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Genetic Gain Trial Design

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EXECUTIVE SUMMARY

The investment in tree breeding hinges on the need to quantify genetic gain. Coupled to this is the need for ongoing development of growth and yield prediction models to forecast changes in product flows. The Radiata Pine Breeding Company (RPBC) and Future Forests Research (FFR) require a common genetic gain trial design to meet these objectives. Forest owners and managers require operational gain trials they can implement within their forests to validate and demonstrate genetic gain

The RPBC and FFR collaborated in the design of a new series of genetic gain (GG) trials planned to take model development and quantification of genetic tree improvement through the next 20 to 40 years. FFR required robust trial for modelling genetic gain and RPBC needed similarly robust designs to evaluate genetic gain in current and future deployment populations. This project report is the outcome of collaboration between FFR and RPBC.

The first team, lead by Des Stackpole and John Lee, examined the current trial designs, objectives and shortcomings, and developed a set of guidelines to meet the objectives of future FFR/RPBC trials. The second team, lead by Elaine Birk, developed the detailed design parameters for new gain trials to meet the joint company objectives. This team also developed two trial designs to enable forest owners to evaluate genetic gain in forest operations, and consolidated the combined outcomes of both projects into a common report for RPBC and FFR.

The report summarises three designs that collectively meet the range of objectives of both the RPBC and FFR, shareholders, members and associated forest managers:

- A growth and yield prediction trial to underpin future model development
- An operational benchmarking or validation trial using ‘paired-plots’ of contrasting genetics
- A demonstration trial

Gain Models: The principal design is the growth, yield prediction trial which will be the foundation of a cooperative Industry programme commencing in 2012 with trials in New Zealand and Australia. This trial series will test the most advanced germplasm and current production population germplasm across a wide range of environmental conditions and examine the relationship between growth and yield and genetic improvement expressed as GF Plus ratings for growth and wood properties. Data from these new trials will enable the development of new regression models and predictive algorithms. Approximately 20 large block trials in the first series will test a common set of germplasm including OP, CP and clonal stock to provide the range of genetic improvement.

The growth and yield trials will be established under the management of the RPBC in cooperation with forest owners. Forest owners have the option to establish additional growth and yield trials to analyses results for specific site types if they wish to do so.

Gain Validation: A complimentary trial design including ‘paired-plot’ comparisons of standard control seedlots against operational plantings is provided to enable forest owners and managers to evaluate and validate genetic gain more extensively across their estates and quantify operational differences in genetic gain under comparable site and silvicultural conditions. The data from these trials can also be evaluated collectively by FFR and RPBC.

Gain Demonstration: A simpler design is provided for managers wishing to examine and demonstrate visual differences between specific genotypes that may be considered for, or deployed in forest operations.

Trial protocols and management requirements are included in this report. Standard protocols are required for several reasons: to improve the probability of successful trial outcomes, to ensure that trial data can be combined for analysis; and to inform parties of their responsibilities. One key requirement is that all growth and yield trials be managed under the same silvicultural regime to avoid the confounding effects of stocking on wood properties and log quality.

New trial designs will provide the means to quantify and predict genetic gain (yield) and performance of key traits (growth, wood properties) using common methodologies and standard genetic controls. An extensive network of trials established by RPBC and forest owners/managers across the range of site types characterising shareholder estates will provide a sound basis for validating and demonstrating genetic gain, and enable shareholders to evaluate the benefits of alternative deployment decisions.

INTRODUCTION

There is an ongoing need to demonstrate and quantify genetic gain to justify ongoing investment in genetic improvement and provide data to build and modify growth and yield prediction models which forecast product flows resulting from the deployment of new and improved germplasm. The needs range from snapshots and demonstrations of differences between genotypes, to forecasts of future wood flows, quantification of comparative yields and validation of assumptions for strategic decisions at the shareholder and national level.

The Radiata Pine Breeding Company (RPBC) and Future Forests Research (FFR) have a major interest in the implementation of a new series of genetic gain trials, and in upgrading their design. These companies have collaborated on a project to provide a common starting point to ensure that data from new experimental trials will be suitable for future model development and validation. New trials are required to predict gains for leading edge germplasm, to validate gains achieved through operational deployment of improved germplasm, and to provide demonstrations of gain for shareholders and forest managers.

Genetic gain in trials established between the 1970's and 1990's in N.Z. were primarily limited to comparisons of unimproved seedlots of unknown pedigrees (GF1 – 7) and open pollinated seed orchard seedlots (GF 8 – 20), with some early control pollinated family crosses between parents with outstanding growth rates e.g. 850-55 x 850-96 (GF23). Significantly, the current suite of genetic gain trials was installed prior to the development of the GF Plus scheme in which germplasm is rated for each trait independently. Trials to date do not include higher levels of improvement, more recent family selections, or clonal selections.

