Living Lab Program for Climate Change and Conservation - Final Report

Project title: An approach to mitigate climate change-induced Yellow cedar decline in the Great Bear Rainforest; identification of survivors for reforestation and selection stocks

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Research findings

In year one, we visited more than 20 parks, conservancies and protected sites in the Great Bear Rainforest (GBR) to collect yellow-cedar twigs primarily from 3-50 m altitude. In addition, JM was able to join helicopter-based visits to 100-300 m elevation sites over three days in the Prince Rupert and Work channel areas. All sites showed signs of yellow cedar decline, and we collected twigs primarily from healthy individuals. When available, we collected naturally rooted twigs at soil level. Many of these individuals were growing in wetlands and had a bush rather than tree-like growth pattern. In total, we collected from approximately ~150 trees/genotypes at various sites (collected 20-100m apart at each site to avoid clones and half siblings).

In year 2, covid-related travel restrictions prevented travel to GBR and north Vancouver Island parks. We instead focused our attention on five provincial parks in the mountains of Southern BC. In these mountains we found yellow-cedar trees from about 700m altitude and upwards. In contrast to the individuals sampled in year one, we also had access to large trees growing close to but not in wet sites. These trees were in the 10-30 height and 0.3-1m diameter range, providing evidence of growth potential of the collected genotypes. In total, we collected twigs from about 130 individual trees. In both years, we took GPS coordinates and altitude data for most plants collected.

At SFU, we built two high-humidity greenhouses with automatic misting system and used them for rooting of cuttings from collected twigs. As indicated in the original proposal, we also have worked on identifying yellow-cedar genes that may play a role in freezing tolerance with the intention of generating genetic markers that may facilitate easier screens for trees with freezing-tolerant roots.

Ecological observations:

- Yellow-cedars could not be found in many of the visited parks/conservancies/protected areas in the Douglas channel and in the Terrace area despite being included in maps of yellow-cedar growth range. In some parks, a few isolated yellow-cedars were found. Both low and higher altitude (skiing hill with subalpine vegetation) lacked yellow cedars.
- Yellow cedars stands were observed only in the Eastern area between the Skeena river and Southern tip of Alaska.

- All these sites contained extensive evidence of yellow cedar decline as evidenced by trees that died 15-30 years ago (lacking most branches), more recent deaths, and dying trees with clustered branches and dead branches in between as well as trees with dying foliage.
- Yellow-cedars growing as bushes in boggy areas appear less affected as foliage was mostly living. On the other hand, no seed cones were observed on these plants.
- Several visited sites in the Douglas channel marked by aerial surveys as containing yellow-cedar decline did not contain yellow cedars but rather stands of dead Western redcedars and alders.
- During the travels in GBR, a total of three seedlings (20-30 cm height) were observed. Seedlings were NOT observed under larger seed-producing trees. Taken together, these observations suggest a lack of reproduction/regeneration by seeds, possibly due to early decline/death of germinated seedlings.
- We found many yellow cedars above 700 m altitude in the Tetrahedron, Spipiyus, Cypress, Mount Seymour and Golden Ears Provincial Parks. Searches at lower altitudes along roads leading up to these parks were unsuccessful, indicating that in Southern BC, Yellow cedar is confined to higher altitudes.
- We saw an area from distance north of Spipiyus provincial park with many dead trees. That these dead trees are yellow cedars and that death can be attributed to YCD needs to be assessed by a forest tree ecologist, but if confirmed, it would be the Southern-most case of YCD in BC.

Collection and screening for freezing tolerance:

In total, we have collected foliage from over 300 trees, which all have been cut up for rooting using 30-100 segments per genotype. We currently have rooted cuttings (Figure 1) from > 150 trees, of which those collected in 2020 are still growing larger root systems before testing. Of those collected in 2019, about 40 genotypes have been tested, and another 20 are being cold-acclimated. For freezing tolerance testing, we have use cooling water baths that allow incubation of roots at precise sub-zero temperatures as per published procedure to assess cellular electrolyte leakage due to freezing damage. Testing at -4°C revealed substantial variation between genotypes (Figure 2) with the most tolerant roots leaking 40% less electrolytes than the average 75% leakage (index of injury), relative to unfrozen controls. Differently from the set-up in year 1, the new set up avoids super cooling without freezing in some samples, which reduces variation between replicates. As a consequence, we find that many of the obtained averages have statistically significant differences in pair-wise comparisons using ANOVA. In addition, clones of one individual (ALE6; Figure 1) has been used as internal control to allow comparison of separate freezing trials. The trial to trial variation is limited, indicating that observed differences are not likely caused by variation in cold hardening, growth of plants and parameters other than genotype differences.

