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Pacific Forestry Centre assists Province and industry in assessing efficacy of breeding programs in southern Vancouver Island seed orchards

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Credit: Taikoko Shoin Co. Ltd

Western redcedar seedling.

The Natural Resources Canada, Pacific Forestry Centre (PFC), Analytical Chemistry Lab is performing DNA microsatellite marker analysis of select foliage and seed to assess self-pollination rates in Western redcedar (*Thuja plicata*) and external pollen contamination rates in Coastal Douglas-fir (*Pseudotsuga menziesii* var. menziesii) in southern Vancouver Island seed orchards.

Approximately 5,000 individual tests of Western redcedar and Douglas-fir are being conducted at the PFC lab to help orchardists ensure sufficient seed of high genetic value is produced through tree breeding, seed-orchard production, and related activities to meet reforestation objectives and enhance timber supply and quality.

Self-pollination (selfing) is a problem for orchardists who produce redcedar seed used for reforestation, as it results in in-breeding depression. Pollination by non-orchard Douglas-fir trees may result in losses in volume and wood quality gains for which orchard trees are selected. Manual pollination using select pollen, a common practice to avoid selfing and pollen contamination, is labour intensive.

The multi-year study will help the provincial tree breeding and seed production programs assess and promote long-term sustainability and viability of these two species and the associated benefits (economic, social, environmental). Seed and seedlings of confirmed genetic makeup are valuable for the producers, seed users, and the Province of British Columbia where this seed is deployed.

PFC Chemistry Services Lab

The PFC Chemistry Services Lab is collaborating with Western Forest Products, TimberWest and B.C. Ministry of Forests, Lands, Natural Resource Operations and Rural Development in developing practical industry-driven experiments to address their concerns for two of the most economically important tree orchard species. Ultimately, these studies directly impact the management of seed orchards which are crucial to the B.C. Provincial forestry industry, providing seed stock bred for valuable characteristics such as disease resistance, drought resistance and wood quality for trade.

Western redcedar and Coastal Douglas-fir are major coastal regeneration species for which orchard seed is used. Seed orchards are comprised of parent trees originating from coastal forests or from multiple generation offspring of breeding the original forest selections. Western redcedar parent trees are selected for favourable traits, such as growth and needle leaf blight resistance whilst parent tree scores are based on field test performances. Douglas-fir parent trees are selected through field trials for volume growth gains, wood density scores and overall wood quality. In British Columbia, genetic modification is not practiced in seed and seedling production.

Western redcedar

Technician Robert Kowbel and UVic Biochemistry student Allison Griffiths check growth of western redcedar seedlings prior to DNA extraction.

The incidence of self-pollination or "selfing" is higher in redcedar than in other conifer species, both in orchards and in the wild. Although early performance of selfed seed may be acceptable, inbreeding depression becomes apparent over time. Seed lot registration practices do not currently account for losses of genetic gain due to self-pollination. When aiming for 20% volume gain by rotation age 60, self-pollination effects can become quite significant.

As part of this selfing study, four different pollination management schemes are examined at three Vancouver Island orchards:

