

Early-height variation between full-sibling families of Sitka spruce growing in Ireland

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Abstract: Progeny trials are an essential form of genetic testing required in every tree breeding programme. In Ireland, Sitka spruce (*Picea sitchensis* (Bong.) Carr) progeny created by controlled pollinations between 41 phenotypically superior parent trees have been planted in different full-sibling trials to evaluate the genetic value of family and parent material. Establishing full-sibling field trials is an expensive and labour intensive process and regular assessment of the progeny is required to evaluate the success of such trials. This study presents the first detailed examination of the Irish Sitka spruce full-sibling field trials, comprising 69 families measured after six to seven growing seasons. From the 69 families used, 25 were significantly taller compared to unimproved control material. For the most successful trial, a mean height gain of 10% was achieved by the improved material. Regression showed that height increment over a year was partly dependent on initial height at the beginning of the year, indicating that early selection of material might be an option. Additionally, one poorly performing female parent which produced generally low yielding progeny was identified. This parent should be removed from the breeding population to avoid future wasted investment and thus to save costs within the programme.

Keywords: *Tree breeding, early selection, progeny tests.*

Introduction

Sitka spruce (*Picea sitchensis* (Bong.) Carr) is the main commercially used tree species in Irish forestry, accounting for about 52% of the total forest estate (National Forest Inventory 2012, Farrelly et al. 2009). As with most other commercially used tree species, breeders focused heavily on improving productivity. Hence, since the early 1970s, a tree breeding programme has been established for Sitka spruce in Ireland using an initial selection of 747 superior phenotypes (plus-trees) based on several morphological characters (Thompson et al. 2013). Open-pollinated seed material from 505 of those plus-trees were collected and tested in replicated progeny trials across 41 locations in Ireland (Lee et al. 2013). Based on stem form and height of the progeny after six growing seasons (9 years from seed), a re-selection was conducted, which reduced the number of parental trees to 86. As earlier tests have shown a negative relationship ($r = -0.66$, Lee 2001, Wu et al. 2007) between 15-year stem diameter and wood density, wood density was also assessed. Forty-one plus trees with rapid growth rates that did not demonstrate a decrease in wood density were then chosen as the basis of the Irish breeding population, based on the performance of their half-sibling progeny.

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As during open-pollination any male tree can be the potential gene donor, progeny (half-siblings) can be very diverse in traits like height. To reduce the degree of this variation, specific crossing of superior parents (one male $\times$ one female) is used to create full-sibling families. Half- and full-sibling progeny were deployed in trials throughout Ireland to determine the levels of genetic gain that could be achieved. This process of progeny testing is essential for genetic testing but involves high costs for monitoring and maintenance (Lowe and Van Buijtenen 1989). The number of years progeny has to be monitored varies from species to species and is between six to 15 years for Sitka spruce in Ireland (Gill 1987, Thompson 2013).

Considering the time, labour and costs necessary for the progeny to be produced and tested with the objective of finding well performing families for future propagation, regular detailed examination of existing full-sib trials is of crucial importance. A preliminary evaluation of a six year old full-sib field trial that included 34 families of Sitka spruce plus trees showed that 18 were more than 15% taller than the commercially used Washington control material (unimproved), six were 10-14% taller and an additional five were 5-9% taller (Thompson 2013). However, NATFOREX (*Establishing a National Resource of Field Trials and a Database for Research and Demonstration*, Ireland) has made all data collected in these field trials available for more detailed analysis. In the current study, data from three field trials aged six to seven years with material derived from 69 full-sibling families were analyzed to determine the potential for improving the deployed Sitka spruce material in Ireland. Additionally, as one trial was measured for height in two consecutive years, a potential dependency of height increment on initial height was investigated. If such a relationship exists, it might indicate a possibility to reduce selection age and thus decrease time and cost of producing improved material.

Materials and methods

Plant material

Forty-one superior parent trees selected during the Irish Sitka spruce breeding programme were employed in a controlled pollination programme leading to the production and harvesting of seeds from 69 full-sibling families. As seed availability was greatly dependent on flowering, not all seeds could be collected in the same year, which is why seed germination was conducted in a sequence of three successive years from 2005-2007 (11 families in 2005, 34 families in 2006 and 24 families in 2007). For each of the 69 families, 36 trees were grown in pots under greenhouse conditions for two years in Kilmacurragh, Co. Wicklow, until the material had reached a size of about 60 cm and was considered ready for field planting.