The older trial designs had also limitations: gains over unimproved seedlots have been well demonstrated, but confirmation of gains among improved seedlots are less evident and may be variably confounded by mixed genetic seedlots, silvicultural regimes, site factors, plot sizes and limited replication. This project provides an opportunity to reconsider these aspects of trial design. Specific tree measurements required to ensure that gain reflected in log and wood quality attributes can be incorporated in future models also need to be documented, so as to provide distributions as well as average values.

A spectrum of trial designs is required to meet Industry needs ranging from statistically designed well replicated trials suitable for model development, through to more basic designs that can be incorporated into routine forest operations to provide forest managers with the opportunity to measure differences in performance and yield under comparable operational conditions.

Aim

The objectives of this project report are:

a) to summarise the range and features of the existing genetic gain trial series noting their genetic and design details, limitations and scope for improvement
b) to summarise the various objectives for genetic gain trials and their implications for trial design
c) to present design criteria for three new genetic gain trials including genetic entities to be tested, trial layouts, statistical models, replications, silviculture, measurements, documentation, protocols and responsibilities.

- The principal trial (GG 1) is designed for model development and future growth and yield prediction
- An operational validation trial (GG2) design is included for routine implementation in forest operations
- A demonstration trial design (GG3) is included for simple, visual comparisons

EXISTING GENETIC GAIN TRIALS

Formal testing of genetic gains has been addressed historically through six trial series established between 1978 and 2004, summarised in Table 1 (Burger, 2011). The establishment and ownership of the trials has variously been through FRI/industry cooperatives, SCION and private forest companies (Table 2). The programme commenced with the 1978-Genetic Gains trials, based on seedlots rated using the GF system of improvement 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. Data from these trials derived from half rotation measurements also formed the basis of the genetic gain multipliers developed by Carson *et al.* (1999) used to adjust national radiata growth models which were based largely on GF7 material.

Table 1. Previous N.Z. genetic gains trial series^

Year established	Name	Sites	Reports	Description
1978-80 (84)	1978 Genetic gains trials	12	Carson *et al.* 1991, 1999; van de Colff *et al.* 2010 Witehira 1997, Witehira & Jefferson 1999	First large plot GG radiata trials; 10x10 block+ 6-tree row-plot trials
1987-1991	Silviculture Breed series	22	van der Colff *et al.* 2010, Hayes et al. 1995	Second set of large block radiata trials
1988	Australasian Breeds series	9 NZ 15 Aust.	Sorensson 1995 Low l*et al.* 2003	Demonstration trial with single or paired row plots
1992-94	Special Purpose Breeds Series	8	Low and Miller 2003	Developed for wood properties and other special purposes
2003	GF Plus international trials	18	Concheyro 2005	Single or paired row plots.
2001-2004	Response surface	~10	Dean 2005	Genotype × thin × prune factorial, large plots

^ For additional trial series details see Appendix 1

Unlike the 1978 trial series, subsequent trial series have not been reported in such a sophisticated manner. 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. This trial series should be further evaluated for the genetic gain of the highest GF Plus 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 would be complementary to the new trial series with regard to wood and log property traits in yield plots. Some sites of the GF Plus international trial series have been measured at around age 7-8 years but more data is required prior to full reporting.

The design of these prior trial series appears to have in general, been reasonably well suited to their designed purpose (M. O. Kimberley pers. comm.). However management of trials across the various companies has not been consistent, resulting in temporal gaps in the datasets. 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 Carson *et al.* (2004), and that future trials can therefore omit differing silvicultural treatments.

NEW GENETIC GAIN TRIALS: OBJECTIVES, BLOCKS, PLOTS AND APPLICATIONS

It is important to understand the applicability of different trial designs and align designs with trial objectives. There is clearly a range of requirements for genetic gain trials from quantifying the gain achieved in the breeding programme to measuring improved yields in forest operations on various site types, predicting future yields, to providing simple visual comparisons. The range of objectives and applications for the new genetic gain trials are summarised in Table 2.

Table 2. Genetic Gain trial objectives for Radiata Pine

Entity	Objective	Activity
FFR	Quantify gain from genetic improvement and develop new algorithms and models	Establish large block trials testing a wide range of genetic improvement across a wide range of site conditions
RPBC	Demonstrate long term gain and performance of leading edge germplasm and production population genotypes	Establish large block genetic gain trials with a wide range of genetic improvement aligned with FFR objectives
	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
Shareholders and Forest Managers	Compare, measure and validate differences in performance, yield and stand value	Establish 'Paired-plots' of control genotype and an operational genotype. The control may be a standard OP seedlot within a CP stand; or a control CP seedlot (or clone) within an OP stand
Shareholders and Forest Managers	Demonstrate genetic gain focusing on visual differences between genotypes	Establish easily accessed rows or blocks of contrasting genotypes

Trial objectives have a large bearing on design. Of key importance is competition between trees; for example, in small plots and single tree plot designs, the 'true' differences between seedlots of contrasting growth potential are exaggerated sooner than in large plots. In investigating genetic gains for growth, yield and (to a lesser extent) wood properties with quantifiable gains and development of significant regressions against GF Plus, it is necessary to develop designs with large to 'medium' size plots (Table 3).

