RPBC Trial Structure and Resource

Workshop, 9 May 2018, Rotorua
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Why do we do experiments?

We have a question and we need an answer that has statistical robustness.

Statistical robustness requires good trial design.

1. Plot Type
2. Replication
3. Design Type
4. Siting

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Plot Type

Considerations when deciding on a plot type:

- How long will the trial be used
- Will the trial need thinning
- Will destructive sampling be required
- What is the purpose of the trial
  - Breeding population
  - Species (hybrid)
  - Genetic gain
  - Demonstration
- What is the output required
  - ➢ Growth and yield over time
  - ➢ Ranking of individual genotypes

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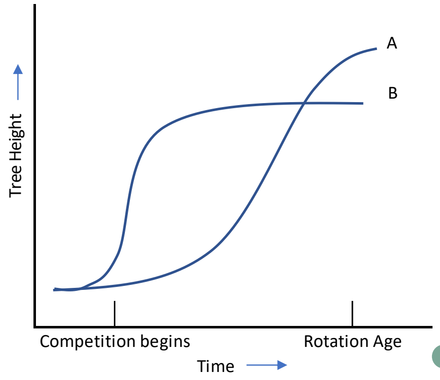
Blocks vs rows vs single tree plots

- **Blocks** are preferred when the goal is to measure yield of genetic entries per unit area / growth response over time.
- When there are large differences in the growth curves among the genetic material being tested.

   eg. Genetic gain seedlots



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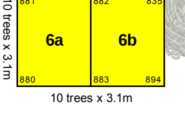
Genetic Gain Trial Layout

Rep 1	Rep 2	Rep 3	Rep 4
881 882 **GF19**	895 896 **G20**	909 910 **GF19**	923 924 937 **G20**
880 883 **Stiffness**	894 897 **G24**	908 911 **Stiffness**	922 925 936 **G24**
879 884 **Density**	893 898 **G29**	907 912 **Density**	921 926 935 **G29**
878 885 **G29**	892 899 **Density**	906 913 **G29**	920 927 934 **Density**
877 886 **G24**	891 900 **Stiffness**	905 914 **G24**	919 928 933 **Stiffness**
876 887 **G20** 875 888	890 901 **GF19** 889 902	904 915 **G20** 903 916	918 929 932 **GF19** 917 930 931

- Rep 1
- Rep 2
- Rep 3
- Rep 4



TREE POSITIONS

X X X

10	11	30	31	50	51	70	71	90	91
9	12	29	32	49	52	69	72	89	92
8	13	28	33	48	53	68	73	88	93
7	14	27	34	47	54	67	74	87	94
6	15	26	35	46	55	66	75	86	95
5	16	25	36	45	56	65	76	85	96
4	17	24	37	44	57	64	77	84	97
3	18	23	38	43	58	63	78	83	98
2	19	22	39	42	59	62	79	82	99
1	20	21	40	41	60	61	80	81	100

X X X

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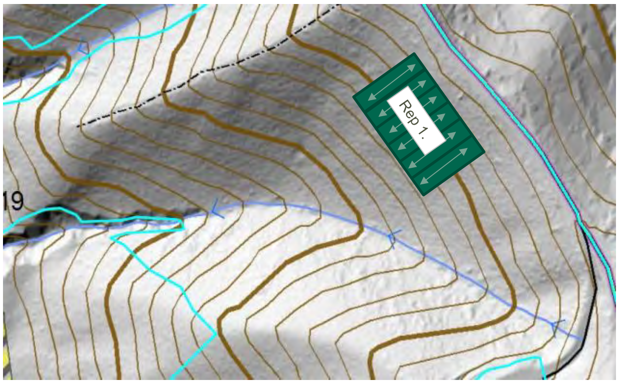
- **Row plots** are similarly **unsuitable** when there are large differences in the growth curves among the genetic material being tested.
- Suitable for demonstration or short term evaluation
- Arrangement of row plots in each replication extend along the elevational gradient



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- **Single tree plots** are the most efficient type of plot when there are many treatments and early rankings are the output.
- Increased precision on very non-uniform sites.
- Require accurate labelling in the nursery and mapping in the field.
- The effect of missing trees at measurement can be managed by spatial analysis

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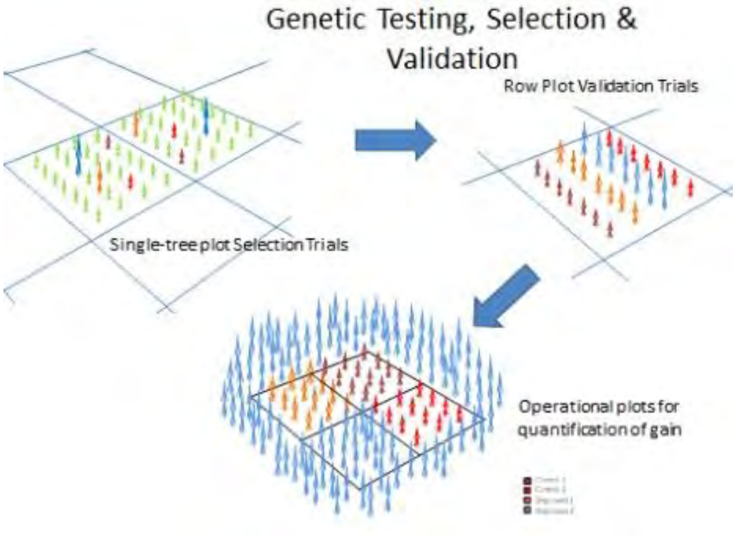