Field trials and experimental design

Three field trials were established in Ballynoe Forest located in south Ireland (52° 11' N, – 8° 30' E, 180 m a.s.l.). These sites were located close together on a moderately exposed gentle sloping afforestation site. The sites had a brown podzol soil and had been previously used for agricultural grazing. Long-term meteorological data (1981-2010, data collected by Met Éireann, Ireland) show a mean annual precipitation of 1,227 mm with the heaviest rain occurring in December and January (133.1 and 131.4 mm). Temperatures reach an average daily maximum of 18.7 °C in August and an average daily minimum of 8.2 °C in January with an annual mean daily maximum of 12.9 °C as well as an annual mean of 2,090 degree days (2007-2014).

The number of families planted in each site was determined by the amount of available seed material for the years 2005-2007, so 11 families were planted at site 1 in 2007, 34 families in 2008 at site 2 and 24 families at site 3 in 2009 (Table 1). Washington origin seedling material of the same age as the families was planted at each site as a control. This material represents seedlings that have not been through the improvement programme. Plants were arranged in a randomized block design with three (site 2 and 3) to four (site 1) replicate blocks per site, with each replicate block consisting of plots of 10 (site 1) or 12 (site 2 and 3) trees planted in a line at a 2 m spacing with one plot representing one family (see Table 1).

Measurements and data analysis

Tree height was measured at the end of 2011 for site 1. The trees on site 2 were measured in both 2011 and 2012, while those on site 3 were assessed only in 2012. One-way ANOVA was used to test for height differences between families where normality and homogeneity of variance was given. Fisher's Least Significant Differences (LSD) test was then performed to identify which families differed from each other. As the trees on site 2 were measured in two consecutive years, the potential impact of initial height on subsequent height growth could be assessed. Therefore, relationships for data from 2011 and 2012 for site 2 were assessed using linear regression analysis and relative height increment (RHI), the increase in height in 2011 from height in 2012 divided by the height in 2011.

Table 1: *Planting details for all sites.*

Site	No. of improved families	Planting date	No. of replicate blocks	No. of trees per plot	Measurement date	Age (years)
1	11	Mar. 2007	4	10	2011	7
2	34	Mar. 2008	3	12	2011 and 2012	6 and 7
3	24	Dec. 2008	3	12	2012	6

As during the production of the families used in this study certain trees were used multiple times as a male or a female parent, the contribution of specific parents towards a positive or negative influence on height performance was analysed. For this, we compared the height performance of families with a specific father or mother tree, used multiple times, with height performance of other families. All data analysis was carried out using the R statistical package (R Core Team 2013).

Results

General height performance

The size of the full-sibling families compared to Washington control material (WC) differed greatly for the three evaluated sites. For site 1, only one cross out of 11 was significantly taller than the control, having a mean height of 2.47 m (± 0.062 m) while WC was 9% smaller with 2.26 m (± 0.054 m) ($P < 0.001$, Figure 1a). One cross was shown to be significantly smaller than WC at the end of the 7th growing season (2.09 m ± 0.068 m, $P < 0.001$). Different results were found for site 2, where 34 families were present. In 2011, when trees were aged six years, 25 families were significantly taller compared to WC, with the tallest family 230 $\times$ 286 being 1.66 m (± 0.066 m, $P < 0.001$) compared to WC which was 32% smaller (1.26 m ± 0.06 m). From the nine remaining families, only 190 $\times$ 547 was found to be significantly smaller (1.07 ± 0.048 m, $P < 0.001$) than the WC. Similar results were found one year later (2012) on the same site, when the number of significantly taller families was reduced to 21, while just one significantly smaller family could be identified (190 $\times$ 547, $P < 0.001$, Figure 1b). The last site comprised of 24 families and showed similarities to site 1. Three families were found to be significantly taller, while two families showed a reduced height compared to WC ($P < 0.0001$, Figure 1c). On average, improved families were 10% taller compared to WC in 2012 at site 2 and less than 1% taller at sites 1 and 3. Overall, from 69 families used in this study 25 were significantly superior to the control (WC).

