident in the subsequent analysis. Thus very advanced levels of gain, if such levels are likely to represent future deployment, should be produced and tested in the current series.

## Frequency with which New Gains Trials are Established

Two main options are available

- Rolling front as new material becomes available over the deployment cycle.
- One hit per breeding cycle (as per RPBC breeding plan)

Consideration is needed as to whether a combined approach is possible or desirable. The big plot trials for growth should go across, in GF Plus terms from G19 to G30, and be established in one hit. While smaller plot trials could be established simultaneously, include the above seedlots, and also all the advanced density and stiffness seedlots. These smaller plots could also be regularly planted on a rolling front regardless of the breeding cycle.

## Number of Trials per Region, and per Site Category

Classically these trials are established into the eight or nine *P. radiata* growing regions across NZ, and at least two regions in Australia. The RPBC breeding plan calls for fewer installations of big plot trials, with two trial sites to be planted in each of four NZ districts, presumably Northland, Kaingaroa and East Coast; and either/or Golden Downs and Southland. In Australia, trials should be placed on both high and low elevation sites. Company benchmark plots could be used to fill out these data sets if (i) they contain relevant seedlots, and (ii) the management and monitoring can be conducted to a similar rigour as the main big plot trials. If more growth regions/site types are required to be included, then they should meet these trial specifications. Single or twin row small plot trials could be used to obtain 1/3 rotation inference on the genetic gains, at much higher intensity across the country.

Inclusion of silvicultural treatments such as establishment stocking, thinning and pruning (as opposed to uniform tending) will increase trial size and/or restrict number of seedlot treatments. Previous research has identified that inclusion of silvicultural treatments will not normally be necessary. Committees should review whether response surface trial series address current needs and how the proposed new series might be linked to them, perhaps with a common seedlot.

## Measurements and Measurement Interval

Survival assessments of all plots should be measured in the first growing season, and this should form the basis of subsequent assessments. Suggest establishment of square growth plots 2 rows in from the treatment edge; with GPS start point recorded, plus direction of measurement trees indicated before thinning. Every tree is allocated a status variable, including live healthy ones. Each tree should be individually numbered and its identity retained after thinning, whether square or circular measurement plots are subsequently adopted. Diameter as DBH (1.4 m above ground level) on all trees is required at each measurement. Mean top height of plots is required, and a measure of green crown height; and some sort of volume function, perhaps site or seedlot-specific. Growth measurements could be annual if around the time of anticipated peak CAI peak; and less regular later in the rotation.

Wood property sampling from the big plot trials runs the risk of influencing subsequent performance of the tree (although trials can be designed to permit sampling from buffer trees), however it should be considered as the 5 or 12 mm diameter cores damages a small portion of the cambium, which rapidly heals so the penalty is brief. Pilodyn windows 5 cm wide cut into a selection of 8 year old *Eucalyptus globulus* in a Tasmanian trial could not be detected in growth of the trees at age 15 years (D. Stackpole pers. comm.). Stiffness as measured by PME is less destructive. Measurement period for density and stiffness could be based on juvenile wood (up to 8 years of age) and harvest age measures (20+ years).

## Protocols

### Implementing the Trial Programme

This trial series needs to be a coordinated and cooperative series across FFR and RPBC. Land, site preparation, planting and maintenance of the trials would be provided by the Cooperating Companies, the germplasm planted should be developed and provided by RPBC; final trials will be designed by both RPBC and FFR. Direction and supervision of establishment; ongoing maintenance; and scheduling and marking for thinning would be closely managed by an FFR/RPBC appointee.

**Trial Establishment**

As the success of this complex project depends on being able to clearly infer differences between seedlots, all efforts to minimise environmental noise should be taken. This includes a trial design to take into account any known site problems, and this in turn requires plenty of lead time for trial sites to be offered, inspected and assessed against eligibility criteria. Planting and early weed control needs to be of excellent quality to minimise planter and weed competition effects. Plants established at regular spacing's are preferred, hence mounding of rows rather than spot cultivation is essential. The project is complex and a dedicated team would be required to assess sites offered and liaise with companies for the best quality outcome.

**Management**

Exemplary weed control, nutrition and thinning practices to a single standard are required to minimise the introduction of environmental variability into the trials. Further, unscheduled harvesting operations in trial sites must be avoided. This management will be provided by landholder co-operators, but active management by FFR and RPBC is needed to ensure operational managers are aware of any test sites (inclusion in their GIS systems is essential), their significance, and the need to avoid unscheduled modification.

