RESFOR

# Workshop #1

# Genomic Applications

September 16, 2020

# RES-FOR Virtual Showcase Agenda 'GENOMICS APPLICATIONS'

**Date: September 16, 2020**

**Title:** RES-FOR Virtual Showcase - Workshop 1
**Location:** Your Computer
**Date:** 9/16/2020, 9am-1pm
**Chair:** Barb Thomas **IT**: Stacy Bergheim **Speakers:** Yousry El-Kassaby & Blaise Ratcliffe

| Start | End | Time | Item | Contact |
|---|---|---|---|---|
| 9:00 AM | 9:30 AM | 30 minutes | Overview and drivers of RES-FOR project | Barb Thomas |
| 9:30 AM | 9:45 AM | 15 minutes | Genomics experts behind the RES-FOR project | Barb Thomas |
| 9:45 AM | 10:30 AM | 45 minutes | What is 'genomics' and how are the tools useful | Yousry El-Kassaby |
| 10:30 AM | 10:45 AM | 15 minutes | **Break** | **ALL** |
| 10:45 AM | 11:00 AM | 15 minutes | Ultimate goal: Genomic Selection | Yousry El-Kassaby/Barb Thomas |
| 11:00 AM | 12:00 PM | 60 minutes | RES-FOR proof of concept: New breeding values, trait selection choices & rankings - using a program called 'Shiny' for lodgepole pine and white spruce | Blaise Ratcliffe |
| 11:59 AM | 12:45 PM | 45 minutes | Options to incorporating genomics tools &/or genomic selection into your current tree improvement programs | Barb Thomas/Blaise Ratcliffe/Yousry El-Kassaby |
| 12:44 PM | 1:00 PM | 15 minutes | Questions | ALL |
| 1:00 PM | | | Adjournment | |
| **Total** | | **4 hours** | | |

## RES-FOR Background and Key Achievements, Fall 2020

Prepared by: Barb Thomas, Project Lead: bthomas@ualberta.ca and
Stacy Bergheim, Project Manager: sberghei@ualberta.ca Website: https://resfor.ualberta.ca/

The RES-FOR project, *Resilient Forests – Climate, pests and policy, genomic applications* – was funded in 2016 through the 2015 Genome Canada LSARP program – (Large Scale Applied Research Project) – for $5.6M.

1. Funding and co-funding has been diverse across sectors. There are 4 Universities, 13 investigators and 4 company partners.
2. This is a 4-year project, of which we are in the 4th year, with a roll-out of deliverables in a series of workshops starting September 16, 2020 (#1), & December 2, 2020 (#2) in conjunction with Tree Improvement Alberta, in Edmonton.

The goals of this project were to take two tree improvement programs in Alberta, one spruce (Region D1, GoA), one pine (Region C, Industry), and apply the tools and state of the art analysis to pilot the application of genomics and genomic selection. This project has also included many components well beyond just genomics such as:

- A comprehensive economic analysis with an add-on module for tree improvement to GYPSY
- A conjoint study of trait trade-offs for mill use
- An optimization study for maximizing fibre use
- Social science surveys of community perceptions to genomics technology
- Internal interviews with the members of the RES-FOR team
- Development of communication tools and scenario mapping of uptake outcomes

**The core genomics work, has included the following:**

- Sampling at 3-4 progeny trials for each program acting as the 'training populations' for development of new genomics based models
- Taking approximately 17,000 measurements of phenotypic data from the progeny trials across traditional and non-traditional traits (eg: height, wood density, resin ducts, physiology etc.)
- The DNA sequencing of the ~ 3,200 RES-FOR trees in the progeny trials (a subset of low, medium & high productivity families and progeny based on height performance at ~ age 30yrs)
- The reanalysis of all the progeny trial data incorporating an enhanced spatial analysis, and refined pedigree information that was revealed through the DNA sequencing
- Building of genomic models linking phenotype (eg: height) with genotype (ie: the genetic DNA sequence)
- Conducting a large greenhouse trial to test both drought and insect resistance in both species (ie: MPB and spruce budworm), using selected families from the programs – matched to the field trials, providing the 'validation population' for the models
- For the pine, 15 phenotypes are being analyzed in various combinations to provide new rankings for forward selections based on genomic estimated breeding values
- For the spruce, the most recent reference genome was accessed and all the data is being reanalyzed now using this additional high quality DNA sequencing information – ready later this fall.

<u>This program will provide information as follows:</u>

- Recommendations will be made for the 2nd generation forward selections in each program – based on a series of different rankings (based on selected traits) – **end-users can decide how they want to implement and can run their own scenarios for selection options**.
- Genomic models and new breeding values will be available for future selection in these programs.
- Advancement to the 3rd generation orchards can be made much more rapidly by conducting breeding of selected progeny trial trees, and then sequencing the seedlings from those crosses and applying the genomic models to predict their phenotypes – THIS IS THE STEP IN GENOMIC SELECTION THAT ELIMINATES OR REDUCES THE NEED FOR A GENERATION OF PROGENY TESTING. The selected individuals can then be grown for a period of time and subsequently grafted into an advanced 3rd generation orchard.

There is a need for understanding policy in light of these potential changes, that allows for rankings to be made through genomic based models with new genomic estimated breeding values, application of genomic selection and location, management and access to the digital DNA etc. An investment in people also needs to be recognized.

## Barb Thomas – RES-FOR Lead

Barb is a Professor in the Department of Renewable Resources at the University of Alberta (UofA), Edmonton, Canada. She holds a BSc and MSc from UBC (agriculture/forest genetics) and a PhD in Forest Biology and Management from the UofA. Prior to her current position, Barb worked in the Alberta forest industry for ~20 years as an industrial scientist working with poplars, in government policy and in knowledge exchange.

Barb came to the UofA in 2014 with an NSERC Industrial Research Chair in Tree Improvement, which is now in its second 5-year term. The research in Barb's lab is focused on answering primarily applied questions addressing challenges in tree improvement. The first term of the IRC tackled eight projects in conjunction with the forest industry and Alberta Government addressing provincial and local scale projects including:

- ✓ Determining how to increase the seed yield in a high elevation lodgepole pine orchard and understanding the impact of parent origin on cone serotiny
- ✓ Understanding ecophysiological responses to drought stress in hybrid pine to assist with selection of parents and progeny adapted to climate change (eg: drought)
- ✓ Evaluating the economic impact of the current tree improvement structure of recognizing genetic gain relative to fibre flow and corporate investments
- ✓ Understanding the underpinning of hybrid vigour in balsam poplar through disparate population breeding within a species
- ✓ Developing new models to incorporate genetic gain into growth and yield
- ✓ More fundamental research interests include understanding the trade-offs in the distribution of trembling aspen on the landscape relative to gender and resource availability. This question is being addressed through phenotypic, ecophysiological and genomic assessments.
- ✓ RES-FOR projects include the ecophysiological response of pine and spruce to drought and climate change

## Yousry El-Kassaby – RES-FOR Co-lead

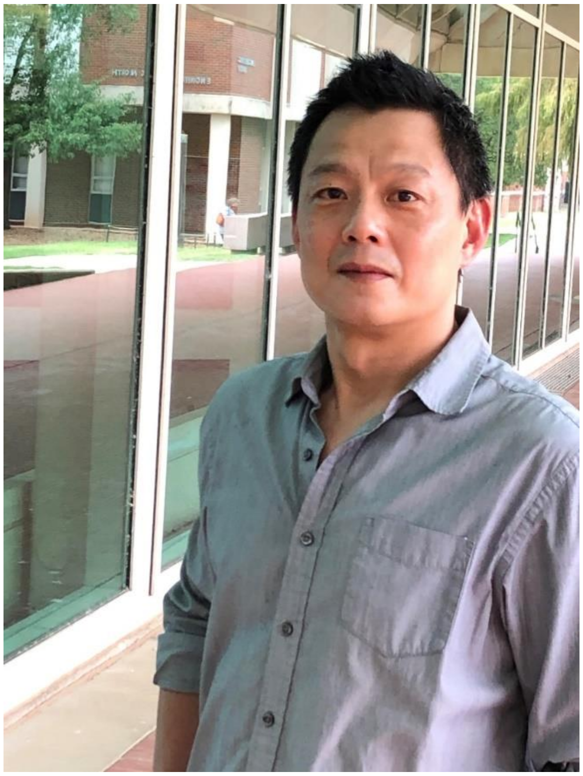
Yousry is currently a Professor and Associate Dean, Graduate and Postdoctoral Studies, Faculty of Forestry, The University of British Columbia, Vancouver, Canada. He has a PhD in population and quantitative genetics (University of British Columbia, Canada). Prior to his current post, Yousry worked in the industry as Director, Genetics and Product Development, CellFor Inc., and as Director, Applied Forest Research, Pacific Forest Products Ltd, both based in Victoria, British Columbia.

Yousry has received numerous research grants in the area of Forest Genetics and Tree Improvement and has authored and co-authored more than 250 refereed publications, book chapters and invited papers. His research interest is diverse and covers tree domestication, tree improvement delivery system, seed and seedling biology and production, gene conservation, biotechnology and genomics.

Yousry is a member of the IUFRO Extended Board and is Division 2 (Physiology and Genetics) Coordinator. He serves as an Associate Editor to the Canadian Journal of Forest Research, a member of the Editorial Review Board for the International Journal of Forestry Research. Yousry is an Adjunct Professor at Fujian University and Nanjing University (China) and the recipient of the IUFRO Scientific Achievement Award (2010) and Professor Honoris causa at the Czech University of Life Sciences Prague (Czech Republic).

- ✓ BSc Genetics (1970), MSc Quantitative Genetics (1977), PhD Population and Quantitative Genetics (1980)
- ✓ 21 years of forest industry and biotech start-up senior management experience
- ✓ 40 years of academic expertise (lecturer, Adjunct Professor, Professor, Department Head, and Associate Dean)

## Charles Chen – RES-FOR Co-applicant



- ✓ PhD from UBC in population and quantitative genetics
- ✓ Postdoc at Cornell University as a developer and statistical analyst for [GRAMENE.ORG](GRAMENE.ORG)
- ✓ Charles was also part of the largest seed bank sequencing project, Seeds of Discovery, with the Gates Foundation & the Food & Agriculture Organization of the United Nations
- ✓ Currently - Faculty at Oklahoma State University
- ✓ Leads an interdisciplinary Translational Genomics Laboratory which capitalizes on the wealth of molecular genetic knowledge, prioritizes discovery, and creates exciting translational and convergent research opportunities in public health, natural resource conservation, and agriculture.

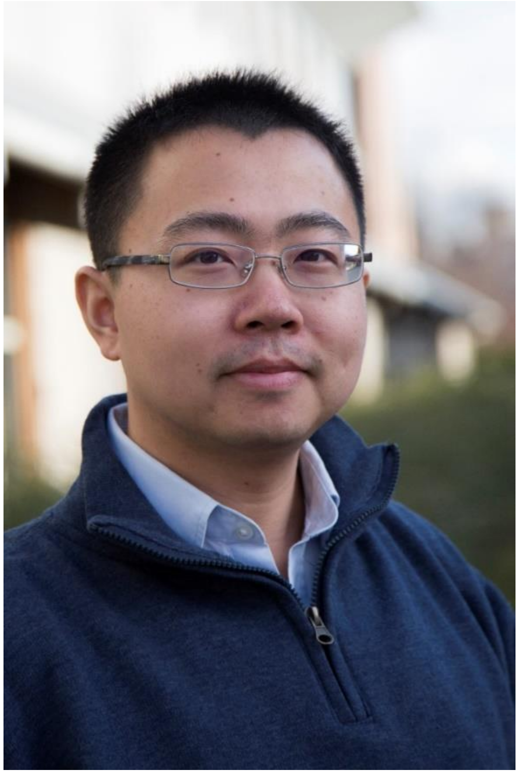
## Eduardo Pablo Cappa – RES-FOR Research Associate

- ✓ PhD (2007) in Agricultural Sciences - School of Agronomy, University of Buenos Aires, Argentina.
- ✓ He is a quantitative forest geneticist working with:
    - ✓ Mixed linear models,
    - ✓ The statistical methods and quantitative genetics for genetic evaluation and molecular information in tree breeding,
    - ✓ The Bayesian inference about parameters of linear models, and
    - ✓ The computational aspects with applications to forest genetic evaluations.
- ✓ Currently Eduardo works at the National Agricultural Technology Institute in Buenos Aires (Argentina).
- ✓ Eduardo is also an adjunct researcher of the National Scientific and Technical Research Council.
- ✓ Eduardo has 32 published papers in peer reviewed international journals, more than 70 articles in national and international conference proceedings, and is a co-author on 4 book chapters.
- ✓ He has participated in more than 15 national and international funded research projects.