Parental contribution

As only site 2 was shown to have a substantial difference in height between WC and the improved families as well as between the improved families themselves, just this site was assessed for parental contribution. Parent 190 when used as a female parent produced four average or below average families compared to other combinations (Figure 2). One of the male parents combined with female 190, parent 547, yielded taller progeny when used in combination with 286, 500, 547 and 575. Male parent 286, for instance, was used nine times as a male parent and progeny reached heights from 1.93 m (± 0.067 m) to 2.14 m (± 0.079 m) except when combined with female 190, where an average height of 1.72 m (± 0.072 m) was observed. The two remaining male parents combined with female 190 showed similar results, producing smaller progeny than in other combinations. Even

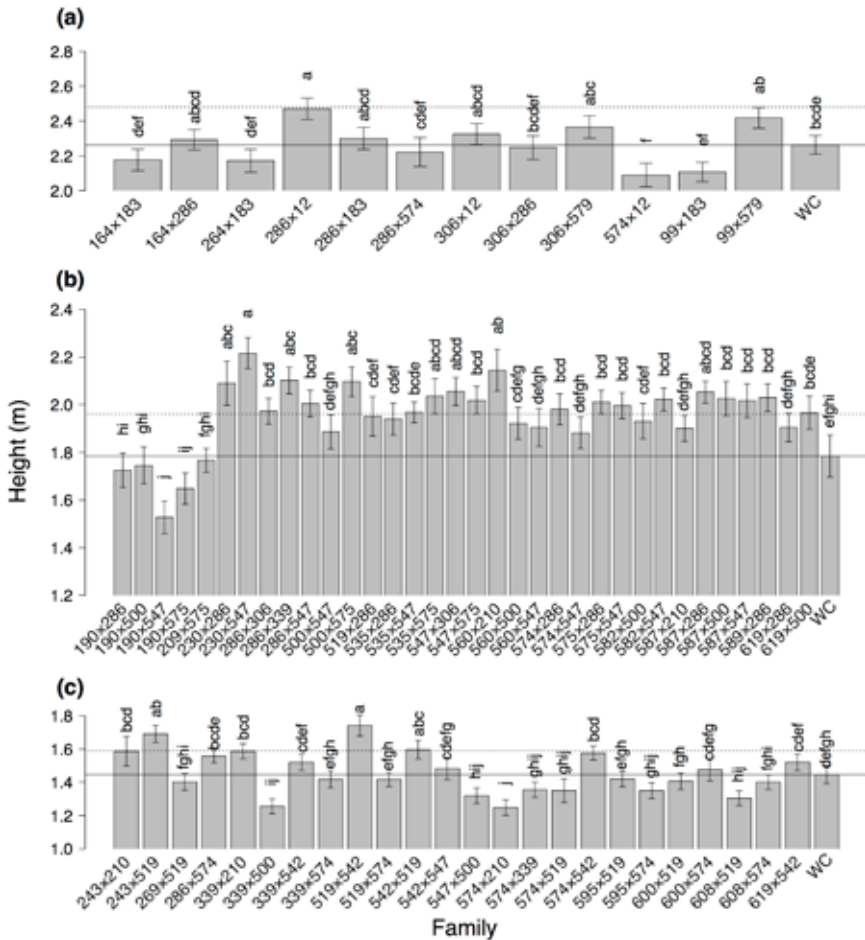


Figure 1: Height performance of improved families and control at site 1 (a), site 2 (b) and site 3 (c). The graphs show means $\pm$ standard error of the mean. Means with the same letters are not significantly different. The solid line represents the average height of control (WC) and the dotted line shows a 10% improvement compared to WC.

though male gene donors 286 and 547 were used 9 and 10 times to produce families, no specific influence on the height performance of the progeny could be found. Overall, female parent 190 could be identified to produce poorly performing progeny.