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

Plot size	Purpose	Genetic entities
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+ trees, medium =64 -100 trees, and small = 6 -10 trees i.e. row plots

Measurements of rotation length yield that accurately reflect the yield potential of the seedlots under test are best obtained with replicated, large plot designs. The space occupied by trees is considerable such that large block trials tend to occupy large land areas. When appropriate replication is included, and buffers allowed for, the large area means that few seedlots (typically up about 6) can be tested, hence considerable care must be taken in the selection and formulation of those seedlots.

At the other extreme, small plots with less replication can be used for demonstration; for example, paired rows or single row plot designs, which can also be used for early age growth measurements as well as for demonstration purposes. They also appear to be appropriate for early age wood property comparisons. Smaller trials mean that it may be possible to get estimates of genetic differences 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 1), with any extant smaller plot plantings containing the same seedlots.

Future genetic gain trials can be categorized into 3 types: Growth & Yield Prediction trials, Operational Paired-Plot trials and Genetic Gain Demonstration trials. Their designs are presented in this report. The principle trial of interest to the RPBC and FFR is designed to enable development of new algorithms and predictive models (GG 1, Growth and Yield Prediction) based on a wide range of genetic improvement and a wide range of sites conditions. Two designs are included for forest managers and owners: GG 2 is a simple Paired-Plots design suitable for routine establishment and comparison of growth and yield of operational seedlots under comparable operational conditions. GG3 is a design aimed at visual demonstrations of differences between genotypes.

NEW GENETIC GAIN TRIALS: DESIGNS

Earlier genetic gain trial series largely tested different levels of GF using mixed seedlots and demonstrated the benefits of open pollinated over unimproved control germplasm (Carson *et al*. 1999, 2004, Burger 2011). Their designs and genetics do not permit the development of predictive models reflecting the range of breeding values or GF Plus values representative of improved germplasm being deployed today or into the future. The new trial designs incorporate learnings from prior trial series, requirements for model development, and levels of genetic improvement reflecting current and future deployment populations.

Trials to Develop Growth and Yield Prediction Models (GG1)

Model, Sites, Replication and Frequency

Models

Both RPBC and FFR are forward planning on a 20 to 40 year horizon with a focus on growth modelling. Predictive models derived from at least the first series of genetic gain trials will be developed from regression relationships between performance attributes (growth, yield) and genetic improvement expressed as GF Plus. DBH is the primary trait but genotypes representing top genetics for wood properties (density and stiffness) will also be included as yield will be defined, increasingly, by wood quality. Trial assessments will include log quality and the wood quality contribution to product value.

The germplasm selected for these trials will be planted in each replicate trial established over several years across a wide range of environmental conditions. It is a fundamental requirement that a wide range of genetic improvement be tested as the basis for future algorithms predicting genetic improvement. Some range of control materials tested in prior test series will not be included, for example, GF14, because the focus is on improved germplasm; GF19 will become the benchmark control germplasm against which to test the most improved genetic materials available, including clones which represent the leading edge of genetic improvement.

Sites

The historic genetic gain trials (Table 1) were established into the eight or nine *P. radiata* growing regions across N.Z., and some trials were located in Australia. RPBC has projects underway to examine the performance of genotypes, including genetic x environment interactions, in all suitable trials in N.Z. and Australia as a means of identifying key site types. A distribution of trials across different sites types will permit a comprehensive analysis of genetic gain. At this stage it is anticipated that there could be about 10 site types once the analyses have been completed and rationalised; it needs to be a manageable number for future trial installations.

FFR recently reported that the level of genetic gain achieved is greater in the higher growth rate regions of N.Z. (Kimberley and van der Colff, in prep) so it makes sense to incorporate site quality into future trial designs. Understanding the drivers of site to site differences in genetic performance will improve predictability of genetic gain in future models.

The full range of site types over which this series of genetic gain trials is established will include New Zealand and eastern Australia; trials will be established in areas of the radiata pine estate owned by shareholders of the RPBC. However, only the N.Z. trial data will be used by FFR for future model development.