## Blaise Ratcliffe – RES-FOR Postdoctoral Fellow

- ✓ Blaise Ratcliffe obtained a PhD degree from the University of British Columbia in Applied Forest Genetics.
- ✓ Since 2018, Blaise is employed as a post-doctoral fellow for Resilient Forests (RES-FOR), a large-scale genomic project between the Universities of British Columbia and Alberta and the Alberta forest industry.
- ✓ His research focuses on the integration and use of genomic information in conifer tree improvement programs. Genomic selection tools have the potential to accelerate rates of genetic gain for complex, quantitative traits through early prediction of phenotypes and increased selection intensity. These new tools enable breeding programs to respond rapidly to the changing market demands of forest products as well as emerging abiotic and biotic threats.

## Yang Lui – RES-FOR Postdoctoral Fellow



- ✓ PhD from UBC (2016)
- ✓ Postdoc at UBC in the Forest & Conservation Sciences Department
- ✓ Interested in the eco-evolutionary dynamics of plant populations that have undergone environmental heterogeneity over various spatiotemporal scales.

Yang aims to provide a holistic understanding of major episodes in plant demographic and life histories and to inform conservation interventions.

He tackles research questions at the interface of ecology and evolutionary biology, which integrates genetics and genomics to elucidate:

(1) signal and energy flux in the course of “Environment → Functional/ Life-history Traits → Ecosystem Functioning” (issues in global change); and

(2) the causes and consequences of such dynamic changes through the lens of evolution (topics in adaptive evolution, population genetics, and quantitative genomics).

# RES-FOR HIGHLIGHT #1

## Jan 2019

## *The impact of policy on the benefits of adopting genomic technology in the Alberta forest sector*

### Overview

The competitiveness of the Alberta forest sector is facing threats from multiple directions including supply constraints due to the Mountain Pine Beetle devastation and unusually high fire losses. The adoption of genomic technology and the use of improved seeds are expected to improve timber productivity in Alberta. Traditional tree breeding of boreal conifer species takes about 30 years to complete one breeding cycle, making it difficult to respond quickly to external changes (e.g., climate change, new regulations and changes in the market). With the adoption of genomic technology, the tree breeding cycle can be significantly shortened: by up to 20 years in some cases. Moreover, genomic selection delivers more accurate breeding values and allows for a higher intensity of selection than traditional breeding methods. However, the use of genomic technology will need to take place within the confines of the public-private nature of the sector where 93% of the total forest area is publicly owned. In particular, the role of the “allowable cut effect" (ACE) has significant impacts on the incentive to invest in new productivity-enhancing technologies.

### Goals & Objectives

What are the economic benefits of adopting genomic technology in the Alberta forest sector under different policy scenarios?

### Outcomes & Deliverables

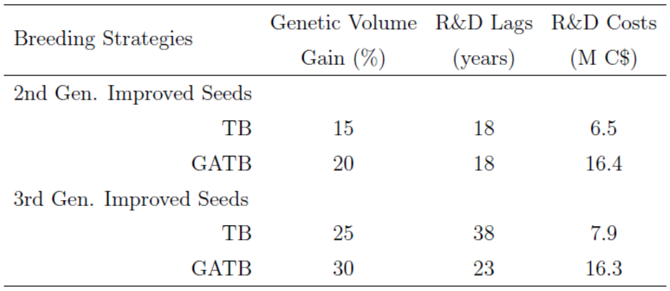
The results of this study will be useful in guiding public investment decisions on genomic research and policy decisions on the use of the ACE in Alberta. The contributions of this paper go beyond its role as a case study of adopting genomic technology in Alberta. Forest genomics research is being undertaken all over the world, especially in Canada, U.S., and Sweden where forestry is a key sector. Lessons learned from this study could provide guidance to other regions interested in adopting genomic technology on how best to formulate policy to encourage the adoption of productivity-enhancing technologies.

### Methods & Scenarios

**Two-step approach:**

1. Quantify the increase in harvest volume attributable to the use of improved seed through a timber supply simulation model.
2. Integrate the timber supply change information into a global forest product trade model which is used to measure the economic surplus gain.

**Scenarios:**



| Breeding Strategies | Genetic Volume Gain (%) | R&D Lags (years) | R&D Costs (M C$) |
|---|---|---|---|
| 2nd Gen. Improved Seeds | | | |
| TB | 15 | 18 | 6.5 |
| GATB | 20 | 18 | 16.4 |
| 3rd Gen. Improved Seeds | | | |
| TB | 25 | 38 | 7.9 |
| GATB | 30 | 23 | 16.3 |

*Table 1: Elicited parameters for different R&D (research & development) scenarios. Note: TB = traditional breeding; GATB = genomics-assisted tree breeding; Gen. = generation.*

## Conclusions

1. It is beneficial to use the ACE policy instrument to encourage investment in tree improvement in Alberta because the simulated ACEs in all breeding scenarios are positive (Table 2).

| Scenarios | AAC(M $m^3$) | ACE(M $m^3$) | Timber supply change (%) | SWL supply change (%) |
|---|---|---|---|---|
| Baseline | 18.5 | 0 | 0.0 | 0.0 |
| BAU | 19.2 | 0.7 | 3.8 | 3.8 |
| 2nd Gen. Improved Seeds | | | | |
| TB | 20.7 | 2.2 | 11.9 | 11.9 |
| GATB | 21.3 | 2.8 | 15.1 | 15.1 |
| 3rd Gen. Improved Seeds | | | | |
| TB | 22.6 | 4.1 | 22.2 | 22.2 |
| GATB | 23.3 | 4.8 | 25.9 | 25.9 |

*Table 2: Simulated allowable cut effects (ACEs). Note: Gen.= generation; TB = traditional breeding; GATB = genomics-assisted tree breeding.*
*Note: The R&D lag for the 2nd Gen. is 18 yrs for TB & GATB, and 38 yrs for TB & 23 yrs for GATB in the 3rd Gen.*

2. Using 3rd generation improved seeds produced through genomics-assisted tree breeding can generate significantly more economic benefits, indicating that the main driving factor of the economic returns is the time saved during the breeding process (Figure 1).

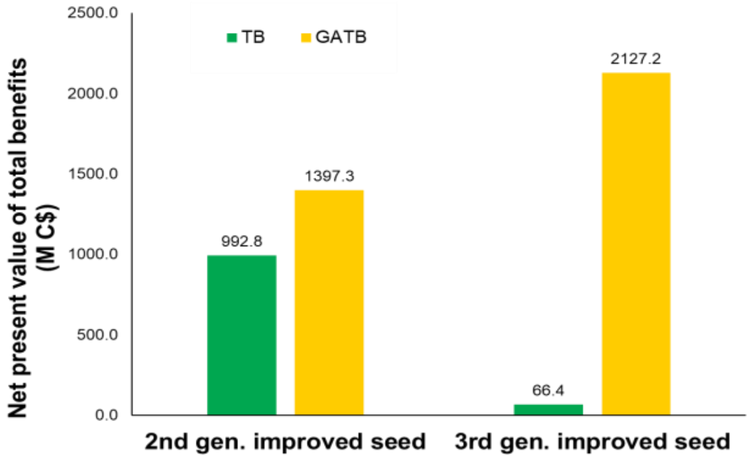


*Figure 1: Discounted total benefits of adopting improved seed produced using TB and GATB, 2016-2053, r=4%.*

3. The size and opportunity to realize the economic benefit of adopting genomic technology is controlled by government policy (i.e., allowable deployment area). In certain scenarios, the economic benefits associated with GATB are not able to offset the R&D costs (Table 3).

| Adoption rate (%) | Genetic Volume Gain at Rotation (%) | | | | | |
|---|---|---|---|---|---|---|
| | 6 | 10 | 15 | 20 | 25 | 30 |
| 100 | 126 | 325 | 724 | 1031 | 1741 | 2127 |
| 80 | 28 | 171 | 518 | 677 | 1197 | 1516 |
| 60 | -16 | 28 | 325 | 416 | 724 | 983 |
| 40 | -113 | -69 | 126 | 225 | 325 | 472 |

*Table 3: Discounted economic returns (Millions of C$), under various genetic volume gains and adoption rates (i.e., allowable deployment area) with the 3rd gen. GATB improved seeds (2016-2053, r=4%).*

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# RES-FOR HIGHLIGHT #2

## March 2019

## *Economic Evaluations of Tree Improvement for Planted Forests: A Systematic Review*

### Overview

Planted forests play an important role in sustainable forest management and can help to fulfil a wide variety of social, economic, and environmental objectives. As planted forests become more common, it makes economic sense to improve the quality of seed and subsequent seedling stock used, which includes planting improved (superior) trees rather than planting unimproved seed and seedling stock year after year. Using improved seedling stock also creates economic incentives for investors to pursue plant domestication and tree improvement activities to capture the benefits of these improvements and innovations.

Tree improvement is the application of genetic principles to increase the value of trees. Many traditional breeding programs around the world were initiated in the 1950s and involved selecting superior (plus) trees, breeding or simply collecting seed from these selected trees, and testing the progeny for the desired traits. Since the 1990s, biotechnologies, such as tissue culture, cloning, marker assisted selection, and genetic modification/transgenics programs, have been developed and introduced to forestry and more recently, the use of genomic selection. While the use of biotechnology has the potential to improve trees by enabling foresters to achieve gains in selected traits more quickly and effectively, such as greater volumes or better wood quality, the key question before any specific program can be implemented is "*What exactly will the benefits be*?" Private woodlot owners and investors may be more interested in the potential (financial) profitability of the program, while policy makers will also consider the societal benefits.

### Research Goal

In order to consolidate the knowledge that exists about the financial and societal benefits generated from tree improvement programs, we conducted a systematic review of existing literature on the economic analysis of employing tree improvement as a management option for planted forests.

### Research Questions

1. Are there economic/financial benefits of using improved reforestation stock from tree breeding and improvement programs over the use of unimproved reforestation stock?

2. What are the most common evaluation methods for assessing the economic returns of using improved planting stock for planted forests?

3. What are the knowledge gaps in the existing literature, and what are the challenges and issues that may affect the economic evaluation of employing tree improvement for planted forests?

## Document Acquisition

In total, 15 studies were identified through systematic web-based searches. The studies were located in seven countries, including Canada (5 studies), the United States (1), Finland (3), Sweden (2), the United Kingdom (2), New Zealand (1), and Australia (1). Under the category of tree species, most studies (14 articles, >90%) focused on the examination of softwood species and only one study focused on a hardwood species. Among all of the studies investigated, the majority (11 articles, or 78%) focused on the economic analysis of the use of improved tree stock generated from conventional seed orchards versus unimproved material (wild seeds); four studies (22%) investigated the financial incentives in comparisons of alternative tree breeding strategies (e.g., seed orchard, rooted cuttings, or genetic marker approaches) for planted forests. For the economic evaluation methods, cost-benefit analysis is the primary empirical approach for estimating the economic effects of tree improvement for planted forests, which was employed in all of these studies and tended to focus on assessing the derived market (financial) benefits.