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Cost

Single tree plots	$$$$$$
Row plots	$$$
Blocks	$

However, the long term cost is greater for rows and blocks and the outcome is less

- Fewer genotypes can be tested.
- Interactions within site are greater.

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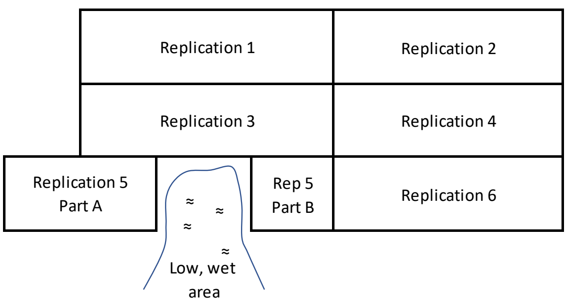
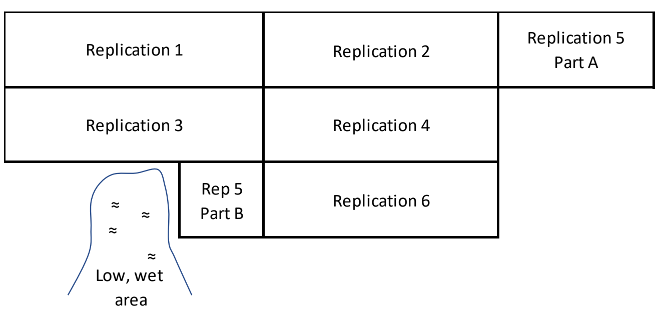
Replication

- To provide an estimate of experimental error. When there is no method of estimating experimental error, there is no way to determine whether observed differences indicate real differences or are due to inherent variability.
- To reduce the standard deviation of the treatment mean.
- More replication = more precision = more cost
- Trial size is a combination of the number of treatments, plot type and the number of replications.
- Minimise variation within replication.





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More replication per site or more sites

GxE

Trials are planted to estimate genetic and **environmental effects** on genotype performance.

Design Type

To retain a level of precision and limit trial size requires a reduction in the number of treatments or efficient trial design.

Incomplete Block Design

- This experimental design is very common in forestry when there is a large number of genetic entries.
- The complete block is subdivided into incomplete blocks.
- All treatments occur randomly within each block, a group of blocks makes up a replication.
- Treatments do not occur together in the same block across replications.
- The blocks are not assigned randomly.

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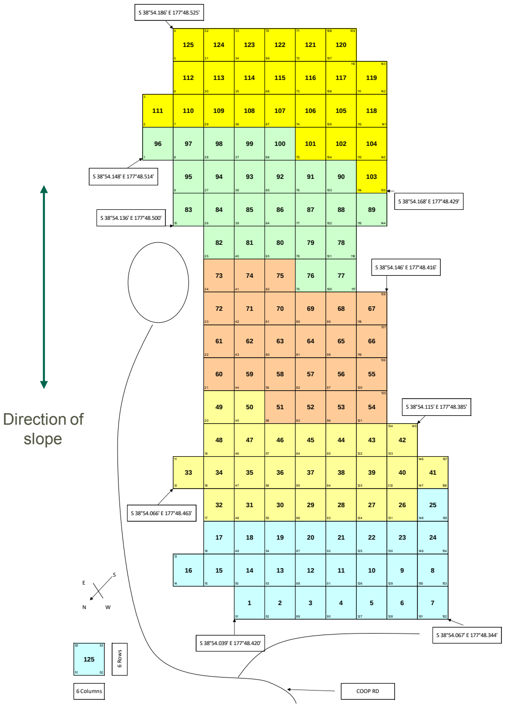

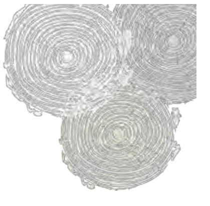

Incomplete Block Design

- 180 treatments
- 5 incomplete blocks
- 125 blocks of 36 trees
- 25 replications
- 5 blocks per replication

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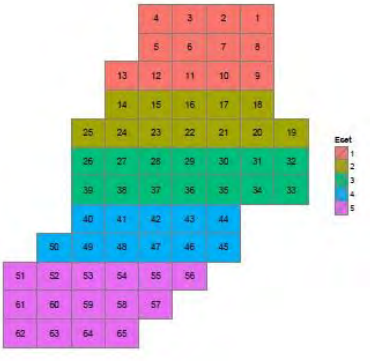
Optimal Design

- Used for trials established 2013 onwards (Cullis et al).
- Efficient use of small numbers of plants. Particularly when using ramets per clone.
- Treatments repeated across Esets.
- Location of treatments within blocks is predetermined to control placement of related genotypes.