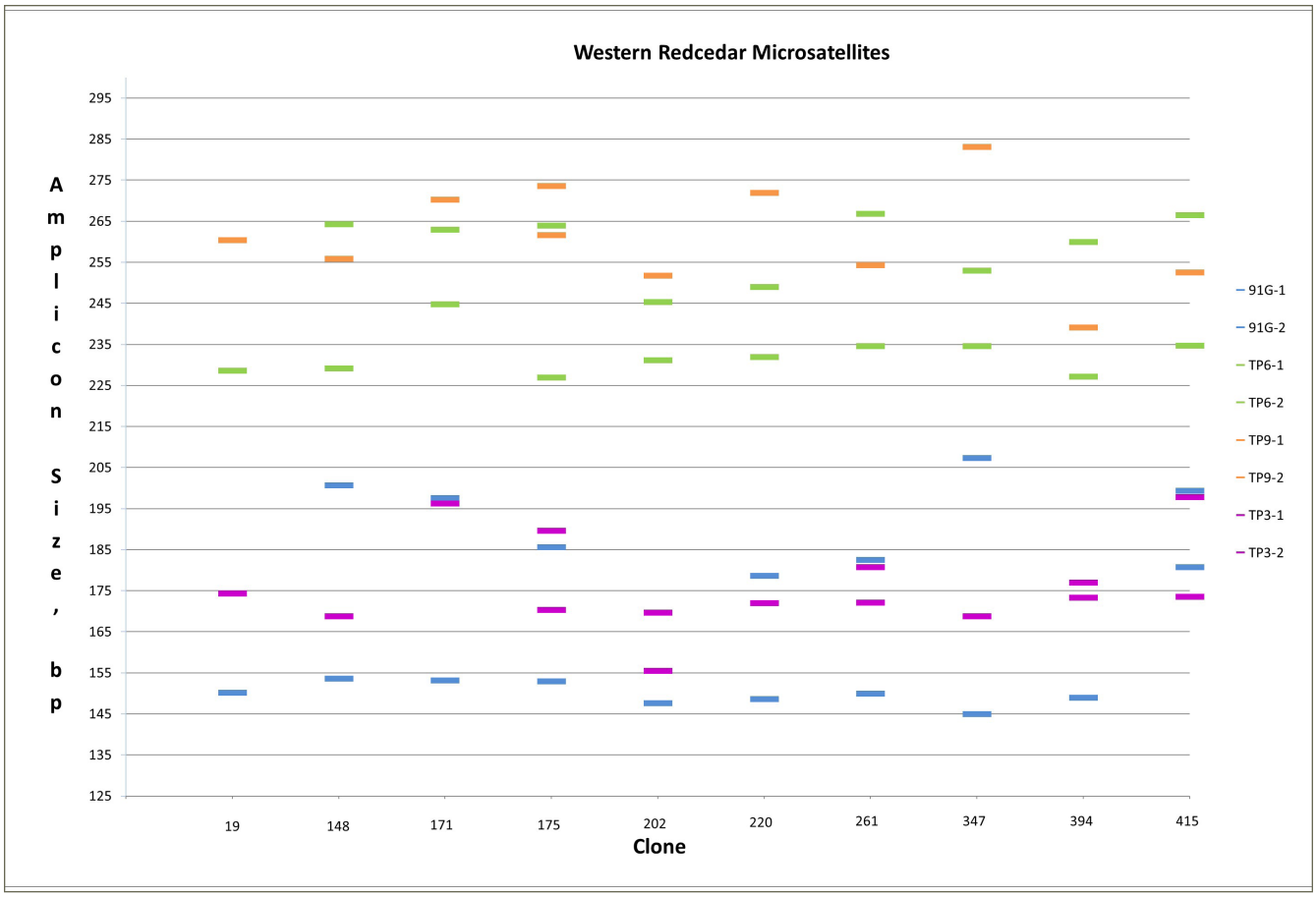
I) supplemental mass pollination (SMP), where pollen from unrelated parents is harvested and applied to receptive cone flowers on crop trees to compete with pollen cloud;

II) pollen removal, where pollen flowers are removed from crop trees prior to pollen release, and only pollen from unrelated parents is available in the local pollen cloud;

III) air-blast spraying, where pollen may be dispersed further from source; and

IV) control, where no pollen management is applied (non-SMP).

Orchard staff provided samples to the lab for analysis. Once germinated, seeds were transferred to water agar tubes to grow for a few weeks to increase biomass for extraction purposes. The seedlings were homogenized and DNA was extracted using standard extraction procedures. DNA from seed was genotyped using multiple primer pairs and compared with the foliar mother's observed genotype to establish whether they were deemed "selfed" or outcrossed. The data was organized by treatment type to estimate inbreeding rates among pollination management schemes.



Consensus DNA "fingerprints" of 10 western redcedar clones across three separate orchards, obtained using four pairs of microsatellite primers. Each sample (x-axis) has a unique combination of size or number of DNA fragments produced (y-axis) during the amplification process.

Microsatellites

A microsatellite is a repeating region of 2-5 base pairs in usually non-coding areas of genomes, due to the repeating nature of microsatellites they are vulnerable to mutation, and are heritable, thus can be used for parental determination.