## Main Findings

1. Tree improvement is an effective tool to improve forest productivity and to realize financial returns.
2. Economic gains from wood production with selection for breeding traits (e.g., high volume yield or height growth) are the main reasons forest managers adopt new biotechnologies in tree improvement.
3. Cost-benefit analysis is the primary empirical approach for estimating the economic effects of tree improvement for planted forests.
4. There is very little literature on estimating the non-market benefits (e.g., improved watershed protection, amenities, or conservation of genetic diversity) that tree improvement brings using non-market valuation techniques.

## Conclusion and Future Prospects

*We suggest that future research should:*

1. Consider the additional benefit, extra research and development costs, and time saved by applying new biotechnologies in tree improvement (e.g., genomics-assisted tree breeding leading to genomic selection) in the cost-benefit analysis;
2. Investigate the trade-offs between timber volume and wood quality traits and assess the economic effects of new biotechnologies in tree improvement along different stages of the forestry supply chain; and
3. Explicitly account for the non-market trait values for the targeted breeding traits (e.g., drought/pest resistance) so that tree improvement programs can contribute to sustainable production systems.

Economic analyses along these lines could help policy makers, forest managers, and forest company owners better understand the trade-offs of alternative breeding objectives and make economically efficient investment decisions for planted forests.

## Reference

Chang, W.-Y., Wang, S., Gaston, C., Cool, J., An, H. and Thomas, B. R. 2019. Economic evaluation of tree improvement for planted forests: A systematic review. *BioProducts Business* **4**(1): 1-14.

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# RES-FOR HIGHLIGHT #3

## March 2019

## *A Financial Analysis Using Improved White Spruce and Lodgepole Pine Planting Stock in Alberta: Genomic Selection* vs. *Traditional Breeding*

## Introduction

Genomics-assisted tree breeding (GATB) is an emerging biotechnology method that has the potential to produce improved planting stock in selected traits, such as greater volume or higher wood quality, more quickly and effectively compared to using traditional breeding (TB) methods. In this study, we conducted an *ex-ante* stand-level financial benefit-cost analysis that is linked to a provincial tree growth and yield projection (GYPSY) model to investigate the potential financial benefits of using improved white spruce (*Picea glauca*) and lodgepole pine (*Pinus contorta var. latifolia*) planting stock identified via alternative tree improvement strategies (i.e. GATB *vs* TB methods) for forest companies in the province of Alberta, Canada.

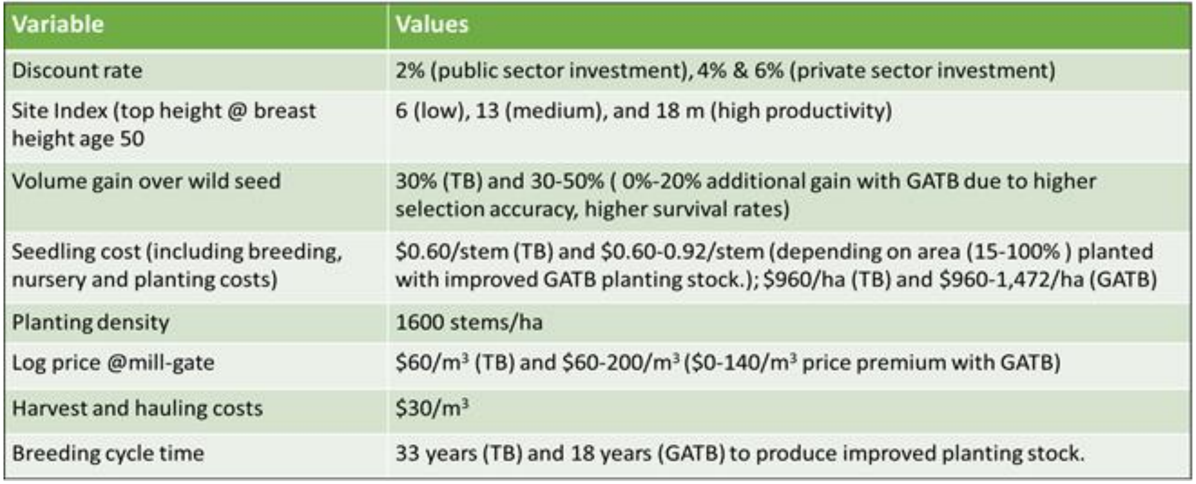
## Goals and Objectives

1. Investigate whether there are additional financial benefits of planting stock derived from selecting parents using the GATB approach compared with selecting parents for planting stock using the traditional field-based tree breeding method for 3rd generation improved white spruce and lodgepole pine programs in Alberta.
2. Develop a decision matrix to help forest managers financially justify the use of the GATB method under varying biophysical and market circumstances, including: (1) various levels of site productivity; (2) higher genetic gain in volume at rotation as a result of higher genomic selection accuracy; (3) a shorter breeding cycle; (4) a change in potential seedling costs for GATB planting stock at the time of planting; and (5) a potential price premium for better- quality logs at harvest.

## Financial model, Data and Assumptions

For comparison, a Faustmann formula was used to determine the maximum land expectation value (*LEV*) generated by each breeding strategy at the optimal economic rotation age.

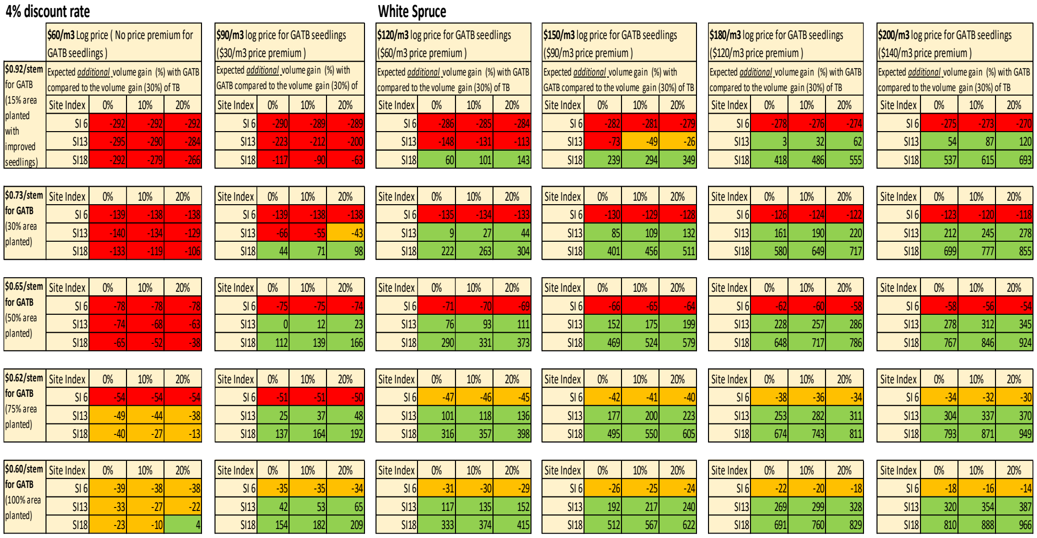
**Table 1.** Values of input variables used in the financial model.



| Variable | Values |
|---|---|
| Discount rate | 2% (public sector investment), 4% & 6% (private sector investment) |
| Site Index (top height @ breast height age 50 | 6 (low), 13 (medium), and 18 m (high productivity) |
| Volume gain over wild seed | 30% (TB) and 30-50% ( 0%-20% additional gain with GATB due to higher selection accuracy, higher survival rates) |
| Seedling cost (including breeding, nursery and planting costs) | \$0.60/stem (TB) and \$0.60-0.92/stem (depending on area (15-100% ) planted with improved GATB planting stock.); \$960/ha (TB) and \$960-1,472/ha (GATB) |
| Planting density | 1600 stems/ha |
| Log price @mill-gate | \$60/m³ (TB) and \$60-200/m³ (\$0-140/m³ price premium with GATB) |
| Harvest and hauling costs | \$30/m³ |
| Breeding cycle time | 33 years (TB) and 18 years (GATB) to produce improved planting stock. |

## Results

**Table 2.** Financial justification for using genomics-assisted tree breeding (GATB) derived planting stock relative to planting stock derived from traditional breeding (TB) method. A red cell represents an additional loss of more than CAD\$-50/ha; a yellow cell represents an additional loss between \$0 and \$-50/ha; and a green cell indicates an additional benefit (greater than \$0) of using GATB seedlings compared with the net present value of using TB seedlings at a 4% discount rate for both white spruce (top) and lodgepole pine (bottom).



**4% discount rate** **White Spruce**

**$60/m3** Log price ( No price premium for GATB seedlings )

Expected *additional* volume gain (%) with GATB compared to the volume gain (30%) of TB

| | Site Index | 0% | 10% | 20% |
|---|---|---|---|---|
| **$0.92/stem** for GATB (15% area planted with improved seedlings) | SI 6 | -292 | -292 | -292 |
| | SI13 | -295 | -290 | -284 |
| | SI18 | -292 | -279 | -266 |
| **$0.73/stem for GATB** (30% area planted) | SI 6 | -139 | -138 | -138 |
| | SI13 | -140 | -134 | -129 |
| | SI18 | -133 | -119 | -106 |
| **$0.65/stem for GATB** (50% area planted) | SI 6 | -78 | -78 | -78 |
| | SI13 | -74 | -68 | -63 |
| | SI18 | -65 | -52 | -38 |
| **$0.62/stem for GATB** (75% area planted) | SI 6 | -54 | -54 | -54 |
| | SI13 | -49 | -44 | -38 |
| | SI18 | -40 | -27 | -13 |
| **$0.60/stem for GATB** (100% area planted) | SI 6 | -39 | -38 | -38 |
| | SI13 | -33 | -27 | -22 |
| | SI18 | -23 | -10 | 4 |

**$90/m3** log price for GATB seedlings ($30/m3 price premium )

Expected *additional* volume gain (%) with GATB compared to the volume gain (30%) of

| | Site Index | 0% | 10% | 20% |
|---|---|---|---|---|
| **$0.92/stem** for GATB (15% area planted with improved seedlings) | SI 6 | -290 | -289 | -289 |
| | SI13 | -223 | -212 | -200 |
| | SI18 | -117 | -90 | -63 |
| **$0.73/stem for GATB** (30% area planted) | SI 6 | -139 | -138 | -138 |
| | SI13 | -66 | -55 | -43 |
| | SI18 | 44 | 71 | 98 |
| **$0.65/stem for GATB** (50% area planted) | SI 6 | -75 | -75 | -74 |
| | SI13 | 0 | 12 | 23 |
| | SI18 | 112 | 139 | 166 |
| **$0.62/stem for GATB** (75% area planted) | SI 6 | -51 | -51 | -50 |
| | SI13 | 25 | 37 | 48 |
| | SI18 | 137 | 164 | 192 |
| **$0.60/stem for GATB** (100% area planted) | SI 6 | -35 | -35 | -34 |
| | SI13 | 42 | 53 | 65 |
| | SI18 | 154 | 182 | 209 |