	Eset 1	Eset2	Eset3	Eset 4	Eset5
Eset1	48	47	48	46	48
Eset2	47	47	47	45	47
Eset3	48	47	48	46	48
Eset4	46	45	46	47	47
Eset5	48	47	48	47	49

Diagonal elements are number of families per Eset, off diagonals are number of common families between Esets.



Sparse Multi Environment Trial (MET) Design

- Increase the number of sites and treatments, decrease the replication of treatments within each site
- Not all treatments are represented at every site.
- Design is compiled in 2 stages:
  - Initially allocate treatments across sites
  - Allocate treatments to plots within sites
    Rows and Columns eg. 1-6, A-F
- Maintain a high level of connectivity between sites.

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Choosing Test Sites

Forest sites are highly heterogeneous – finding a truly uniform site is like seeking the holy grail.

Some of the challenges

- Uniformity
- Access
- Aspect, altitude
- Slash, Weed problems
- Site Preparation
- Protection – wind, animals, disease

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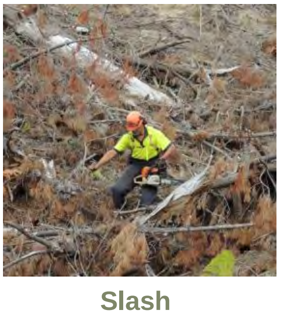
Site Preparation

Uniformity

Access



Slash

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Capturing microsite variation

- Mapping plot layout prior preparing the trial design to assist with blocking

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Maintenance

- Weed control – promote optimal growth, improve access during assessments
- Timely and thorough removal of regen– no competition, no confusion with plot trees
- Identify trial boundaries to avoid 'mishaps' during silvicultural operations
- Protection from pests and disease (unless this is a measurement trait)

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Trial types and objectives

Trial Type	Ha	Objective
Main Population	4	Generate next breeding cycle
Production Population	4	Test (backward) selected orchard parents across different site types, and improve BVs of the Production Population parents
Cloned Elites	4	Generate candidates for the Production Population from tested clones
Dothistroma Resistance	4	Provide GF+ values for Dothistroma and a validation population for the Genomics project
Genetic Gain	2.5	Demonstrate long term gain and performance of leading edge and production population genotypes
Conservation Archive	2	Retain clones of a wider group of genotypes

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Evolution of Trial Design

Incomplete Block Design	
Plant Type	Seedling
No. Treatments	100-150 families
Replication	30 trees per family
No. Blocks	100-125 blocks
No. Sites	3
Trial area	4.0ha

→ Past

Optimal/ Sparse MET Design	
Plant Type	Clone
No. Treatments	≈ 750 clones
Replication	1-5 cuttings per clone
No. Blocks	65
No. Sites	6
Trial area	2.0ha

→ Present

Genomics

→ Future

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Data collection and Analysis

The mating design, trial design and implementation are the main components in a genetic programme.

However the value of the tests depends on precise data collection and analysis

Database

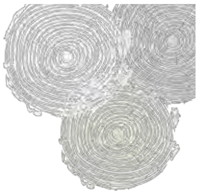
Genetic fields and design factors

Expt	Eg.BC65_1
Plantdate	Year of establishment
Tid	Unique number for the experimental unit
Tree	Used as link to pedigree, clones have the same number
Eset	Blocking structure, usually in the range 1-10
Pset	No. of blocks, 1-100
Prow	Row position, 1-6
Pcol	Column position, A-F
Tpos	Boustrophedon tree position within the block, 1-36
Family	Unique to either a mother and father, or mother in open-pollination
Fcln	Female clone
Mcln	Male clone

RPBC trial resource

The trials are the engine room of the breeding programme

Trial Type	Establishment year	Nthld	BOP/Coromadel	CNI	East Coast	Hawkes Bay	Wairarapa	Nelson/Marl	Canterbury	Otago Coast	Southland	NSW	Tasmania
Main Population	2008	•		•	•			•			•	•••	
	2009	•	•	•				•					•••
Genetic Gain	2011							•		•			
	2012	•		•				•					
	2013				•		•		•	•			
	2014												
	2015			•	••								
	2016		•			•							
Production Population	2011											•••	
	2012	•	•		•								
	2013		•	••		•							
	2014				•		•						
	2015				•			•					
	2016							•			•		
Cloned Elites	2013	•	•	••		•		•					
	2014	•		••			•						
	2015			•	•						•		
	2016	•								•		••	••
	2017		•			•						••	
Dothistroma	2014			•••									
	2015			•••									



Good Trials

Good Data

Good Genetic Gain

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