Replication

Developing a regression model across a range of site types and genetics requires more replication across the *P. radiata* estate than replication within any one site type and trial location. The aim is to establish approximately 20 trials in one series with a minimum of 2 trials per site type and 3-4 replicate plots per treatment in each trial. Trials will not be allocated to site types in proportion to the area represented by each site type because relative area is not relevant to the regression analyses.

Frequency

It is expected that the first series of trials well be established within 5 years. Keeping the establishment period short will reduce the extended time required to measure all trials. However, subsequent series testing further new selections of genotypes and extending the upper range of improvement (GF Plus) will be initiated with each new breeding cycle.

Statistical Power

The limitation of the regression model approach is that it does not allow for within-site analyses of genetic gain. Different sites are required for the regression model to be representative and widely applicable. To also be able to analyse responses for specific sites types would require much larger trials (e.g., at least 6 replications of each treatment per trial) and therefore fewer trials overall, which would expose the series to consequent risks (e.g., due to partial or complete failure of trials at one or more sites).

Although trials in the proposed series theoretically could be analysed individually, the statistical power of such analyses would be poor. Based on an analysis of historic trials, for a single 4-replicate trial, a difference in mean 300 Index[1] between genetic treatments would have to exceed 10% to be judged statistically significant (least significant difference at the 5% level of significance) (Kimberley pers. comm.). However, subsets of trials in the series could provide meaningful results for specific site types. For example, data from four trials on a particular site type should detect differences of 5% between treatments[2]. For the full series of 20 trials, it is estimated that a difference greater than 2.2% between any two treatments will be statistically detectable (Kimberley pers. comm.).

Silviculture

The new growth and yield prediction trial series will be established with a common silvicultural regime across all sites. Significant effects of stocking on wood properties (Lasserre *et al*. 2004, Carson *et al*. 2004, Cown at al. 2006) mean it is important to implement a standard regime across all trials at planting and thinning. Variations in regimes between sites would inevitably confound the genetic and site effects on logs and value. Results from the 1987 Silviculture x breed trial series showed no interaction between genetics and silviculture at 8 years (Carson *et al*. 2004, Carson *et al*. 1997)

The silvicultural regime will be:

- Stocking at 1000 sph, preferably with a square spacing (3.16m x 3.16 m)
- Final crop stocking of 450 sph, unpruned. This is a requirement; no variations permitted to avoid confounding effects on wood properties

[1] 3000 Index is the stem volume mean annual increment at age 30 for a defined reference regime of 300 stems ha^{-1} (Kimberley *et al*. 2005)

[2] Note that as currently planned there will be only 2 trials per site type in one series, assuming 10 site types

- Thinning at a mean top height of 12 metres
- Access pruning prior to measurement
- Where mechanical land preparation is required, cultivation will be limited to line ripping/mounding to ensure even spacing. It is difficult to achieve even spacing with spot mounding so this land prep method is undesirable for the GG1 trials.

Genetic Requirements - Populations under Test

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 analyses. Thus the aim is once again to focus on genetic materials that demonstrate advanced levels of gain, which now includes clones, as well as RPBC seedling genotypes. Clones represent the top end of the market; they are being deployed on an operational scale and enable a wider range of genetic improvement to be tested above the GF19 control and CP families.

Control Seedlot

Three seedlots have previously been considered 'automatic inclusions' in genetic gain or seedlot trials because they provided connectivity between trials. The seedlots were climbing select GF7, GF14 (seed orchard open pollinated) and GF Plus23. More recently, the GF7 seedlot was omitted and the GF14 seedlot replaced by a bulked sample of GF19 seed. In addition, higher-rated CP seedlots were introduced as they became available, in a bulk of up to five specific 'clone-clone' crosses of specific parents.

In future genetic gain trials, connectivity with existing trial series will be maintained through the inclusion of an open pollinated, bulked GF19 seedlot. Although multiple seedlot mixes have been rated as GF19 over the years, it is very important going forward that the same GF19 seedlot be tested repeatedly in all trials to ensure connectivity between all trials.

The selected GF19 seedlot will be the genetic control in the new trials. See Appendix 2A for the genetic composition

Test Seedlots and Clonal Mixes

The importance of including a wide range of GF Plus ratings for diameter to convincingly establish quantitative differences is underscored by analyses of 1978 and 1988 genetic gains trials (van der Colff 2010, Stackpole and Stovold 2009) in these trials it was difficult to separate closely ranked seedlots for growth at the higher GF range.

The range of GF Plus values required for density or stiffness traits is less well understood. Although density was used as a selection trait in the 268 series, it has not been a key trait in the selection of seedlots for genetic gain trials. 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.

One important difference between the existing and new trial series is that genetic improvement will be expressed at GF Plus rather than GF. Each genetic entity included in the trial will be rated (GF Plus) for all traits but they will be selected specifically for their DBH rating, wood density rating, or stiffness rating.