**$120/m3** log price for GATB seedlings ($60/m3 price premium )

Expected *additional* volume gain (%) with GATB compared to the volume gain (30%) of TB

| | Site Index | 0% | 10% | 20% |
|---|---|---|---|---|
| **$0.92/stem** for GATB (15% area planted with improved seedlings) | SI 6 | -286 | -285 | -284 |
| | SI13 | -148 | -131 | -113 |
| | SI18 | 60 | 101 | 143 |
| **$0.73/stem for GATB** (30% area planted) | SI 6 | -135 | -134 | -133 |
| | SI13 | 9 | 27 | 44 |
| | SI18 | 222 | 263 | 304 |
| **$0.65/stem for GATB** (50% area planted) | SI 6 | -71 | -70 | -69 |
| | SI13 | 76 | 93 | 111 |
| | SI18 | 290 | 331 | 373 |
| **$0.62/stem for GATB** (75% area planted) | SI 6 | -47 | -46 | -45 |
| | SI13 | 101 | 118 | 136 |
| | SI18 | 316 | 357 | 398 |
| **$0.60/stem for GATB** (100% area planted) | SI 6 | -31 | -30 | -29 |
| | SI13 | 117 | 135 | 152 |
| | SI18 | 333 | 374 | 415 |

**$150/m3** log price for GATB seedlings ($90/m3 price premium )

Expected *additional* volume gain (%) with GATB compared to the volume gain (30%) of TB

| | Site Index | 0% | 10% | 20% |
|---|---|---|---|---|
| **$0.92/stem** for GATB (15% area planted with improved seedlings) | SI 6 | -282 | -281 | -279 |
| | SI13 | -73 | -49 | -26 |
| | SI18 | 239 | 294 | 349 |
| **$0.73/stem for GATB** (30% area planted) | SI 6 | -130 | -129 | -128 |
| | SI13 | 85 | 109 | 132 |
| | SI18 | 401 | 456 | 511 |
| **$0.65/stem for GATB** (50% area planted) | SI 6 | -66 | -65 | -64 |
| | SI13 | 152 | 175 | 199 |
| | SI18 | 469 | 524 | 579 |
| **$0.62/stem for GATB** (75% area planted) | SI 6 | -42 | -41 | -40 |
| | SI13 | 177 | 200 | 223 |
| | SI18 | 495 | 550 | 605 |
| **$0.60/stem for GATB** (100% area planted) | SI 6 | -26 | -25 | -24 |
| | SI13 | 192 | 217 | 240 |
| | SI18 | 512 | 567 | 622 |

**$180/m3** log price for GATB seedlings ($120/m3 price premium )

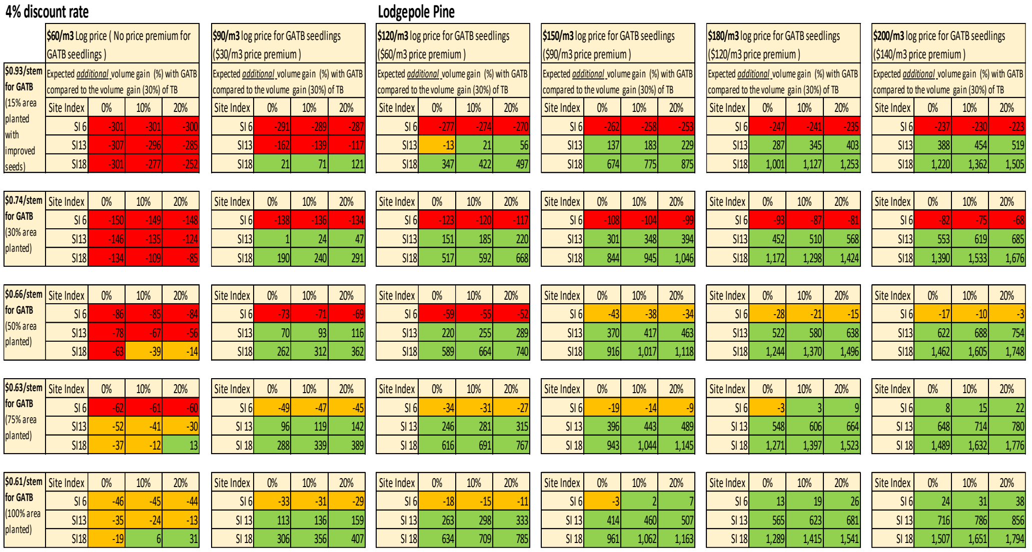
Expected *additional* volume gain (%) with GATB compared to the volume gain (30%) of TB

| | Site Index | 0% | 10% | 20% |
|---|---|---|---|---|
| **$0.92/stem** for GATB (15% area planted with improved seedlings) | SI 6 | -278 | -276 | -274 |
| | SI13 | 3 | 32 | 62 |
| | SI18 | 418 | 486 | 555 |
| **$0.73/stem for GATB** (30% area planted) | SI 6 | -126 | -124 | -122 |
| | SI13 | 161 | 190 | 220 |
| | SI18 | 580 | 649 | 717 |
| **$0.65/stem for GATB** (50% area planted) | SI 6 | -62 | -60 | -58 |
| | SI13 | 228 | 257 | 286 |
| | SI18 | 648 | 717 | 786 |
| **$0.62/stem for GATB** (75% area planted) | SI 6 | -38 | -36 | -34 |
| | SI13 | 253 | 282 | 311 |
| | SI18 | 674 | 743 | 811 |
| **$0.60/stem for GATB** (100% area planted) | SI 6 | -22 | -20 | -18 |
| | SI13 | 269 | 299 | 328 |
| | SI18 | 691 | 760 | 829 |

**$200/m3** log price for GATB seedlings ($140/m3 price premium )

Expected *additional* volume gain (%) with GATB compared to the volume gain (30%) of TB

| | Site Index | 0% | 10% | 20% |
|---|---|---|---|---|
| **$0.92/stem** for GATB (15% area planted with improved seedlings) | SI 6 | -275 | -273 | -270 |
| | SI13 | 54 | 87 | 120 |
| | SI18 | 537 | 615 | 693 |
| **$0.73/stem for GATB** (30% area planted) | SI 6 | -123 | -120 | -118 |
| | SI13 | 212 | 245 | 278 |
| | SI18 | 699 | 777 | 855 |
| **$0.65/stem for GATB** (50% area planted) | SI 6 | -58 | -56 | -54 |
| | SI13 | 278 | 312 | 345 |
| | SI18 | 767 | 846 | 924 |
| **$0.62/stem for GATB** (75% area planted) | SI 6 | -34 | -32 | -30 |
| | SI13 | 304 | 337 | 370 |
| | SI18 | 793 | 871 | 949 |
| **$0.60/stem for GATB** (100% area planted) | SI 6 | -18 | -16 | -14 |
| | SI13 | 320 | 354 | 387 |
| | SI18 | 810 | 888 | 966 |



**4% discount rate** **Lodgepole Pine**

**$60/m3** Log price ( No price premium for GATB seedlings )

Expected *additional* volume gain (%) with GATB compared to the volume gain (30%) of TB

| | Site Index | 0% | 10% | 20% |
|---|---|---|---|---|
| **$0.93/stem for GATB** (15% area planted with improved seeds) | SI 6 | -301 | -301 | -300 |
| | SI13 | -307 | -296 | -285 |
| | SI18 | -301 | -277 | -252 |
| **$0.74/stem for GATB** (30% area planted) | SI 6 | -150 | -149 | -148 |
| | SI13 | -146 | -135 | -124 |
| | SI18 | -134 | -109 | -85 |
| **$0.66/stem for GATB** (50% area planted) | SI 6 | -86 | -85 | -84 |
| | SI13 | -78 | -67 | -56 |
| | SI18 | -63 | -39 | -14 |
| **$0.63/stem for GATB** (75% area planted) | SI 6 | -62 | -61 | -60 |
| | SI13 | -52 | -41 | -30 |
| | SI18 | -37 | -12 | 13 |
| **$0.61/stem for GATB** (100% area planted) | SI 6 | -46 | -45 | -44 |
| | SI13 | -35 | -24 | -13 |
| | SI18 | -19 | 6 | 31 |

**$90/m3** log price for GATB seedlings ($30/m3 price premium )

Expected *additional* volume gain (%) with GATB compared to the volume gain (30%) of TB

| | Site Index | 0% | 10% | 20% |
|---|---|---|---|---|
| **$0.93/stem for GATB** (15% area planted with improved seeds) | SI 6 | -291 | -289 | -287 |
| | SI13 | -162 | -139 | -117 |
| | SI18 | 21 | 71 | 121 |
| **$0.74/stem for GATB** (30% area planted) | SI 6 | -138 | -136 | -134 |
| | SI13 | 1 | 24 | 47 |
| | SI18 | 190 | 240 | 291 |
| **$0.66/stem for GATB** (50% area planted) | SI 6 | -73 | -71 | -69 |
| | SI13 | 70 | 93 | 116 |
| | SI18 | 262 | 312 | 362 |
| **$0.63/stem for GATB** (75% area planted) | SI 6 | -49 | -47 | -45 |
| | SI 13 | 96 | 119 | 142 |
| | SI 18 | 288 | 339 | 389 |
| **$0.61/stem for GATB** (100% area planted) | SI 6 | -33 | -31 | -29 |
| | SI 13 | 113 | 136 | 159 |
| | SI 18 | 306 | 356 | 407 |

**$120/m3** log price for GATB seedlings ($60/m3 price premium )

Expected *additional* volume gain (%) with GATB compared to the volume gain (30%) of TB

| | Site Index | 0% | 10% | 20% |
|---|---|---|---|---|
| **$0.93/stem for GATB** (15% area planted with improved seeds) | SI 6 | -277 | -274 | -270 |
| | SI13 | -13 | 21 | 56 |
| | SI18 | 347 | 422 | 497 |
| **$0.74/stem for GATB** (30% area planted) | SI 6 | -123 | -120 | -117 |
| | SI13 | 151 | 185 | 220 |
| | SI18 | 517 | 592 | 668 |
| **$0.66/stem for GATB** (50% area planted) | SI 6 | -59 | -55 | -52 |
| | SI13 | 220 | 255 | 289 |
| | SI18 | 589 | 664 | 740 |
| **$0.63/stem for GATB** (75% area planted) | SI 6 | -34 | -31 | -27 |
| | SI 13 | 246 | 281 | 315 |
| | SI 18 | 616 | 691 | 767 |
| **$0.61/stem for GATB** (100% area planted) | SI 6 | -18 | -15 | -11 |
| | SI 13 | 263 | 298 | 333 |
| | SI 18 | 634 | 709 | 785 |

**$150/m3** log price for GATB seedlings ($90/m3 price premium )

Expected *additional* volume gain (%) with GATB compared to the volume gain (30%) of TB

| | Site Index | 0% | 10% | 20% |
|---|---|---|---|---|
| **$0.93/stem for GATB** (15% area planted with improved seeds) | SI 6 | -262 | -258 | -253 |
| | SI13 | 137 | 183 | 229 |
| | SI18 | 674 | 775 | 875 |
| **$0.74/stem for GATB** (30% area planted) | SI 6 | -108 | -104 | -99 |
| | SI13 | 301 | 348 | 394 |
| | SI18 | 844 | 945 | 1,046 |
| **$0.66/stem for GATB** (50% area planted) | SI 6 | -43 | -38 | -34 |
| | SI13 | 370 | 417 | 463 |
| | SI18 | 916 | 1,017 | 1,118 |
| **$0.63/stem for GATB** (75% area planted) | SI 6 | -19 | -14 | -9 |
| | SI 13 | 396 | 443 | 489 |
| | SI 18 | 943 | 1,044 | 1,145 |
| **$0.61/stem for GATB** (100% area planted) | SI 6 | -3 | 2 | 7 |
| | SI 13 | 414 | 460 | 507 |
| | SI 18 | 961 | 1,062 | 1,163 |

