From seed to harvest – factors affecting genetic worth of a radiata pine stand over its rotation

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Nursery bed where seedlings of known genetic background can be raised to a planting age

Abstract

The genetic worth of radiata pine (*Pinus radiata*) seedlots in New Zealand can be described using the Growth & Form Plus (GF Plus™) rating system (Vincent, 1998). This rating system is issued for controlled-cross material based on the relative merit and relative parental contributions. This paper looks at how between the sowing in the nursery to the point of final harvest, the proportional representation of individual parents within the mix (and therefore the genetic worth) can change (Figure 1).

A seedlot's life-cycle affects its genetic make-up

The extent to which events in a seedlot's life-cycle affect the final genetic make-up of a stand and original rating is largely unknown. However, genetic analysis technologies are now at a point where the pedigree of individual trees can be determined using DNA markers if the potential parents are known (Telfer et al., 2015; Kess & El-Kassaby, 2015; Doerksen & Herbinger, 2008; Vidal et al., 2017).

If the parentage of a harvested stand can be determined then it will be possible to recreate a GF

Table 1: Seedlot composition

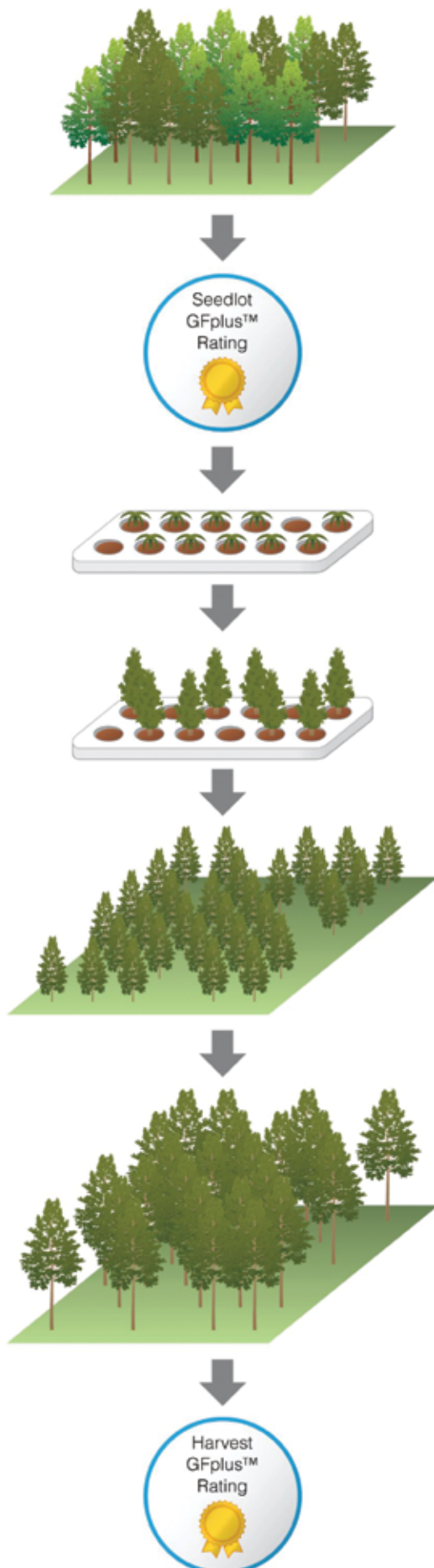| ID	Seedlot no.	Name	GF rating	GF Plus™[1]	Female parents	Male parents	Total unique parents	Total progeny collected
A	6/3/86/46	Amberley '268'	21	20.9	9	21[2]	26	143
B	9/3/86/166	870	13	17.3	4	4	5	137

[1] GF Plus™ rating generated retrospectively as the system was not in place at the time these seedlots were compiled (Grattapaglia et al, 2004) as summarised below.

[2] 1984 equal contributions pollen mix.

What is a seedlot?

A seedlot can be formed from a single genetic cross of two known parents or up to as many as 80 crosses. The genetic variation within a seedlot can vary from low to high depending on number of parents. A seedlot with a wide mix of genotypes is seen as a countermeasure to the impact of genotype by environment (G x E) (Li et al., 2017). Genetic diversity is not yet included in the classification of a seedlot (Stoehr et al., 2004) in New Zealand, but it is in other countries.