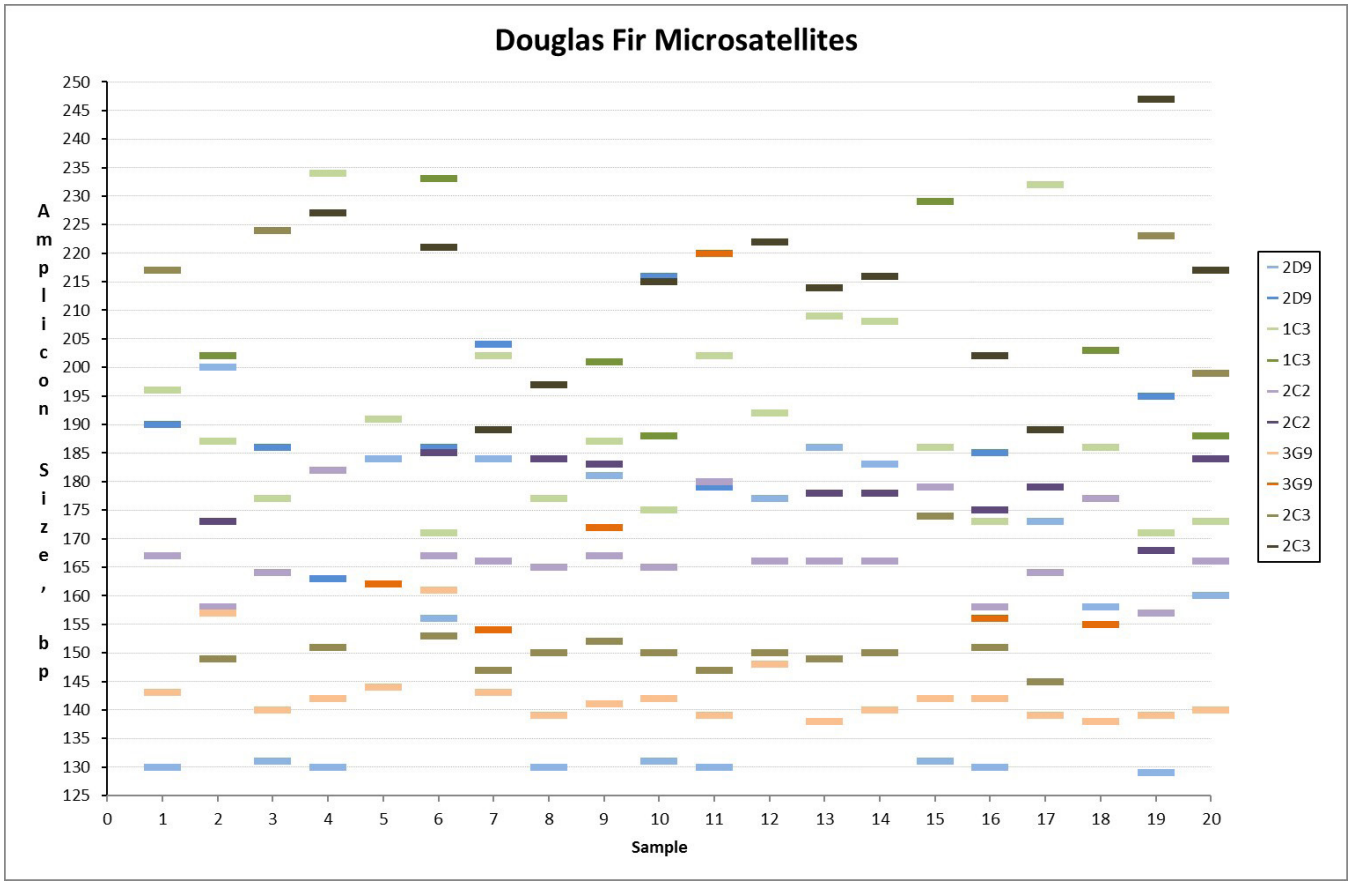
After pollination, the resulting gametophyte receives a copy of the microsatellite locus from each parent. Using specific detection primers for the microsatellite region, the mother and offspring's microsatellite DNA are compared for the determination of self-pollination or outcrossing. If the offspring contains genetic information not found in the mother, the seedling was a result of outcrossing. If the offspring has an identical banding pattern (amplicon size + heterozygous or homozygous) as the mother, the offspring may be a result of self-pollination.

Coastal Douglas-fir

Although Coastal Douglas-fir also experiences inbreeding depression due to selfing, its incidence is much lower. The primary concern here is pollination from "wild" trees situated just outside the orchard; their pollen is unlikely to deliver high value for traits of interest to the offspring.