**$180/m3** log price for GATB seedlings ($120/m3 price premium )

Expected *additional* volume gain (%) with GATB compared to the volume gain (30%) of TB

| | Site Index | 0% | 10% | 20% |
|---|---|---|---|---|
| **$0.93/stem for GATB** (15% area planted with improved seeds) | SI 6 | -247 | -241 | -235 |
| | SI13 | 287 | 345 | 403 |
| | SI18 | 1,001 | 1,127 | 1,253 |
| **$0.74/stem for GATB** (30% area planted) | SI 6 | -93 | -87 | -81 |
| | SI13 | 452 | 510 | 568 |
| | SI18 | 1,172 | 1,298 | 1,424 |
| **$0.66/stem for GATB** (50% area planted) | SI 6 | -28 | -21 | -15 |
| | SI13 | 522 | 580 | 638 |
| | SI18 | 1,244 | 1,370 | 1,496 |
| **$0.63/stem for GATB** (75% area planted) | SI 6 | -3 | 3 | 9 |
| | SI 13 | 548 | 606 | 664 |
| | SI 18 | 1,271 | 1,397 | 1,523 |
| **$0.61/stem for GATB** (100% area planted) | SI 6 | 13 | 19 | 26 |
| | SI 13 | 565 | 623 | 681 |
| | SI 18 | 1,289 | 1,415 | 1,541 |

**$200/m3** log price for GATB seedlings ($140/m3 price premium )

Expected *additional* volume gain (%) with GATB compared to the volume gain (30%) of TB

| | Site Index | 0% | 10% | 20% |
|---|---|---|---|---|
| **$0.93/stem for GATB** (15% area planted with improved seeds) | SI 6 | -237 | -230 | -223 |
| | SI13 | 388 | 454 | 519 |
| | SI18 | 1,220 | 1,362 | 1,505 |
| **$0.74/stem for GATB** (30% area planted) | SI 6 | -82 | -75 | -68 |
| | SI13 | 553 | 619 | 685 |
| | SI18 | 1,390 | 1,533 | 1,676 |
| **$0.66/stem for GATB** (50% area planted) | SI 6 | -17 | -10 | -3 |
| | SI13 | 622 | 688 | 754 |
| | SI18 | 1,462 | 1,605 | 1,748 |
| **$0.63/stem for GATB** (75% area planted) | SI 6 | 8 | 15 | 22 |
| | SI 13 | 648 | 714 | 780 |
| | SI 18 | 1,489 | 1,632 | 1,776 |
| **$0.61/stem for GATB** (100% area planted) | SI 6 | 24 | 31 | 38 |
| | SI 13 | 716 | 786 | 856 |
| | SI 18 | 1,507 | 1,651 | 1,794 |

## Conclusions

1. The use of GATB-selected 3rd generation planting stock was difficult to justify on economic grounds alone compared with TB-selected planting stock under the current sawlog market value and the current planting rate (~15%) with improved stock in Alberta for both white spruce and lodgepole pine.
2. The stand-level financial performance of lodgepole pine GATB planting stock consistently outperformed that of the white spruce GATB stock. Thus, if budgets are limited, investing in lodgepole pine improvement programs would generate more revenue per hectare than investing in white spruce in Alberta.
3. Results from the sensitivity analysis revealed that the GATB method is:
    a) more financially supported at lower discount rates;
    b) strongly supported through decreased seedling costs with increased areas planted with improved stock;
    c) strongly supported through an increase in log price premiums at harvest resulting from potentially better wood quality and higher volume production relative to TB-selected stock.

## Reference

Chang, W.-Y., Gaston, C., Cool, J., & Thomas, B.R. 2019. A financial analysis of using improved planting stocks of white spruce and lodgepole pine in Alberta, Canada: Genomic selection *versus* traditional breeding. *Forestry* doi:10.1093/forestry/cpz011

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RES FOR

# RES-FOR HIGHLIGHT #4

## December 2019

## *Perceived Risks of Genomic Selection Among Forestry Stakeholders*

### Overview

Controversies surrounding new technologies can often be attributed in part to differing perceptions of the risks and benefits posed by those technologies. A better understanding of perceived risks associated with new technologies prior to implementation can offer managers and decision-makers important information that can be used in planning and communication. While the perceived risks of biotechnologies in agriculture have been the subject of extensive research, reactions to technologies introduced into the forest sector have received less attention. Recently however, researchers have also been exploring the use of genomic tools such as genomic selection in tree breeding. These tools would provide breeders with more information to select desirable traits, provide a more thorough understanding of a trees underlying genome, and in doing so, potentially increase the rate of tree selection compared to current breeding strategies. Because forests support multiple public uses and values, understanding stakeholder perceptions of genomic selection in tree breeding is essential for technology adoption and decision-making.

### Methods

This pilot study presents one of the first assessments of stakeholder attitudes toward genomic selection or genomics assisted tree breeding in forestry. Due to the lack of previous research, and the relatively low level of public knowledge regarding this technology, we adopted a qualitative methodology approach. This method allows for identification of the underlying values and levels of understanding that influence perceptions and attitudes. We focused on forest stakeholders, based on the supposition that they would be most likely to be informed, and interested, in the topic (see Table 1 for sample description). Interview participants either worked in the forestry sector (both government & industry) or were involved in a recreational group. An initial list of participants was gathered from the Foothills Research Institute, and additional participants were selected via ‘snowball sampling’, in which interview participants suggested additional potential interviewees. All participants were first contacted through email, and were offered $20. Most interviews were audio recorded and conducted in person, although some took place by phone. Each interview was transcribed and imported into a qualitative software program for analysis. Stakeholders were asked how knowledgeable they thought they were and to describe what they thought genomic selection and tree breeding entailed. In each interview, a script was read providing an overview of genomic selection which highlighted potential benefits.

### Research Goal

The goals of this research were to understand the attitudes and concerns that forest stakeholders may hold regarding the use of genomic assisted tree breeding, in order to inform planning and management decision-making in a manner that would minimize the potential for conflict, and meet the expectations of stakeholders.

### Research Questions

1. What is the relative awareness of tree breeding efforts and genomic selection among those working in the forestry sector and recreational stakeholders living in forest-based rural communities?
2. What are the risks that various stakeholders associate with genomic selection in forestry?

## Overview of Sample

Table 1: Overview of Sample Demographics

| Location | Number of Participants | Stakeholder Type | | | Age Class | | | Gender | |
|---|---|---|---|---|---|---|---|---|---|
| | | Forestry Sector[1] | Recreational Group[2]+ Forestry | Recreational Group[2] | <40 | 40-60 | >60 | Male | Female |
| Edmonton | 4 | 4 | | | | 4 | | 3 | 1 |
| Hinton | 8 | 5 | 2 | 1 | 2 | 4 | 2 | 5 | 3 |
| Whitecourt | 11 | 7 | 3 | 1 | 2 | 8 | 1 | 8 | 3 |

1. Including ecologists, forest health officers, contractors, and foresters (assistant, planning, operations, silviculture).
2. Recreational users were members of at least one of the following groups: Whitecourt Trailblazers, Whitecourt Fish and Game Association, Hinton Mountain Bike Association, Hinton Nordic Ski Club, and the Hinton ATV Society.

## Main Findings

1. Participants were all fairly aware, supportive, and confident they could describe tree breeding. However, stakeholders had many different understandings of what tree breeding actually entails. Participants were less aware and less certain they could describe genomic selection.
2. Some risk characteristics that were most prominent included: uncontrollability of potential outcomes, in-equitability in distribution of benefits, delayed consequences, being unknown to science, and being unnatural.
3. Despite perceiving risks, few participants expressed outright rejection. As such, stakeholders expressed some acceptance of risk, and were supportive of a cautious approach to implementation.
4. Genomic selection was also perceived by respondents as potentially reducing biological diversity, which departs from claims made about this technology in some scientific literature. This presents one potential difference in how experts and stakeholders perceive risks.

## Conclusions and Future Prospects

Not all participants were confident in the anticipated benefits of genomic selection, nor accepting of the perceived risks. Some concerns stemmed from participants' familiarity with forests as complex ecosystems, and with forestry's long rotation cycles. Overall, most interview participants expressed cautious support, and none expressed outright objection to genomic selection in tree breeding.

### *Implications for planning and management:*

1. This research identifies a number of topics that warrant attention in stakeholder dialogues, such as the potential impact of genomic selection on biological and genetic diversity, and the current standards in place to maintain that diversity.
2. This research also indicates that stakeholders are not necessarily unwilling to accept risk. Meaningful discussions of uncertainty in current projections of intended and unintended outcomes are warranted.
3. More research is needed to include additional regions and stakeholder groups, and to assess shifts in views over time.

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RES-FOR

# RES-FOR HIGHLIGHT #5

## May 2020

## *“Spatial Analysis”*
## *a good opportunity to revise genetic evaluations*

### Overview

Progeny test trials are typically large and located throughout the deployment region established for a tree improvement program. These trials are often established on heterogeneous sites to mimic future reforestation sites. However, to maximize the information from these trials, including a precise genetic evaluation, these trials should provide performance information without confounding factors such as soil fertility, moisture availability, and terrain variation (i.e., spatial variation). The magnitude and direction of the spatial variation should, in theory, be accounted for before (*a priori*) trial establishment. However, progeny test designs can only account for the obvious, identifiable causes of site variation and this is accomplished through a statistical term called “blocking”. However, unknown sources of variation often confound the results which can lead to the incorrect identification of superior trees. Therefore, ‘after-the-fact’ (*a posteriori*) analysis is often needed to adjust for these unknown factors and this is known as “spatial analysis”. In the RES-FOR[1] project, three mature (>30-year-old) progeny tests from one of Alberta’s first generation white spruce (*Picea glauca* (Moench) Voss) breeding programs were established across a range of sites. Previous analyses based on an *a priori* blocked design, offered a good opportunity for reanalysis using an *a posteriori* spatial approach.

### Objective

To compare the performance of the classical *a priori* block design with the *a posteriori* bidimensional autoregressive spatial model on 30-year-old height and diameter measurements.

### Methods

Data from 150 half-sib open-pollinated families from three progeny trials associated with the Region D1 first generation white spruce breeding program were used for analysis. The experimental design employed a random complete block design with six-tree family row plots and six blocks per site. Total height and diameter at breast height (1.3m) were measured at year 30 (HT30 and DBH30, respectively). Two individual-tree models were evaluated. Both models included a fixed effect of genetic group to account for the means of the different origins of parents, a random plot design effect, random additive genetic effects (breeding values), and random errors. The standard *a priori* block model also included random block effects, while the *a posteriori* spatial approach included a random autoregressive correlated residual at the individual level.

### Results

As an example, Figure 1 displays the estimates of the block effects for the block model (left), and the estimated surface for the spatial model (right) for HT30 at the Red Earth progeny test site. The fit of the *a priori* block model is expectedly abrupt as block effects are parameters for a categorical variable. On the other hand, the estimated surfaces with the spatial model shows continuous spatial variation resulting in a more accurate modeling of environmental variability. Note also that the environmental variability accounted for in the *a priori* block analysis is in the opposite direction to that shown in the *a posteriori* spatial analysis, based on HT30.

[1] RES-FOR = Resilient Forests: Climate, pests & policy – genomic applications (Genome Canada 2015 LSARP)

Additionally, the *a posteriori* analyses, using a multiple-trait multiple-site mixed model, showed that differences between the *a priori* standard (without spatial consideration) and *a posteriori* spatial analyses were evident by the change in ranking of 132 of the top selected trees (Figure 2).