Pollination

Controlled pollination is subject to both human error and environmental contamination. Mislabelled pollen or contamination from airborne pollen can result in seeds of uncertain paternal parentage (Grattapaglia et al., 2004). Levels of mislabelled seed in the forest industry are known to range from none detected (Hansen & Kjær, 2006), up to 15-35% mislabelled material (Gömöry et al., 2000).

Germination

Germination rates vary with the mechanism of pollination. Rates of 96% have been reported for open-pollinated seed, dropping to 90% in control-pollinated seed (Rimbawanto et al. 1988). There is also evidence of genetic variation in germination rate in conifers (Wahid & Bounoua 2013; Singh et al., 2015; Stoehr & Farmer, 1986), and environmental effects (Stoehr et al., 1998), which could affect the genetic makeup of a seedlot.

Nursery/uplifting

Post-germination, selection occurs in nurseries carrying out manual lifting, with malformed or small seedlings being left behind (Dun, 1956). Other environmental factors within a nursery, such as rain-splash, feeding rabbits or birds, the presence of diseases and the quality standards of individual nurseries will affect the makeup of a seedlot before it leaves the nursery. Commercial nurseries plan for 80-85% of germinated seedlings to leave their nursery gate (Paul Keech, Nursery Manager, Scion, pers comm.)

Planting/survival

Survival rates after establishment in the field are affected by genotypes and environments, particularly susceptibility to the foliar pathogens such as Dothistroma sp,(Bulman et al., 2016). Foliar diseases can impact trees with different genetics from establishment, and trees affected by the disease may be more likely to be culled during thinning operations (Bulman, 1994; Dungey et al., 2014; Suontama et al., 2018; Ganley et al., 2014). Inbreeding depression can also influence survival rates (Wu et al., 1998; Kumar, 2004).

Silviculture

The final genetic makeup of a harvested stand depends on the type of thinning operation undertaken. For example, wood density is a desirable trait where the target is solid wood. High wood density is unfortunately negatively correlated with growth (Gapare et al., 2009; Jayawickrama, 2001), so that seedlot mixes that have an average of good growth and good wood density may have a mix of faster growing, lower density trees mixed with slower growing, higher density trees. It is not hard to imagine that thinning may drastically change the average wood density, and therefore genetic worth of a stand.

At the point of harvest, considerable impacts to the genetic worth of a stand have occurred which have the potential to alter the GFplus™ rating of a stand.

Figure 1: Points where changes to the genetic make-up of a seedlot can happen

Plus™ rating for the final harvestable crop. Comparing the changes in GF Plus™ rating from seedlot to end rotation could have important implications for our understanding of how seed selection and forest management can be better applied to maintain the maximum genetic potential of any planted forest.

What is a seedlot?

A seedlot can be formed from a single genetic cross of two known parents or up to as many as 80 crosses (the largest number of contributing parents recorded in 2017). The genetic variation within a seedlot can vary from low to high depending on the number of parents. Those with less than 10 crosses tend to have the same proportion of each cross in the mix. Seedlots that have more than 20 crosses may have a wider variation in percentage contributions to the overall mix.

A seedlot with a wide mix of genotypes is seen as a counter-measure to the impact of genotype by environment (G x E) (Li et al., 2017). Genetic diversity is not yet included in the classification of a seedlot (Stoehr et al., 2004) in New Zealand, but it is in other countries. The breeding values of the parent trees are used to calculate the genetic worth of a seedlot. However, from the time a seedlot is formed, to the harvest of trees in a specific stand, there are many factors that can change the genetics and therefore the value of a seedlot.

Comparing the changes in GF Plus™ rating from seedlot to end rotation

To illustrate the differences between two seedlots, we compared one with low and one with high genetic diversity – either thinned to waste or unthinned – to understand how seedlot ratings might change between sowing and harvest.

Methods

Genetic material

The seedlots were planted at Glengarry in 1987 as part of a larger genetic gain trial (Grattapaglia et al., 2004). Seedlot A comprised known female parents fertilised with mixed pollen from known male parents. Seedlot B was control-pollinated with known female and male parents' tissue in storage at Scion (Table 1).