Figure 1. Example of rooted cuttings from foliage of one yellow cedar tree.

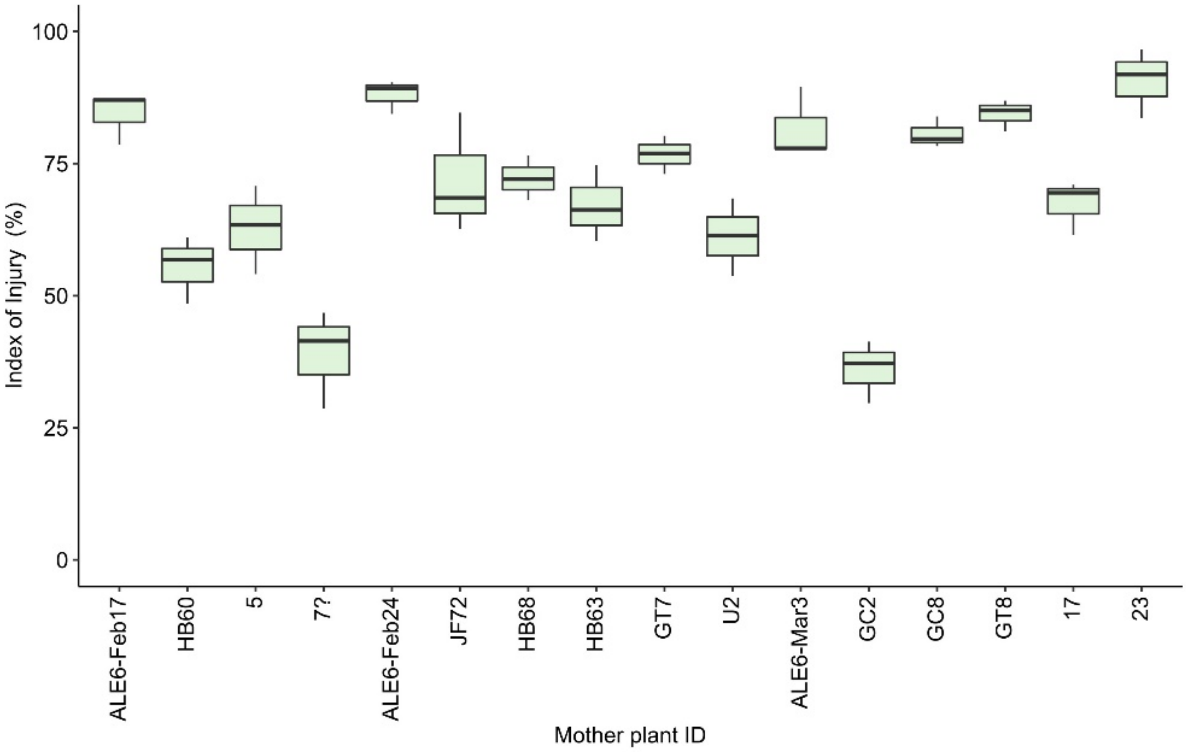

Figure 2. One of four freezing tolerance assays to date. Box indicates one standard deviation above and below the mean of the data (thick line). Observe that several genotypes (HB60, 5, 7, U2, GC2) have averages below the majority of genotypes, indicating higher than average tolerance of freezing.