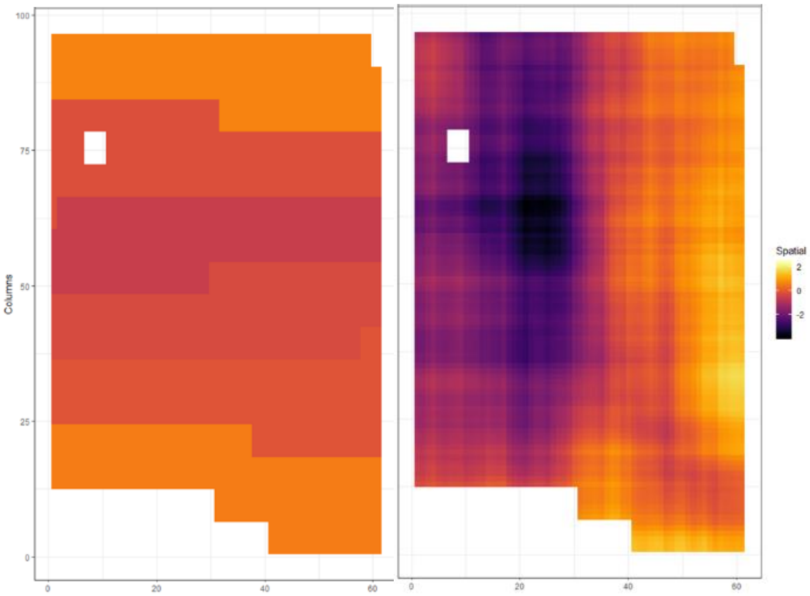

**Figure 1**. Heat maps showing a comparison of predicted spatial effects from *a priori* (left) block and *a posteriori* (right) spatial analysis for each tree from height at age 30 in the white spruce Red Earth progeny test site.

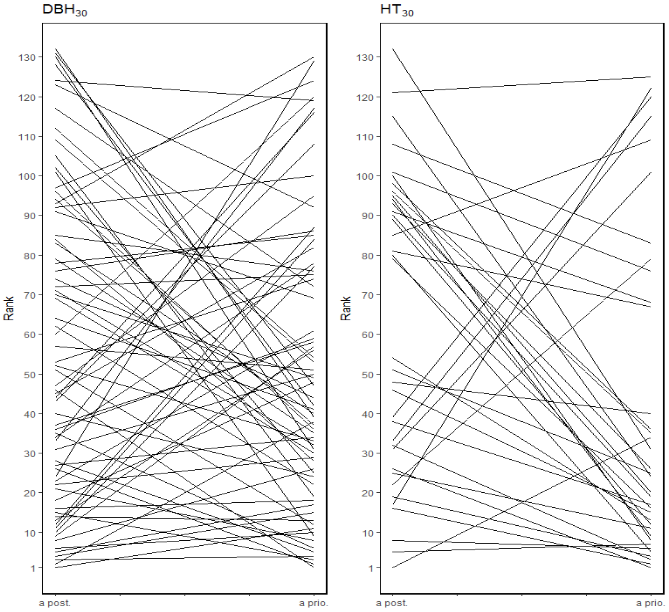

**Figure 2.** Ranking of the top 132 white spruce trees based on *a priori* (a prio.) blocking and *a posteriori* (a post.) spatial analyses in a multi-site multi-trait genetic analysis. Note change of rank for two traits at age 30 years (diameter at breast height ($DBH_{30}$, left) and height ($HT_{30}$, right)).

## Conclusions

Precise genetic evaluation is of critical importance before selection of parents for either second-generation breeding or seed orchard establishment. In this example, the spatial model approach captured the complex environmental spatial variation at the progeny test sites and yielded more precise genetic estimates of performance for height and diameter. This analysis allowed for better ranking and selection of superior trees by removing variation not captured through the blocked design, leading to higher and more reliable estimates of improved performance or genetic gain in this program.

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# RES-FOR HIGHLIGHT #6

## May 2020

### *RF-LIMS – Use & Structure*

## Overview

The RF-LIMS (https://rf-lims.wishartlab.com) is a web-accessible laboratory information management system (LIMS) created to consolidate all of the data from the Resilient Forests[1] (RES-FOR, RF) project, thus making it available for all invited users to view and export long after the project's completion. The RF-LIMS has several measures put in place to ensure data integrity: (1) data is validated before it is imported; (2) edits are logged and can be easily rolled back; and (3) backups are periodically made to recover anything that has been accidentally deleted. This 'Highlight' sheet describes how the data in the RF-LIMS is accessed and stored. Additional in-depth video walkthroughs and tutorials which cover how to navigate the RF-LIMS and how to use its other features are available for download from the "General Files" section accessible from the RF-LIMS' navigation bar.

## Use

To gain initial access to the RF-LIMS, users are invited by email to set up a password which they can use to log in using any internet browser. Anyone with an existing account can invite new users through the "Users" dashboard accessible from the navigation bar's "Admin" dropdown.

The RF-LIMS is primarily used to house the RES-FOR project data. The user interface is particularly well-suited for viewing the data for individual trees, samples, and measurements, each of which can be found by using the filter bar present at the top of their corresponding browse page. All samples and measurements are linked to the RES-FOR coded trees that they were taken from, and these relationships are laid out clearly through the "Tree Summary" navigation on the left-hand side of every entity's page in the RF-LIMS. Samples and measurements are also able to be edited individually through this manner by clicking on the blue "edit" buttons.

For more efficient data upload into the RF-LIMS, there is a "Bulk Import" interface. This feature allows researchers to import all of their data into the RF-LIMS en masse by uploading a single CSV file. Any errors found in the data will stop the import, thus ensuring that researchers make the necessary corrections before they are able to continue. To upload new data types not currently present in the RF-LIMS, a site administrator must first be consulted so that the required headers can be programmed into the system; this allows for data validation upon import, data querying for export, and complete definitions/documentation for all data fields (https://rf-lims.wishartlab.com/references).

All of the data in the RF-LIMS can be exported using the "Advanced Search" interface. This feature allows users to select their desired data fields and to narrow down their results by constructing queries for desired conditions. The results can be previewed before being downloaded as a CSV file.

[1] RES-FOR = Resilient Forests: Climate, pests & policy – genomic applications (Genome Canada 2015 LSARP)

# Structure

Experimental data imported into the RF-LIMS (either through the user interface or through the bulk import feature) is saved into the system's database. This import system allows for the relationship among discrete pieces of data to be preserved (e.g. what measurement value belongs to which tree). In the overall structural hierarchy, a single tree can have many samples and each individual sample is associated with a single measurement type. Only the data stored in the database can be exported using the advanced search function. Alternatively, individual files (including papers, presentations etc.) can be uploaded to the "General Files" section where they are downloadable and viewable by all users. With the exception of the files used for bulk imports, the contents of individual files are not validated, nor associated with other entities in the database, and therefore not exportable using the Advanced Search function. Figure 1 summarizes how data in the RF-LIMS is stored, imported, and exported.

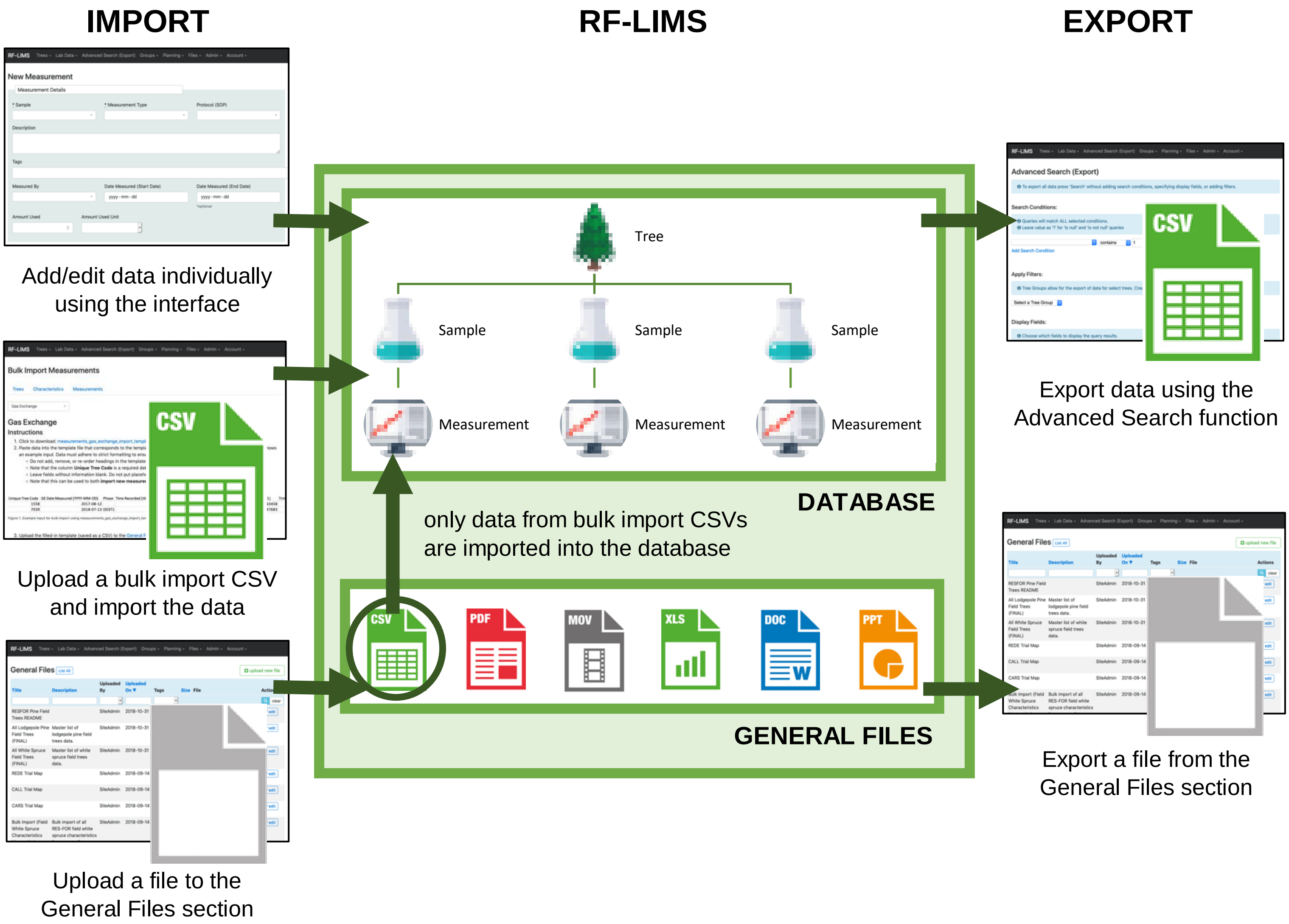

*Figure 1. Overview of the RF-LIMS' import and export methods.*

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RES FOR

# RES-FOR HIGHLIGHT #7a

## June 2020

## *Genetic variation in stomatal sensitivity to atmospheric drought & water use efficiency in lodgepole pine & white spruce progeny trial trees*

## Overview

We examined genetic variation in two drought resistance traits: 1) stomatal sensitivity to atmospheric drought; and 2) water use efficiency in field progeny trials of the Region C lodgepole pine (Pl) & Region D1 white spruce (Sw) tree improvement programs.

## Goals & Objectives

1. A key plant response to drought is to close their stomata to prevent mortality caused by losing too much water. Our **first goal** was to compare families in their stomata sensitivity through measuring stomatal conductance ($g_s$, how fast water is lost on a per area 'leaf' basis) to atmospheric drought measured as vapor pressure deficit (VPD, a metric that combines air temperature and humidity).
2. The closure of stomata comes with a cost of reduction in photosynthesis. Intrinsic water use efficiency, indicated by measuring the stable carbon isotope ratio ($\delta^{13}C$), captures how many carbon molecules a plant uses for a given amount of water lost, and thus has been used as a selection target in breeding for drought resistant crop varieties. Crop breeders realized that focusing on $\delta^{13}C$ *per se* could result in varieties that had low $g_s$ and low growth rate; Ideally, one should select for genotypes with a high $\delta^{13}C$ ratio driven by a high carboxylation capacity, i.e., the ability to achieve greater photosynthesis at a given $g_s$. Our **second goal** was to compare families in their $\delta^{13}C$ ratio and to test if there was a positive genetic correlation between $\delta^{13}C$ and foliar nitrogen content ($N_{area}$), a surrogate for carboxylation capacity.
3. Our **third goal** was to test if genetic values of stomatal sensitivity and $\delta^{13}C$ of the families were correlated with climate conditions at the mothers' location of origin.