Silviculture treatments

Total height was measured at two years after establishment. Experimental plots were established within the trial and underwent thinning to waste at age

five, reducing the stems per hectare from 250 to 100 (2.5 to 1 thinning ratio). The average mortality within Seedlot A up to thinning at five years after establishment was 7%, with additional losses between thinning and harvest at age 28 of 10%. Within Seedlot B, the average mortality up to thinning at age five was 6%, with additional losses between thinning and harvest at age 28 of 15%. The average loss from planting to harvest in all of the unthinned plots at age 28 year was 24%.

Who's your daddy (and your mummy)? Genotyping and parentage assignment

At age 30, actively growing cambium for DNA analysis was extracted from 5 cm diameter bark windows collected from all trees identified as height trees. Needle tissue was collected from Scion germplasm archives for the parents of trees in the Glengarry seedlots. One parent was no longer present in the archive, so we 'recreated' this genotype using the maternal megagametophyte tissue from stored seeds.

DNA was extracted, frozen and submitted to Rapid Genomics LLC in Gainesville, Florida for exome capture-based genotyping by sequencing. The resulting data was analysed using CERVUS software.

Parentage assignments were performed using sequential maternity and paternity assignments. However, where a reciprocal cross is also present in a seedlot, the markers are unable to distinguish between the two.

Results

In Seedlot A, new parentage results confidently identified 100% of the cone parents and 64% of the pollen parents. In Seedlot B, 66% of the parentage assignments were identified for both cone and pollen parents. The remaining progeny were assigned the most likely parent from within the parent trees sampled.

As only the height trees were DNA sampled, we chose to combine seedlot samples across plots within a treatment to estimate the average seedlot parental proportions. There is a chance with this approach that the estimates are slightly inflated, as generally sub-dominant and suppressed trees are not selected for height measurements.

Using identified parents from the parentage reconstruction representing a sample of the original seedlot, new end-of-rotation GF Plus™ ratings were generated for each given parent in the final ratios (Table 2).

Table 2: New end-of-rotation changes to GF Plus™ ratings for thinned and unthinned stands

Seedlot	GF Plus™* seedlot rating	GF Plus™ stand rating after 2.5 to 1 thinning ratio	GF Plus™ unthinned stand rating
A	Growth 21.1 Density 13.7	Growth 19.8 Density 12.6	Growth 20.1 Density 14.3
B[1]	Growth 17.3	Growth 15.5	Growth 15.5
[1] Not all parents in Seedlot B had GF Plus™ density values, so it was not possible to calculate a density value for Seedlot B			

Male cones, or catkins, produce pollen which can be harvested and applied directly to female cones for a controlled-genetic cross

Implications for forest owners

We were able to assign two parents to each tree in the seedlots tested, but the assignments are very reliant on the accuracy of the candidate parental genotypes. However, there were low confidence scores for some parentage assignments, which suggests 'true' parents may not be present in the collection.

We also observed a number of predicted crosses in Seedlot B that were not in alignment with the documented crosses. The Partial Least Square values for Seedlot B were also lower than for Seedlot A, even though we had a smaller number of parents from which to select.

We noted that these parents are all long internode genotype selections, which represent around 10% of New Zealand's germplasm (Hansen & Kjær, 2006). It is possible that the lower confidence scores reflect some hidden relatedness within these individuals, which is compromising the ability of the software to make confident assignments. In the future, we will explore alternative software that can improve this.

We also considered the possibility that the presence of undocumented 'selfed' seed within the seedlot could contribute to a lowering of the phenotypic performance, but the CERVUS software was set to detect self-fertilisation events and none were seen.

The GF Plus™ rating for growth seems fairly stable, similar to the findings of Kimberly et al. (2015). The actual change in the subset of height trees sampled was only a slight, non-significant decrease in the GF Plus™ rating despite the potential for life-cycle events to affect the genetic worth of a seedlot. Of particular interest is the fact that the thinning to waste regime did not appear to change the end-of-rotation GF Plus™ rating.

Interestingly, the ability to identify elite trees within a stand is feasible and offers up the potential to turn any commercial control-pollinated stand into a quantitative genetics experimental site. Additional work to improve parental reconstruction through the inclusion of a wider pool of markers, and progress towards a more accurate and complete parental genotype database, will help New Zealand forestry move towards a goal of implementing operational genomics tools.

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