Standard silviculture specifications are required i.e. final crop stocking of 400 stems per hectare, no pruning (access pruning only); thinning at a mean-top-height of 12m. Initial stocking ideally would also be consistent, but can follow regional best practice.

**Measurement**

To be written upon identification of required measures.

**Treatments**

Treatments should reflect high, medium and OP of growth rate based on current breeding values, and high BV ratings for density, and 1 or 2 company treatments. Treatments with large differences are ideal, i.e. material already in deployment as well as material likely to reflect future deployment trends.

# CONCLUSION

This document is intended to discuss for FFR and RPBC technical committees the issues underlying the design of a series of genetic gains trials. The following recommendations have been made.

## Design Recommendations for Genetic Gain Trials

- Trials to guarantee coverage over a wide range of New Zealand sites
- Abandon row plots in favour of block plots
- Trials to contain sufficiently contrasting treatments
- All seedlots to be included in future genetic gain trials to be selected on actual breeding values of traits of interest; all future analyses to be based upon contrasting treatment breeding values, not GF or GFplus values.
- Contain trial size by limiting the number of treatments (from forest management point of view start with top OP and skip all below Gf19) and the numbers of trees/plot, but number of trees/plot should be still sufficient to model a “stand” effect. Containing trial size will additionally assist in blocking environmental trends and achieving significant treatment differences while reducing risk of trial loss.
- Trials to contain standard RPBC benchmark(s) seedlots.
- **Committees should review whether response surface trial series address current needs and** how the proposed new series might be linked to them, perhaps with a common seedlot.
- A transition strategy is needed to utilise the existing large plot trial series to support genetic gain quantification  while the new trials are established.

# REFERENCES


1. Lee, J., Stovold, T., and Dungey, H., *Next generation tree improvement trials to future proof the next generation of data provision.* Future Forests research; Radiata management note, 2010.
2. Lee, J., *Next generation of tree-improvement trials to future proof the next generation of data provision: Work Plan.* Future Forests Research; November 2010, 2010.
3. Dungey, H.S., Stovold, T., Low, C., Menzies, M., and Burdon, R.D. *The RPBC Breeding Plan.* 2009 Rotorua.
4. Dungey, H., Hayes, J., and Shula, R., *Towards the next generation of modelling trials.* Stand growth Modelling Cooperative report No. 124, 2004.
5. van der Colff, M., Kimberley, M., and Lee, J., *Testing the performance of the 300-Index Growth Model for major genotype groupings across New Zealand* Future Forests Research, 2010.

5.5 Hayes, Judy, D; Carson, S D; Vincent, T G. *Growth, straightness, and branching GFPLUS values for seedlots in large-block genetic gain trials.* SGMC Report No. 69, 1998.

6. Carson, S., Garcia, O., and Hayes, J., *Realized Gain and Prediction of Yield with Genetically Improved Pinus radiata in New Zealand*. Forest Science, 1999. 45(2): p. 186-200.

6.5 Sorensson, C. T and Low, C. B. *Realised genetic gains of radiata pine seed orchard seedlots for diameter, tree form, and wood density at age twelve at fourteen sites in New Zealand.* New Zealand Radiata Pine Breeding Cooperative Confidential report No. 31. 1995.

6.6 Sorensson, C. T. *Genetic gains at age five years from the 1988 Australasian breeds genetic gains trial at Kaingaroa Cpt. 1205.* New Zealand Radiata Pine Breeding Cooperative Confidential report No. 32. 1995.

7. Carson, S., Carson, M., Wilcox, P., and Kimberley, M. *Trials designed to quantify growth and yield gains from genetically improved Pinus radiata seed in New Zealand.* 1991 Stand Growth Modelling Cooperative report No. 24.
8. Low, C., and Miller, M., *First assessments of the 1992 and 1994 Special Purpose Breeds trials June 2003 RPBC 137.* 2003.
9. Dean, M., *Silviculture x traits trials - Establishment report. Plantation Management Cooperative Report No. 87.* 2005.
10. Stackpole, D.J., and Stovold, T. *Validation of genetic predictions: from Breeding Values to realised seedlot Report for RPBC Ltd December 2009 REPORT NO. SG 1.5.* 2009