Each CP mix will include 12 separate crosses. Mixes are preferred over single family crosses (or clones) because they tend of be more stable so the average breeding value (BV) of a mix provides for greater accuracy around the GF Plus value. There is a limit to the accuracy of BVs for a single entity. This means that the GF Plus values for single parent crosses are not suitable for the X-axis in a regression model.

There are limitations with the range of germplasm available for testing in the immediate future as seedlots and clones are limited to materials currently available from commercial suppliers (seed orchards, clonal providers). Nevertheless, it is possible to select materials that represent a reasonable range extended at the top end of genetic improvement for growth and wood properties (density and stiffness) using genotypes from the current production population and clones. Although clones are not yet rated for GF Plus, this rating will be completed shortly. It is expected that the clones will provide the top end for modelling and demonstration of improvement potential.

In summary the GG1 trials will contain 6 treatments:

- An open pollinated GF19 control (see Appendix 2A)
- 3 CP family mixes providing contrasts in improvement represented by GF Plus values for key traits, particular DBH (see Appendix 2B)
- 2 clonal mixes to increase the range of genetic improvement growth, wood density and stiffness

Field Layout

These large block trials will be randomised complete block designs and will include:

- 4 replicates (3 if the site is not large enough for 4)
- 6 common genetic treatments across all trials (extra treatments could be added at some sites)
- 10 x 10 tree blocks including a 1 tree buffer row around each block and 8x8 tree inner measurement plot
- With 4 replicates and 6 genetic treatments (including the control) planted at 1000 sph, each trial will be 2.4 ha. The inner measurement plots will needs to contain a minimum of 20 trees after thinning.
- The inner measurement plot will be laid out as a ‘constructed’ mix. In a constructed mix, whether seedlings or clones, the identity of each tree in the 8 x 8 block is known, and can be mapped. In a typical mixed seedlot, the parental identify of each tree is ‘lost’ when the seedlots are mixed prior to sowing.
- Planting a constructed mix of seedlings (or clones) requires that all seeds from contributing parent crosses, and all clones to be included in an individual genetic treatment, are grown separately and mixed only at time of planting according to the specified layout. This is a new and advantageous approach; in the usual practice the average genetic rating derived from the seedlot is known rather than the rating derived from the actual mix of trees planted because crosses are mixed prior to sowing in the nursery beds.
- The actual layout of the construct will be developed with methods used to create single tree plots (STP) in progeny trials, but these plots will not be STPs in the sense of standard STP trials.
- One key benefit of a constructed seedlot is that the performance of each genetic entity within a plot can be tracked. After thinning the average GF Plus value of the retained trees can be recalculated.
- A constructed mix for GF19 may be possible in future series but it has not been possible to include this in the current series

Measurements and Measurement Interval

Survival assessments in the inner measurement plot of all replicates will be carried out in the first growing season and trees will be numbered and mapped for subsequent assessments. A GPS start point will be recorded at the time of survival assessment and the direction of measurement will be recorded. The condition status of every tree will be recorded: poor, good excellent. The maps will enable the genetic identity of each tree to be retained after thinning.

The first measurement is planned to occur between 8 years and no later than 10 years after planting. Subsequent growth measurements could be annual around the time of anticipated peak CAI and less regular later in the rotation. The scope and timing of measurements is to be reviewed after 5 years (2017). Measurement will include the following:

- Mean top height - minimum of 12 trees x 4 reps = 48 height measurements for each genetic entry
- DBH all trees 1.4 m above ground level (64 x 4 reps = 256 trees per genetic entity
- Wood properties (density, standing tree stiffness) to provide data for juvenile/corewood (up to 8 years) and harvest age outer wood measurements (20+years)
- PSP measurements to start at age 8 years; these measures will provide ongoing survival/mortality data

It is acknowledged that core wood sampling for density in big plot trials runs the risk of influencing subsequent performance of the trees because 5 or 12 mm diameter cores damages a small portion of the cambium. Samples could be taken from buffer trees to avoid damage although the period of affected growth may be 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.

Protocols and Responsibilities

The genetic gain trial series will be planned jointly with FFR and established by RPBC in cooperation with the host company. The land for trial site preparation, planting and maintenance of the trials is to be provided by RPBC shareholder Companies. The trees planted in the trials planted will be provided by RPBC and clonal providers. Direction and supervision of establishment, ongoing maintenance, scheduling and marking for thinning will be managed by an RPBC appointee in cooperation with the hosting company.

Trial Establishment

The project is complex and a dedicated team is required to assess sites offered and liaise with companies for the best quality outcome. Success depends on a lengthy lead time for site selection and inspection against eligible criteria. Site selection a year prior to establishment will ensure that suitable land is set aside by the cooperating companies.