Height increment

Relative height increment (RHI) from 2011 to 2012 at site 2 was not significantly greater in any improved family compared to the control (Figure 3a). Three families (190 $\times$ 286, 209 $\times$ 575 and 230 $\times$ 286) were found to have a lower RHI ($P < 0.001$). Overall, height increment in 2012 was partly dependent on height in 2011 ($R^2 = 0.44$,

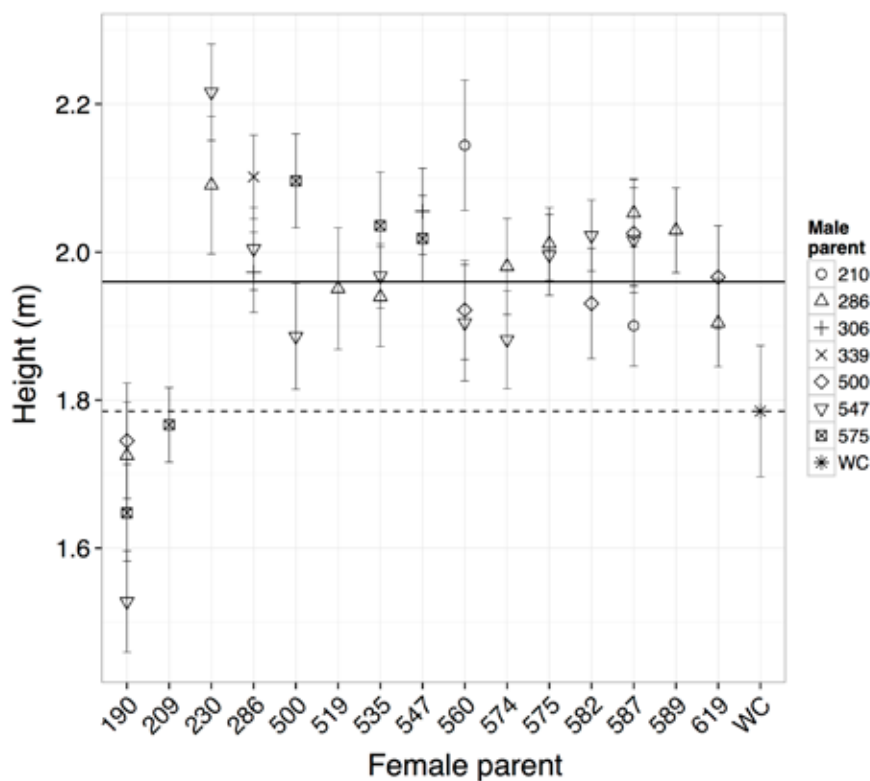


Figure 2: Height performance of site 2 material in 2012, sorted by female parent. Data points show mean $\pm$ standard error of the mean. The dotted line shows the mean height of unimproved material (WC). The solid line represents the average height of all improved families.

$P < 0.001$, Figure 3b).

Discussion

Height performance

Height differences between improved material and WC (Washington control) were present on all sites, but the number of taller families compared to WC varied greatly. Even though the difference in the number of taller families than WC per site is an important indicator of the parental selection success, finding even one superior family is an important outcome. After being shown to perform significantly better than WC, families can be recreated using controlled pollination and used for propagation and then deployed for commercial forestry. The degree of height differences is, to some extent, more important because only with a reasonable gain can commercial forestry profit from a breeding programme (Apiolaza and Greaves 2001). At site 1, the one taller family compared to WC showed an increased height of about 10% and could