We have used *RNA-seq* transcriptome analysis to identify a set of ~ 300 genes that are expressed at higher levels in freezing tolerant Yellow cedar shoots relative to freezing sensitive roots in seedlings 24 hours after removal from cold storage. Among them we have identified putative functions of identified genes based on sequence similarity to genes with known roles in freezing tolerance in other plants species. This includes genes for the complete signal transduction pathway from perception to transcription factor-based cascade and final effector genes whose gene products protect cells from freezing induced damage of plasma membranes, synthesis of osmolytes to prevent ice crystal formation and retain water in cells, chaperones to renaturate proteins, enzymes and metabolites inactivating reactive oxygen species, and more. In year two we put this analysis on hold and focused on rooting of cuttings and freezing tolerance as a primary objective.

Methods summary

Twigs were brought back to SFU in black garbage bags and kept in cold room until use. Multiple (20-100) cuttings per tree were dipped in rooting hormone (0.8% indolebutyric acid) and placed in sunshine mix 4 mixed 1:1 with bark mulch or perlite in plastic pots. Pots were placed in one of two 2x10m greenhouses within SFU large greenhouse with periodical automated misting to keep cuttings from drying. Air temperature is regulated to 15-22°C. Ligh intensity was approximately 500 micro-Einstein (μE) at cutting level with 16-hour day. Rooted plants were transferred to larger pots, fertilized and placed at high intensity (1000-2000 μE) for root and shoot growth. Plants with extensive roots were moved to 4C cold room for cold hardening for 2-3 months. Fine roots and foliage were collected and separated into 300 mg portions and subjected for 24h to 4°C (control) -4°C in calibrated water bath containing glycol antifreeze, with 3-5 biological replicates. Cellular damage from freezing of roots and foliage was assessed by electrolyte leakage assays. This assay assumes that the degree of tissue damage caused by freezing is reflected in the degree of leakage of cell contents, relative to an unfrozen control. Leakage of cell contents was assessed by measuring the electrical conductivity of a solution containing the frozen tissues.

Key outcomes for BC Parks

[e.g., what are the consequences of your research for park values/resources?, bullets are acceptable]

- Substantial genetic variation for freezing tolerance exists in a small (40) set of yellow cedar genotypes collected in the GBR, with the most tolerant genotypes showing 40% less freezing injure based on electrolyte leakage assays.
- We have rooted cuttings from about 150 trees and more genotypes are still in the process of rooting. This population is likely to reveal additional genetic variation once tested.
- We have collected more than 300 genotypes from both North and South BC coast, spanning altitudes from a few meter to 1250 m above sea level.
- This population provides the first set of trees from which selection for freezing tolerant genotypes can begin. Once field tested, such genotypes can be used to, for the first time, mitigate the spread of yellow cedar decline.
- Existing maps of yellow-cedar distribution overestimate the distribution and frequency of yellow-cedar trees
- Visited low altitude protected areas in the Douglas channel that overlap with aerial surveys identifying yellow cedar decline (our target areas) contained few or no yellow cedar trees, indicating (a) that air surveys are not necessarily correct (b) that these areas

do not provide protection to this species. Higher altitude areas in the Douglas channel were not assessed.
- All visited areas on the North coast of BC in which yellow cedar trees were found in larger numbers also showed extensive decline with old as well as recent damage.
- Natural regeneration by seeds/seedlings appear near absent, indicating that the death of larger yellow-cedar trees, although highly visible, is not the only problem affecting the survival of this species in GBR.
- An area North of Spipiyus Provincial Park on the Sunshine coast contained a large number of dead trees. If YCD is confirmed, this site would be the southernmost site of confirmed YCD on the South coast.

Relevance to BC Parks management

[Provide any recommended steps BC Parks can take to incorporate your project's findings in our day to day management of the park system]

This study provides for the first time evidence that substantial genetic variation for root freezing tolerance exists among yellow cedar trees in the GBR, providing a valuable reservoir for selection and propagation of trees that can be used to mitigate yellow cedar decline.

Most if not all the people that we spoke to during these trips, both First Nations and others, did not know about yellow-cedar decline, despite living in affected areas. Thus, there is an opportunity/need to inform the public about this issue in GBR BC Parks.

Diane Lake Provincial Park and the Gamble Creek Reserve contain small stands of Yellow cedar trees and boggy areas with bush-type yellow-cedar trees and extensive yellow cedar decline – information that may not be part of the description for protection.