## Methods

We measured $g_s$ under naturally varying ambient VPD in 40 Pl families and 34 Sw families in two field progeny trials (one trial per species), and measured $N_{area}$ and $\delta^{13}C$ in a subset of the families that: a) covered the entire range of height breeding values of the trees; and b) were also tested in a greenhouse trial (Highlight Sheet #7b).

## Results

1. The Pl families showed significant variation in $g_s$ (but not in stomatal sensitivity to VPD), which was negatively correlated with $\delta^{13}C$ (Fig. 1). This result suggests that the genetic variation in $\delta^{13}C$ among Pl families was primarily driven by $g_s$.
2. In both species, progenies with higher $N_{area}$ showed higher $\delta^{13}C$, but the two traits showed no positive genetic correlation. This result suggests that $N_{area}$ was not a major driver for genetic variation in $\delta^{13}C$, but it might be possible to boost water use efficiency through manipulating environmental factors such as planting at more fertile sites, that could increase $N_{area}$**.**
3. The Sw families showed significant variation in $\delta^{13}C$ and in stomatal sensitivity to VPD. The families of mothers that originated from drier, warmer habitats had more sensitive stomata and higher water use efficiency (Fig. 2). No such climate- trait correlations were found among the Pl families.

# Conclusions

We found significant genetic variation in $\delta^{13}C$, and positive phenotypic correlations between $\delta^{13}C$ and foliar nitrogen content ($N_{area}$) in both species. In addition, we found warmer, drier habitats were occupied by Sw individuals with higher water use efficiency and more sensitive stomatal responses to atmospheric drought.

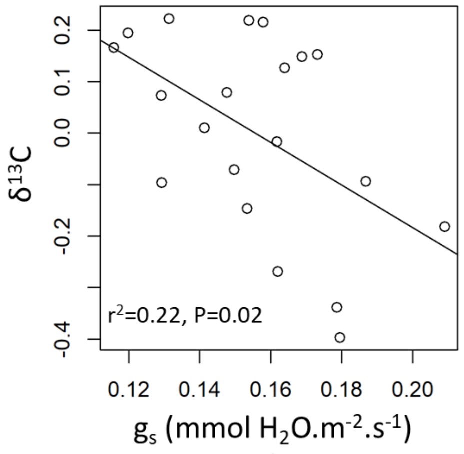
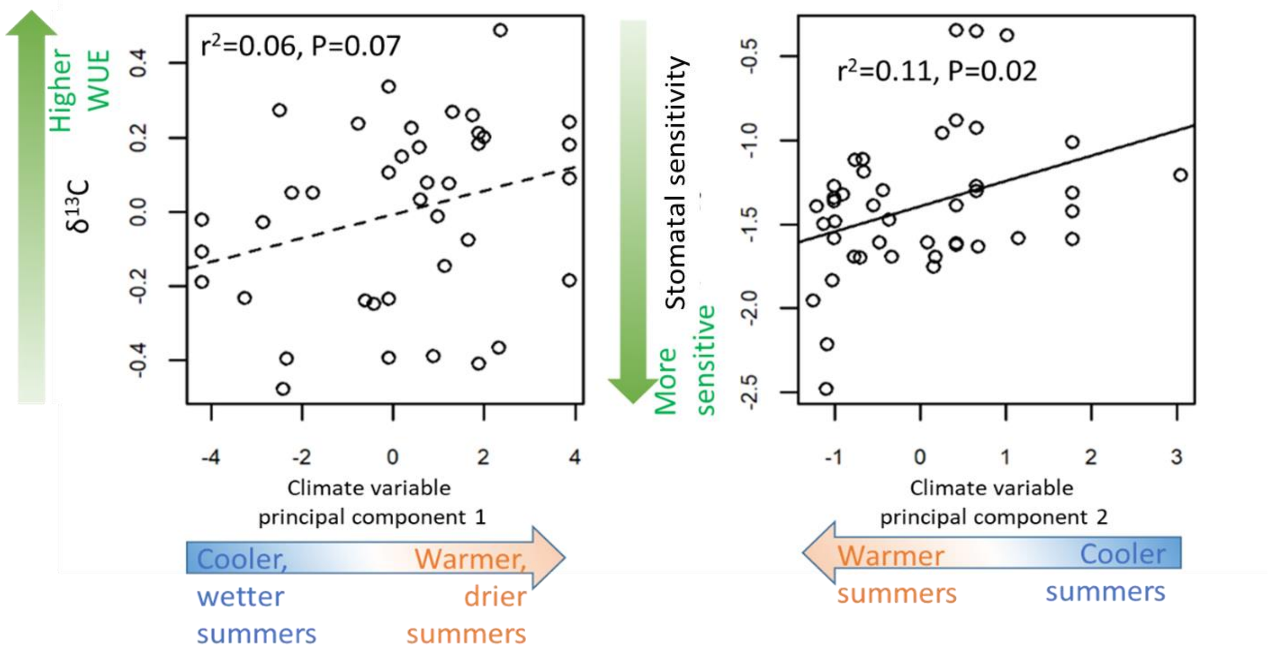


Fig. 1 Breeding values of $\delta^{13}C$ (centered around 0) and $g_s$ of the Pl families. Solid line is the regression line.

Fig. 2 Principle component analysis of breeding values of $\delta^{13}C$ (left) and stomatal sensitivity (slope of $g_s$ against VPD) vs. climate conditions (right) at the mothers' location of origin. Dash and solid lines are the regression lines.



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# RES-FOR HIGHLIGHT #8

## June 2020

## *Development of Mass Spectrometric Methods for the Absolute Quantification of Trace Metals & Polyphenols from Conifer Needles*

### Overview

Coniferous trees are known to have a complex defense system involving a range of physiological and chemical responses to both abiotic and biotic stress conditions. In times of drought, plants often adjust their amino acid, sugar, and inorganic salt levels to protect the integrity and stability of plant cells. When attacked by insects, plants often release a range of noxious secondary metabolites such as polyphenols, terpenes, and alkaloids which can drive away or even kill insect attackers. While much is known about the chemical defense systems of crop plants, very little is known about the chemical defense systems of trees, in particular conifers. By monitoring the chemical changes that occur in conifers due to drought, or insect attack (e.g.: spruce budworm or mountain pine beetle), it may be possible to identify chemicals that make certain trees (or seedlings) susceptible or resistant to these biotic or abiotic stressors. The identification of these chemicals could assist tree breeders in selecting trees that are more resilient to stress.

### Goals & Objectives

This project had two objectives: 1) develop a targeted, fully quantitative, liquid chromatography-mass spectrometry (LC-MS) method to measure ~ 20 polyphenols in conifer needles; and 2) develop a fast, fully quantitative inductively coupled plasma mass spectrometry (ICP-MS) method to measure ~ 38 inorganic salts or trace metals in conifer needles. *Note: Methods manuscript in preparation for submission to Journal of Analytical and Atomic Spectrometry.*

### Method-Absolute quantification of polyphenols by LC-MS

With conifer needle samples collected from seedlings as part of the RES-FOR[1] project, we first froze and then ground the samples to a fine powder using a Geno/Grinder. Next, a quick solvent extraction protocol was used that allowed us to selectively extract polyphenols in high yield using less than 100 mg of needle tissue. A reversed-phase liquid chromatography method was subsequently developed to ensure the effective separation of the polyphenols. By adding (spiking) a known amount of the corresponding $^{13}C$/deuterated polyphenol internal standards (ISTDS) to each needle extract, we accurately quantify 19 polyphenols in both white spruce and lodgepole pine needle samples. The accuracy and reproducibility (intraday and interday) of the methods were assessed by analyzing the National Institute of Standards and Technology (NIST)-Certified Standard Reference Material (SRM) for pine needles (1575a, NIST assigned code) on three different days.

### Results-Absolute quantification of polyphenols by LC-MS

Matrix-matched calibration curves were determined for all 19 polyphenols and the correlation coefficient ($R^2$) values were >0.98. The accuracy of the method was evaluated at two different concentrations and calculated as the average of three replicates and expressed as %, while reproducibility was expressed as a relative standard deviation (% RSD) in Table 1. The precision of the method is quite promising with the % RSD less than 9 % for 16 polyphenols. The method we have developed is far superior to any other method developed for the quantitative measurement of polyphenols in conifer needles. It is substantially (2-5X) more sensitive, faster, and more quantitative than any other published method.

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[1] RES-FOR = Resilient Forests: Climate, pests & policy – genomic applications (Genome Canada 2015 LSARP)

**Table 1**. Spiking recovery test: Percent (%) accuracy and reproducibility (% RSD) of the method at two levels of spiking, LS = low spiking, MS = medium spiking for 15 polyphenols. Bold values indicate responses outside the expected range of 100±20%.

| **Compound** | **% Accuracy (% RSD); n=3** | | **Compound** | **% Accuracy (% RSD); n=3** | | **Compound** | **% Accuracy (% RSD); n=3** | |
|---|---|---|---|---|---|---|---|---|
| | LS | MS | | LS | MS | | LS | MS |
| Apigenin | 77 (14) | 86 (3) | Taxifolin* | **173** (6) | 127 (8) | Quercetin | **66** (4) | 75 (5) |
| Gallic acid | 73 (3) | 83 (4) | Vanillic acid | 115 (2) | 82 (2) | Piceol | 104 (3) | 88 (1) |
| p-Coumaric acid | 79 (1) | 78 (6) | Gallocatechin | 112 (3) | 98 (4) | 3-4-DHBA | 104 (3) | 103 (3) |
| Caffeic acid | 84 (3) | 84 (2) | Pungenol | 93 (3) | 117 (3) | Vanillin | 76 (6) | 81 (2) |
| Protocatechuic aldehyde | 108 (3) | 111 (2) | Ferulic acid | 85 (5) | 84 (3) | Catechin | 101 (4) | 89 (2) |
| LS: Low Spike, MS: Mid Spike | | | | | *Quercetin used as an ISTD | | | |

## Method-Absolute quantification of inorganic salts by ICP-MS

The method we developed avoids microwave-based sample digestion and instead uses a simple water-bath based method, which minimizes the amount of time required for each sample. In order to minimize the amount of sample needed from each tree, optimization of the amount required was critical. By optimizing the composition of the digestion solvent (concentration of nitric acid and hydrogen peroxide), and digestion time and temperature, sample requirements were reduced. The method was validated with a spiked-recovery test and the accuracy and the precision of the method were calculated.

## Results-Absolute quantification of inorganic salts by ICP-MS

Using the method described above, a total of 46 metal ions could be detected, with 38 measured quantitatively, and eight additional metals being measured qualitatively (Ga, Nd, Pd, Nb, Gd, Ho, Hf, and Hg). Calibration curves for the 38 elements/metals yielded $R^2$ values of 0.99-0.98. The percentage of recovery and precision (% RSD) among the measurements were in an acceptable range (80-120%). Furthermore, the accuracy of the method was substantiated by analyzing NIST-Certified SRM, where the concentrations of the 18 metals/elements were compared to reference concentrations. The accuracy of most of the elements/metals was in the range of 75.1% to 117.7%. Thus, the current method enables the accurate quantification of 38 inorganic salts including several essential micronutrients (cobalt, iron, manganese, molybdenum, nickel, zinc, and copper) which are needed for normal plant growth.