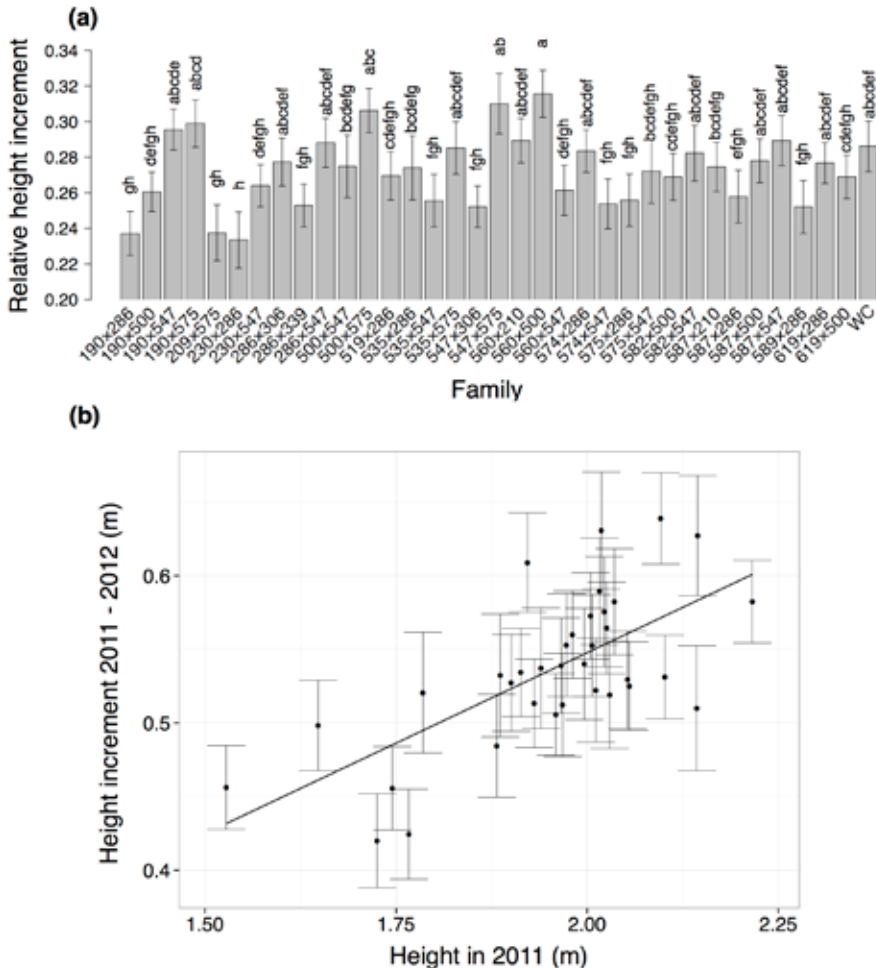


Figure 3: (a) Relative height increment for site 2 between 2011 and 2012. The graph shows means $\pm$ standard error of the mean, means with different letters are significantly different. (b) Relationship between height increment and height in 2011 for site 2. Data points show means $\pm$ standard error of the mean. The solid line represents a linear regression.

therefore be considered for inclusion in the vegetative propagation programme. However, the greater the number of superior families in a breeding programme, the higher the potential gain. At site 2, over 20 families were shown to be significantly taller than WC, reaching a height gain of over 24% for the best family. Across all families, site 2 showed an average gain of 10% in height.

Such a gain is similar or even higher than what has been achieved in other tree breeding programmes. In the USA, the *N.C. State University-Industry Cooperative Tree Improvement Program* (NCSU-ICTIP) has worked on the improvement of

loblolly pine (*Pinus taeda* L.) since the late 1950s and has predicted height gains of about 6% to 10% after eight years of growth (Li, McKeand and Weir 1999). A gain in stem diameter of 15% to 18% at the end of rotation using full-sibling families was predicted in the breeding programme in Britain (Forestry Commission 2015). A recent review from Lee et al. (2013) showed that the use of cuttings from improved Sitka spruce could increase the genetic gain in height from about 15% to 20% in Ireland, but stated that data from Irish-based field trials are needed to confirm this. The results in this study agree with this estimate, being a first and promising full-sibling analysis. It is important to note that the sites analysed in this study were not replicated over different climatic conditions within Ireland, resulting in the possibility of measured gain being over or underestimated due to a missing calculation of genotype by environment interaction. The same missing genotype by environment interaction assessment has given reason not to calculate heritability estimates, a value expressing the degree of this interaction. A high heritability would indicate that crossing certain plus-trees with high yielding progeny would consistently produce good performing progeny, independent of the planting location. As the material used in this study was not replicated, a measure of heritability would just be meaningful for this location only. Additionally, to achieve a complete assessment of the sites, more traits need to be measured (for example stem form and wood quality).