The living, dying and dead yellow-cedars in the Diane lake provincial park are easily accessed from the road leading from the entrance to the lake beach and could potentially be used for educational purposes to inform the public about the ongoing yellow-cedar decline.

It may be worth training BC Parks rangers to (a) identify yellow-cedar trees and (b) identify areas of yellow cedar decline and types of damage. This will allow them to make observations during their travels. It is not easy to distinguish the rare yellow cedar trees from the much more common Western redcedar trees (which may also contribute to the lack of knowledge of yellow-cedar decline). They are best separated by comparing the scales of foliage and the shape of loose bark. (While cones are easily distinguished, they are rare on yellow cedar trees, and therefore of limited use).

From a larger perspective, our findings suggest that Yellow-cedar decline may be more acute problem in the GBR than currently known. This proposition may not affect day to day management but have bearings on the overall purpose of the ecological and cultural protection of the GBR. We base this proposition on the following observations:

- Current maps of yellow-cedar distribution in the GBR severely overestimate the presence of this species.
- All assessed areas showed extensive Yellow-cedar decline, both low and higher altitude sites.
- Natural regeneration by seeds appear near absent.

Project's challenges/opportunities

1. Yellow-cedar trees are not present in many of the low-altitude parks that we visited in the GBR, despite being included in growth range maps and aerial survey maps of yellow-cedar decline.
2. The probability of finding yellow-cedar trees increase at higher altitude up to the tree line. However, in the GBR these sites are also difficult to reach and require either the use of helicopter, or targeted multi-day hikes in potentially dangerous environments.
3. Cuttings from adult yellow-cedar tree foliage are highly recalcitrant to hormone-induced rooting and takes 4-6 months before responding with calli and root formation. During this time, 40-100% succumb to rot.
4. Freezing tolerance testing is a time-consuming process and the parameters that influence variation is to some extent still unknown to us.

Conclusions/next steps

This study provides for the first time evidence that substantial genetic variation for root freezing tolerance exists among yellow cedar trees in the GBR. To date only about 15% of collected genotypes have tested for root freezing tolerance, with the most tolerant genotypes having 40% less injury at tested temperature than the average. A graduate student will continue the testing of the remaining genotypes and is likely to find other freezing tolerant genotypes. Once a larger population has been tested and observed differences confirmed in independent tests, we will be able to publish our findings. We have also received permission to test the BC Forests, Lands, Natural Resource Operations and Rural Development population of 360 genotypes originating from across the yellow cedar growth range for freezing tolerance. We are planning follow-up grant proposals to increase the testing and also to carry out the first crosses of the most freezing-tolerant genotypes, with the intention to identify offspring that are potentially more freezing tolerant than any of the parents. Field trials will also be required before genotypes can be used as stocks for planting. In summary, the results provide proof of concept that genetic variation for freezing tolerance exists in yellow cedar, providing for the first time a route to mitigate the spread of yellow cedar decline in BC. Since yellow cedar is a species of ecological (key forest tree species at higher altitude and at wet sites), cultural (Coastal First Nations) and economic significance (most expensive lumber of any Canadian species), a proactive approach to mitigate yellow cedar decline is likely to have long-standing and multiple positive effects in BC.

Checklist

- Have you submitted a short blog for BC Parks' website? I have set up a link at my SFU website, but if you point to a link, with instructions, I will be happy to set up the same material at the BC Parks site.
- Have you added any relevant Living Lab project data or reports to the BC Data warehouse and/or EcoCat? First year report was uploaded by Stephen. Please contact Jen Grant or Stephen Ban for assistance.
- Invoice submitted? An invoice is required to receive the final instalment of your Living Lab transfer agreement. The invoice should include:
  - the university address,
  - the Transfer Payment number (as per your agreement),

- a one-line description of what the project is about,
- the amount due (you may need to send this via your financial arm) and indicate that this is the final instalment. The invoice should follow or accompany the completion of this final report template of which both are due on or before March 22nd, 2021. **If we do not receive an invoice from you by this date, we will not be able to issue your final payment.**