## Conclusions

1. The LC-MS method we developed for measuring polyphenols in conifer needles appears to be more sensitive, faster, and more quantitative than any currently published method for polyphenol measurements of needles. The technique is now being applied to the measurement of hundreds of pine/spruce seedling samples and it is expected to yield important new insights into the insect stress response, facilitating the identification of resilient individuals through their chemical response profiles.
2. The ICP-MS method we developed for measuring trace elements and inorganic salts in conifer needles is faster and more sensitive than any currently published method. Our ICP-MS technique is being applied to the measurement of hundreds of pine and spruce seedling needles samples and is expected to yield important new insights into the abiotic (drought) stress response of these trees and help in the identification of informative chemical responses in resilient individuals.

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RES FOR

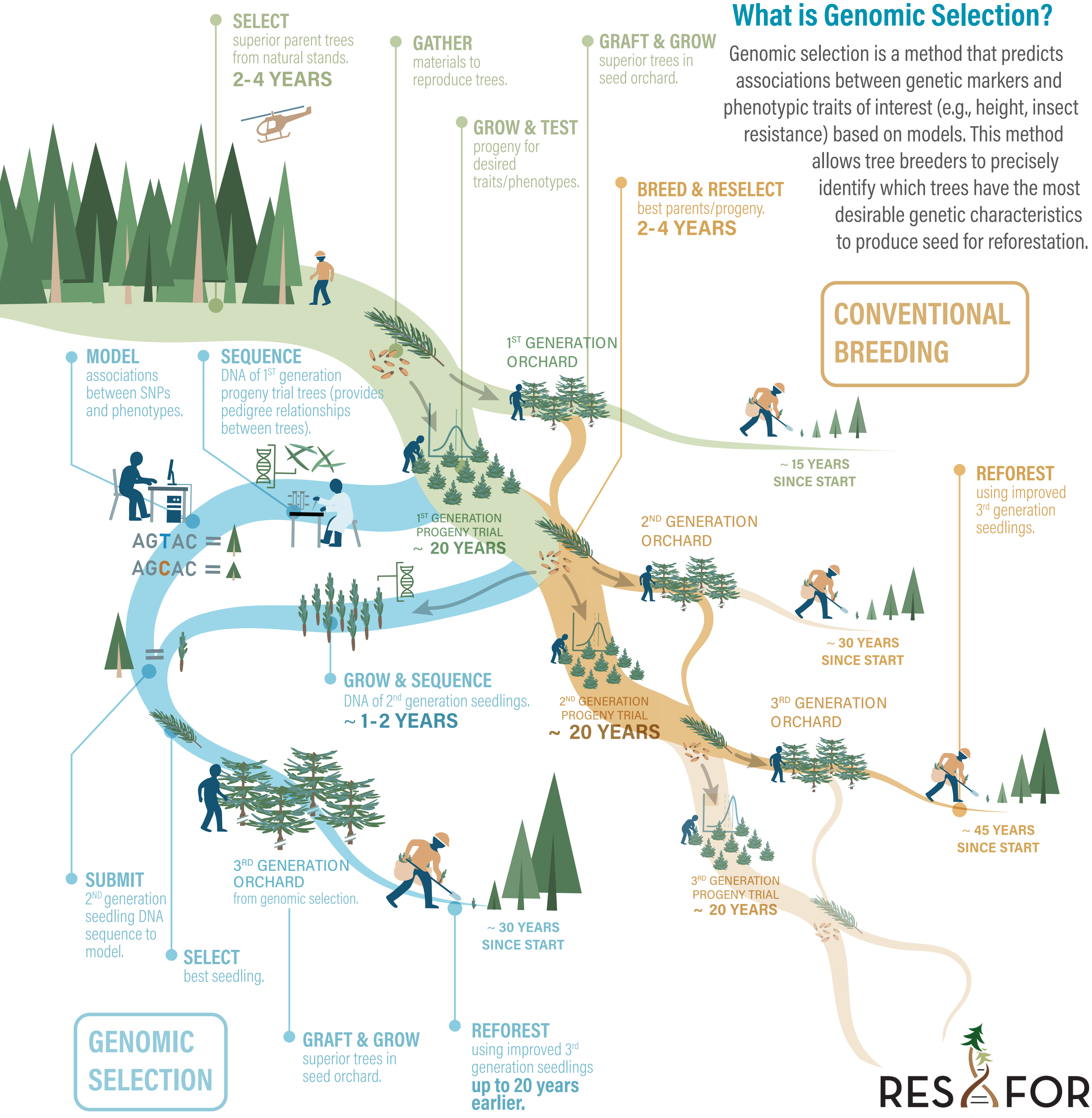
# Using Genomic Selection for Tree Improvement

AUGUST 27, 2020

Breeding healthy and resilient seedlings is a key part of successful reforestation. Selecting the best trees as a seed source is a key part of this process. However, **conventional tree improvement methods** are struggling to keep up with a rapidly-changing climate and frequent insect outbreaks. After decades of genetics research, **genomic selection** has emerged as a tool that could provide well-adapted seedlings in a much shorter time frame. This technology represents a paradigm shift from a phenotype-driven, to a genomics-based data-driven process.

## What is Genomic Selection?

Genomic selection is a method that predicts associations between genetic markers and phenotypic traits of interest (e.g., height, insect resistance) based on models. This method allows tree breeders to precisely identify which trees have the most desirable genetic characteristics to produce seed for reforestation.



**SELECT**
superior parent trees from natural stands.
**2-4 YEARS**

**GATHER**
materials to reproduce trees.

**GROW & TEST**
progeny for desired traits/phenotypes.

**GRAFT & GROW**
superior trees in seed orchard.

**BREED & RESELECT**
best parents/progeny.
**2-4 YEARS**

**CONVENTIONAL BREEDING**

1ST GENERATION ORCHARD

~ 15 YEARS SINCE START

1ST GENERATION PROGENY TRIAL
~ 20 YEARS

2ND GENERATION ORCHARD

~ 30 YEARS SINCE START

**REFOREST**
using improved 3rd generation seedlings.

2ND GENERATION PROGENY TRIAL
~ 20 YEARS

3RD GENERATION ORCHARD

~ 45 YEARS SINCE START

3RD GENERATION PROGENY TRIAL
~ 20 YEARS

**MODEL**
associations between SNPs and phenotypes.

**SEQUENCE**
DNA of 1ST generation progeny trial trees (provides pedigree relationships between trees).

AGTAC =
AGCAC =

**GROW & SEQUENCE**
DNA of 2nd generation seedlings.
**~ 1-2 YEARS**

**SUBMIT**
2ND generation seedling DNA sequence to model.

**SELECT**
best seedling.

3RD GENERATION ORCHARD
from genomic selection.

~ 30 YEARS SINCE START

**GENOMIC SELECTION**

**GRAFT & GROW**
superior trees in seed orchard.

**REFOREST**
using improved 3rd generation seedlings
**up to 20 years earlier.**

RES4FOR
https://resfor.ualberta.ca/

# How Does Genomic Selection Compare to Other Methods of Selecting Trees?

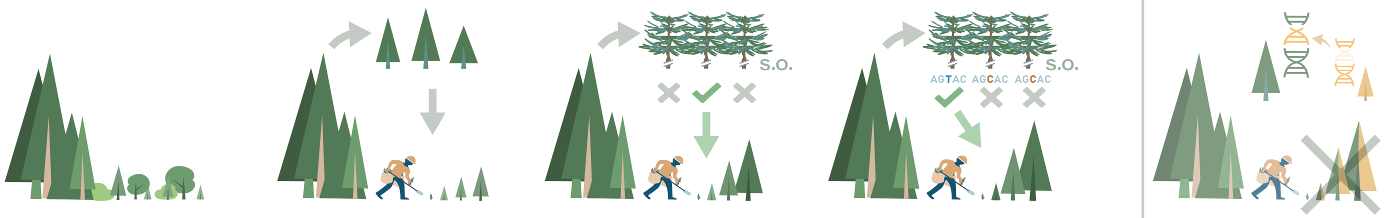
NATURAL REGENERATION

ARTIFICIAL REGENERATION

WILD SEED COLLECTIONS FROM SEED ZONES

IMPROVED SEED COLLECTIONS FROM SEED ORCHARDS (S.O.)

GENETIC ENGINEERING



### NATURAL REGENERATION

- Trees recolonize the site naturally.

### PLANT WILD SEEDS AS-IS

- Seedlings are produced from wild seeds and planted.
- *i.e., Stream 1 seed.*

### CONVENTIONAL BREEDING

- Repeated cycles of breeding and progeny testing to select for desired traits.
- Trees are selected for breeding based on phenotype and statistical models.
- Testing times are long, with breeding, testing and selecting taking decades.
- *i.e., Stream 2 seed.*

### GENOMIC SELECTION

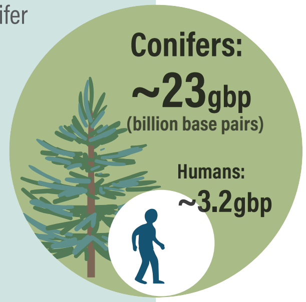
- Uses phenotyping and genotyping to find associations between genetic markers and desired traits.
- Trees are selected based on a more detailed understanding of the genetics in candidate trees.
- Bypasses the additional progeny testing of at least one subsequent generation in the tree improvement cycle.
- *i.e., Stream 2 seed.*

### GENETIC ENGINEERING

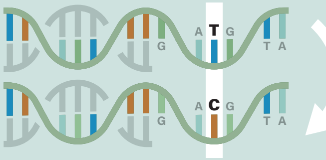
- **NOT** a part of the genomic selection process.
- Involves directly manipulating DNA (e.g., inserting genes from another species) to produce a modified organism.
- Currently not allowed in Alberta for trees.

## How Does Genomic Selection Work?

A genome is the entire genetic material of an organism. As a human, your genome comprises all of the DNA in all of your 23 pairs of chromosomes. Conifer trees typically have fewer chromosomes than humans — e.g., 12 — however, their chromosomes are much larger than ours.



Genomic selection involves examining tree genomes for SNPs: single nucleotide polymorphisms. A SNP is a place in the genome where one nucleotide differs between individuals.



These two DNA strands differ by a single nucleotide — known as a SNP.

While many SNPs have no effect on phenotype, some may affect observable traits directly or, more frequently, be closely associated with nearby regions of DNA that affect a given trait. In a few cases, the presence of a single SNP in an individual's genome might reliably predict their appearance, such as what eye colour they might have. However, most traits of interest in tree improvement, such as height, are the result of the complex interplay of many genes and their environment. This complexity makes it difficult to predict how traits will pass down from one generation to the next. Genomic selection allows us to find out what combinations of SNPs are associated with an individual's genome and trait phenoype.

By modeling these associations through an intensive computer process, we can predict the future phenotype of a seedling (e.g., its height or wood density) simply by knowing what combination of SNPs it has. Similar modeling of SNPs is used in DNA ancestry testing, testing for genetic diseases and in animal and plant breeding.

## What Can Genomic Tools Offer?

- **A dramatically shortened breeding cycle.** By cutting out potentially one generation of progeny testing, genomic selection can accelerate the tree improvement process by up to 20 years. This improved efficiency could result in economic savings over the long term and more rapid selection and deployment of drought resilient and insect resistant seedlings for reforestation.

CONVENTIONAL BREEDING CYCLE:

GENOMIC SELECTION CYCLE:

- **The ability to select for additional traits** we couldn't select for before, particularly those traits that cannot be measured in young trees (e.g., wood density, insect resistance).
- **Increase forest productivity and health** by selection of trees from a larger population of candidate trees.
- **Better understanding of family relationships** among trees (i.e., which trees descended from which parents), can enhance the assessment of genetic traits, genetic progress across generations and genetic diversity.

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