Plant handling, planting and weed control needs to be of excellent quality to minimise planter and weed competition effects. Trees need to be established at an even spacing; this means that rows need to be continuously mounded if cultivation is required; spot cultivation will not result in even spacing.

Other requirements

- Early contact with host companies is essential to ensure that suitable sites are selected before mechanical operations are carried out and to ensure co-ordination between the RPBC contracted personnel responsible for establishing the trials and host company managers
- A work plan is to be prepared by RPBC in advance for each trial established
- RPBC to ensure material is available and ready to plant within the optimal planting season
- Transport to trial sites is to be coordinated with the host company to ensure plant quality is not compromised prior to planting
- RPBC is responsible for mapping the genetic identify of each tree in all inner measurement plots
- The host company is required to GPS survey and protect the trial over the rotation including documenting spatial records on their GIS system

- Disease management – *Dothistroma* assessment and spraying is required by the host company for the trial if *Dothistroma* levels reach 15% infection. The family mixes for DBH materials do not have high *Dothistroma* resistance (GF Plus 11-16)

Management

- Exemplary weed control, nutrition and thinning practices to a single standard are required to minimise the introduction of environmental variability into the trials. Optimal weed control is to achieve and maintain a 'free to grow' environment for the trial trees during crop establishment (3-4 years)
- The host company is required to remove regenerating pine seedlings within first 1-2 years and continue to pull/remove regen over the establishment period (3-4 years).
- Marking for thinning is to be managed by RPBC/FFR jointly in collaboration with the host company. The identify of all retained trees is to be confirmed against the original mapped layout
- Unscheduled harvest operations are to be avoided

Operational Paired-Plot trials (GG2)

Forest managers and owners seek confirmation of genetic gain in forest operations. GG1 genetic gain trials are not well suited for routine implementation in forest operations, they may not represent the range of site conditions planted by all shareholders, and may not represent the genotypes planted or the local silvicultural regimes. Validation of gain outcomes and predictions from GG1 trials can be achieved with side-by-side (paired-plot) comparisons of different genotypes under comparable site, establishment and silvicultural regimes. Multiple objectives for the Paired-Plot trials include:

- Validating gain predicted from models derived from the GG1 trial series
- Confirming operational decisions on the level of genetic improvement being deployed various site types
- Quantifying estate wide yield and value gains associated with genetic improvement
- Evaluating the potential 'slippage' between genetic gain measured in growth and yield trials (GG1) and normal forest operations

The Operational Paired-Plot trial has been designed to be implemented at the discretion of forest owners and managers. The aim is simpler than for the GG1 growth and yield trials; it is to compare markedly different levels of genetic improvement (and investment) such as that between a standard open pollinated (OP) seedlot, for example GF19, and improved, control pollinated (CP) seedlots or clones, managed under comparable operational conditions. Managers planting predominantly CP seedlots would establish Paired-Plot trials using a standard control OP seedlot within routinely established stands of CP (or clonal) stock. A comparable block would be marked out within the CP stand at planting. Together these OP and CP blocks form a 'Paired-Plot' trial[3].

Alternatively, if the estate is primarily planted with OP seedlots the Paired-Plot trial would be reversed such that a block of a standard control CP seedlot would be planted within routinely planted OP stands.

Field Layout

The operational paired-plot trials are made up large blocks; one block is pegged out within the operational planting area while the 'paired' block is planted in the contrasting genetics (standard control). The key requirement is that both blocks are established in *the same* site and silvicultural

[3] For clarity, the trial is referred to as 'Paired-Plot' trial (generic trial type), but the 'plots' in the trial are

conditions. There is no standard requirement for block size or shape, which are at the discretion of the forest manager. However, the following considerations are relevant:

- A block size of approximately 1 ha will permit 2 or more randomly placed PSP plots to be installed. Assume a PSP plot size of 0.04 ha but larger plots could be established.
- It is straightforward to GPS survey both blocks at establishment when comparable site conditions are most clearly visible and the forester responsible for planting is available. After several years vegetative growth will obscure the ground conditions and the forester may have moved on.
- It is not necessary to establish the PSP plots at establishment; this is at the discretion of the forest manager, as is the timing of measurements.

It is tempting to establish the standard control block on the edge of the routine stand, perhaps off a landing, or somewhere easy for planting access. However, edge locations may sustain more damage during harvest operations and on closer inspection may not be a similar site condition to the majority stand area. Aerial photos provide a good check (sometimes in hindsight) of the comparative levels of disturbance within a stand.