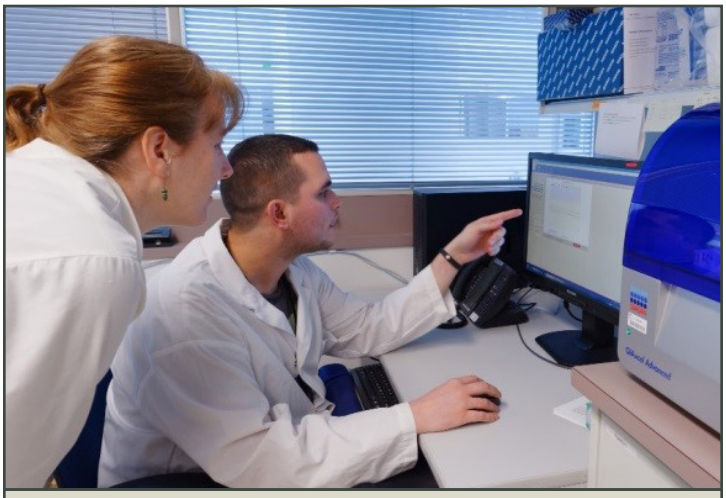
Laboratory analysis offers an improved estimation method compared to pollen traps, which are subject to high levels of variability leading to poor estimates of contamination. Accurate estimates of genetic worth are important to orchardists because they add confidence to the performance expectations of regeneration produced from orchard seed crops.

In the laboratory, Coastal Douglas-fir seeds were cold stratified and germinated in a growth incubator. As with the redcedar, both the foliar and seed gametophyte DNA were extracted, and a number of microsatellite primers were screened with the best candidates chosen for the experiment. Microsatellite genotypes were determined for all the potential parent clones in each orchard and the gametophyte profiles screened



DNA "fingerprints" for a sampling of 20 Coastal Douglas-fir clones, obtained using five pairs of microsatellite primers. Each sample (x-axis) has a unique combination of size or number of DNA fragments produced (y-axis) during the amplification process.

against all the clones in their respective orchards to determine whether they appear: a) outcrossed with local orchard pollen; or b) outcrossed with unknown (external) pollen; or c) self-pollinated.



Technicians Robert Kowbel and Holly Williams perform microsatellite fragment analysis using capillary electrophoresis instrument.

Currently, work is progressing well with years one and two complete for the Western redcedar self-pollination study; preliminary results indicate SMP treatment reduce selfing rates compared to untreated controls. For Douglas-fir, year one data provides a preliminary estimate for both pollen contamination and self-pollination. External contamination rates of Coastal Douglas-fir are lower than existing pollen trap estimates.

Both of these studies have important and direct implications to seed orchards to improve estimates of genetic worth of seed lots to support the reporting on the Forest Genetics Council's goal of 20% volume gain by 2020. The results from three years of this study indicate consistency in our methods of estimation across all three orchards. Investing in this type of research will assist managers in improving pollen management for decreased inbreeding and improving the estimates of genetic worth for their orchards, resulting in generation of higher value seed.

This work is made possible through partnership with the Forest Genetics Council of British Columbia and the Operational Tree Improvement Program, funded by the Land Based Investment Strategy.

Celebrating collaboration

The Pacific Forestry Centre celebrates the collaboration between industry and various levels of government and hopes to continue this important work in improving Canada's forests. The Lab at PFC is a modern facility providing analytical chemistry and molecular biology services to various clients in support of NRCan research priorities, and is an integral part of the research organisation. Principle clients include NRCan research scientists and professional staff, external collaborators from other levels of government, academia and industry. In addition to Robert, Holly and Allison (all pictured), contributors to this work include: Tyler Dyer, Contract research technician, Grace Ross, PFC Laboratory Technician, Maddie Gaucher and Hayleigh Rados, UVic Biochemistry co-op students, and Erin Kim, UBC Biochemistry co-op student.

Pacific Forestry Centre (PFC),
Chemistry Services Molecular Lab

Links

Natural Resources Canada, Pacific Forestry Centre
http://www.nrcan.gc.ca/forests/research-centres/pfc/13489

Forest Genetics Council of British Columbia
http://www.fgcouncil.bc.ca

Western Forest Products Inc.
http://www.westernforest.com

Land Based Investment Strategy
https://www2.gov.bc.ca/assets/gov/environment/natural-resource-stewardship/land-based-investment/2015-16_lbis_strategy.pdf

For more information on this project, or the PFC Chemistry Services Laboratory and its capabilities, please contact David Dunn (david.dunn@canada.ca), Head, Chemistry Services Laboratory at the Pacific Forestry Centre.