Based on the results of this study, some families derived from the parents currently used in the breeding programme can be used to increase the gain of Sitka spruce used in afforestation. However, it is important to note that while all full-sibling families are progeny of improved material, less than half showed a significant improvement over the control. This is a concern with respect to the current available “improved” material. Additionally, while Thompson (2013) pointed out that 29 out of 34 improved families at site 2 had at least 5% greater average heights compared to WC in 2011, it was found that for four of these families the difference was not significant. Moreover, over only one year, the number of improved families that were significantly taller than the control fell from 25 to 21. The trees are, however, still young and could potentially change their growth patterns at an older age.

It is also important to note that, even though site 1 and 2 were the same age at the time of measurement (seven years), site 1 progeny performs generally better than site 2. This includes WC, which was 27% taller at site 1 compared to site 2. As these sites were planted and measured in different years, it might be argued that the plants were exposed to different weather conditions, leading to different growth characteristics. However, as detailed growth data for the sites are not available, this theory could not be verified.

Height increment and parental contribution

To ensure that parents used in breeding programmes are of high genetic quality, progeny of the parent trees must be assessed to either keep or remove certain trees from the breeding programme (Thompson 2013, Hallingbäck and Jansson 2013, Lowe and Van Buijtenen 1989). To detect parents with low genetic value based on progeny performance in a breeding programme, they must be used multiple times as either male or female parent within a progeny trial. Only then is it possible to differentiate between generally bad combiners, which always produce poorly performing progeny and specifically bad or good combiners. Depending on seed production when creating full-sibling families, such an assessment of the progeny is not always possible if each parent is not involved in a number of full-sibling families as either a male or female which makes it difficult to remove badly performing parents from the programme.

In this study, female parent 190 was identified as being of low genetic value, as independent of the male parent, tree heights were generally lower compared to all other families. This information can be of use for the Irish Sitka spruce breeding programme as with the removal of mother tree 190 from the programme resources used for progeny testing or propagation could be saved. Ideally, trials need to be conducted with a wider range of male parents crossed with one female parent and *vice versa*. For example, female parent 230 produced two tall families (site 2), but more crosses with this parent are needed to confirm its genetic worth.

At the age six to seven years at site 2, no family had a significantly higher or lower relative growth rate compared to WC. Considering that 21 families after seven years of growth were significantly taller than WC, this could indicate that families establish their superiority during the early years, i.e. due to higher juvenile growth rates. This assumption is supported by the strongly significant regression between 2012 height increment and height at the end of 2011, indicating that taller families keep getting taller (Figure 3b). It would be of interest to validate this pattern over more years of growth.

Currently, it can take up to 15 years until progeny is assessed for parental selection (Gill 1987, Thompson 2013). This time period is nowadays used in the commercial programme due to concerns that additive variance (and thus heritability) declines with age. However, Gill (1987) found a strong correlation between three-year height and 27-year stem diameter in Sitka spruce and concluded that a reliable height assessment could be carried out at the age of five years. Height measured as early as three years also has been found to correlate strongly with height of older trees (Gill 1987). As rapid juvenile growth rates may be negatively correlated with wood density, height alone should not be used to assess progeny performance. Lee (1997) showed that juvenile wood density at the age of 9 years is a sufficient indicator of mature wood density.

Most studies have focused on half-sibling families. Full-sibling families are expected to show lower within-family variation since all siblings have the same two parents.

Therefore it is of value to investigate traits such as vigour and wood density at an early age in full-sibling field trials. Early selection can, if juvenile-mature correlations are significant, greatly benefit a breeding programme due to a drastic reduction in time, cost and labour spend on progeny trials. The results shown in this study indicate that superiority could be established earlier than seven years, implying the possibility of selecting full-sibling progeny early, which would greatly improve the Irish Sitka spruce breeding programme. However, more research is needed to quantify growth of full-sibling families over more years to support this theory.

Conclusion

This study represents the first detailed examination of full-sibling field trials as part of the Irish Sitka spruce breeding programme. After analysing data from three sites with a total number of 69 families, it can be concluded that most combinations of improved parents resulted in tree height either greater than or equal to that of unimproved material. One family was 24% taller than the control. Within a trial, families with one parent in common can behave quite differently. One particular female parent consistently led to below-average height of the progeny. Additionally, results showed a consistent ranking of families over 2 years of growth.

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