Sites and Replication

It is expected that multiple Paired-Plot trials would be established each year as a routine part of the planting operation. The aim is to be extensive rather than intensive in their distribution, but this is at the Manager's discretion. Key requirements for Paired-Plots plot trials are:

- Representative of operational stands within a region or sub-region; perhaps comparing forest to forest or contrasting site conditions within extensive forests. It may depend on the heterogeneity of an estate and the range of site conditions planted in any one year
- In a reasonably homogeneous estate a minimum of 3 – 5 plots could be established per year per 500 or larger planning programme
- In a heterogeneous estate it would be appropriate to establish more trials; for example there could be 1 per 100 ha

Genetic Requirements - Populations under Test

- The standard control OP seedlot has to be planted repeatedly over time; preferably the same mix used as that planted in the GG1 trials
- In the 2012+ trial series the OP control is a GF19 seedlot which is an appropriate OP control for the Paired-Plot trials
- The RPBC is working with seed providers to ensure an ongoing supply of this seedlot
- A standard control CP seedlot has to be planted repeatedly over time; the GF Plus group 3 seedlot (see Appendix 2B) used in the GG1 trial series is an appropriate CP standard control.
- Unlike the GG1 trials, plots in Paired-Plot trials are not made up of 'constructed' mixes and tree positions will not mapped. These are routinely planted operational blocks of trees. The seedlots in the OP standard control mix would be combined prior to sowing just as it in normal operations. Similarly the crosses in the standard control CP mix would be combined prior to sowing.

Establishment Requirements

The pairs of blocks are established using routine operational practice; one being the routine stand the other being the comparative test block. All planting stock including the control seedlots is to be grown in local nurseries. All stock is to at least meet minimum stock planting specifications and be transported, handled and planted according to best practice.

- A difficulty may arise if the control planting stock and stock for the surrounding stand are not ready to plant the same time. It is nevertheless up to the local manager to manage the planting of the respective stock types.

- If pine regen is significant across the stand it may be appropriate to remove the regen, but if so, this must be done in both across both blocks in the 'Paired-Plot' trial. Note that high levels of pine regen may compromise the comparative estimate of genetic performance.
- GPS survey the blocks and record the trial boundaries on the GIS. The stand control may be recoded in the stand records as a separate stand given that it is a separate genotype depending on company protocols.
- Examples of Paired-Plot trial layouts are provided in Appendix 3.

Measurement and Analysis

- Survival assessment within the first growing season is recommended
- The plots provide side by side visual demonstrations of survival, growth and form at any age
- Establish PSPs in the pair of blocks as early as desired
- Measurement age and frequency is at the forest manager discretion but measurements from about age 8-10 years would align with the GG1 Trials. Comparisons through observation are recommended and can be undertaken at any age
- Performance can be examined on a trial by trial basis but the data can be collectively analysed across 'Paired-plot' trials
- RPBC and FFR would like to be able to include the data of these Paired-Plot trials in their register of genetic gain trials for future quantitative analysis at a national scale

Genetic Gain Demonstrations (GG3)

Forest managers may wish to establish Demonstration trials including several different genetic entities. The aim with Demonstrations is to provide marked visual differences using either row plots or block plots. Demonstration trials are a good way to compare specific visual traits of interest such as *Dothistroma* resistance, Red Needle Cast susceptibility, *Essigella* susceptibility, tree form, internode length or branching habit. Some extreme comparisons are needed to be certain of observing differences.

There is considerable scope and flexibility in design for Demonstration trials which can be established when and as desired with no set frequency or intensity. Site selection is also at the Manager's discretion, however to be effective, Demonstrations are best if readily accessible. They can be located along roadsides providing that areas of harvest disturbance are avoided.

Field Layout

- Double rows, or 2 rows randomly repeated across the trial, OR
- Blocks laid out in squares so 4 different genotypes can be observed standing at the central point (a corner of each block), OR
- Blocks laid out along a road edge so each genotype can be viewed relatively easily
- It may be appropriate to leave a couple of buffer rows at the road edge
- Label the ends of each row or at the corners of each block including details of the material under test so useful comparisons can be made with or without the paperwork

Genetic Requirements – Populations under Test

- Compare approximately 5-6 different materials including a control; though the maximum number is flexible.
- Compare any combination from single family crosses or clones to mixes of OP, CP, or clones.

Management

- Management is at the Manager's discretion but needs to be sufficient to ensure the trees observed and compared are those planted, and not regen.

If managers require statistical significance of differences between genotypes, Demonstration trials could be enhanced with the addition of a single tree plot trial (STP). However, be aware that single tree plot trials are not designed to be rotation length. A STP trial would be used to confirm rankings of the genotypes tested. If this is not the key objective, then the best option would be to consult with RPBC. It may be more appropriate to a establish Growth and Yield trial (GG1).

CONCLUSION

New designs for genetic gain trials for FFR, RPBC and forest owners and managers have been designed to meet different objectives, with associated responsibility and protocols. The principal new design (GG1) is aimed at providing the data requirements for models to predict gain across a wide range of site types for current and future germplasm derived from the RPBC breeding programme. Simpler designs (GG2, GG3) will enable managers to examine gain and compare alternative genotypes across their estates under operational conditions.

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APPENDICES

Appendix 1: Details of previous large block genetic gain trials

Trial series	Group	Year planted	GF range	Gf plus range	GFs of seedlots common across most sites in group	No. sites	No. seedlots per site	No. silviculture treatments	No. reps per site	Rep size (plots)
Genetic Gains	-	78-80	2-22	10-23	2, 14, 22	11	3-5	1	3-6	4-8
	RO1897	84	7-17	11-20	7, 10, 11, 14, 16, 17	1	7	4	2 or 3	14-26
Silviculture Breed	FR7-12	87	7-21	11-21	7, 13, 14, 21	6	4	6	2	24
	FR54-57	88	14-22	17-22	14, 22	4	3	6	2	8-20
	FR121/1-13	90-91	6-25	11-23	7, 13, 14, 16, 25	12	5	7	1	18-25
Special Purpose Breeds	FR172/1-6	92	7-28	11-27	7, 13, 14, 18, 27, 28	5	6	2	1, 2, or 4	4-10
	FR215/1-3	94	7-30	11-27	7, 13, 15, 18, 25, 30	3	6	2	2 (4 at 1 site)	6-17

Appendix 2A: OP (GF19) Standard Control Seedlot

GF19 (OP) standard control: Future (2013+) genetic gain trial composition (GG1, GG2, GG3).

Note: The standard control OP mix used in genetic gain trials established by RPBC in 2012 was Proseed 09/628, which as been used in RPBC and some company trials for several years. There is insufficient seed available for future genetic trials to be established by RPBC (GG1) forest owners (GG2, GG3). This seedlot will be replaced with a seed mix of similar composition (e.g.12/660) as shown below.

clone	%
268054	4%
268065	8%
268248	3%
268262	5%
268315	8%
268405	7%
268531	3%
268543	5%
268547	3%
268556	7%
875076	7%
875262	2%
875293	10%
880656	5%
880660	2%
880729	5%
880739	8%
880740	4%
880754	1%

Appendix 2B: Control Pollinated (CP) Test Seedlots

Parent crosses in three DBH test mixes for the Genetic Gain Growth and Yield Prediction trials (GG1). Group 3 is a standard CP control for GG2 trials established in OP stands.

Female	Pollen	DBH	straightness	Branch Factor	Dothistroma resistance	Wood density	Spiral Grain
DBH GF Plus group 1		**19.7**	**20**	**23**	**13**	**24.9**	**24**
268117	288402	20	21	26	6	23	26
887702	268262	17	20	21	7	26	30
880729	875293	20	23	21	18	23	33
875043	288008	20	20	23	5	28	20
880733	875076	20	20	24	22	26	20
880642	268123	20	20	23	20	25	20
268531	875066	19	18	23	7	24	26
880642	268350	20	20	24	18	21	16
875076	268262	20	21	23	18	26	28
268323	288402	19	25	25	8	21	34
268531	268007	20	16	22	7	28	21
268248	875220	20	23	22	17	28	19
DBH GF Plus group 2		**24.3**	**19**	**23**	**16**	**23.2**	**20**
268248	288101	24	20	22	8	25	18
268543	268123	24	20	21	18	22	15
880754	875242	24	16	24	23	20	20
850533	268007	23	21	25	7	26	25
268054	875242	24	18	23	25	20	20
268532	268007	24	20	23	18	21	19
880692	875220	24	20	23	21	21	22
875066	288101	25	20	25	7	27	26
875293	268123	25	19	22	22	28	26
268041	268007	25	20	23	19	21	16
880741	288304	25	18	22	7	25	24
268131	875242	24	17	22	19	22	14
DBH GF Plus group 3		**28.5**	**19**	**23**	**16**	**22.9**	**23**
268532	875242	28	18	23	25	17	20
288401	288304	29	22	25		19	27
288012	288304	29	20	23		25	26
875242	288101	29	17	24	13	27	22
288407	268609	29	18	23	12	24	34
268609	288008	29	19	22	12	30	24
288202	268609	28	20	23	12	22	17
268547	288304	28	19	23	11	22	20
288106	288304	28	20	23		22	24
268041	268609	31	18	24	24	20	19
268545	268609	28	16	21	18	23	25
875289	268123	27	16	23	21	23	19

Appendix 3: Operational Paired Plots trial examples