March 2020

Living Lab Program for Climate Change and Conservation - Final Report

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

Research findings

We have visited more than 20 parks, conservancies and protected sites in the Great Bear Rainforest to collect yellow-cedar twigs, primarily from 3-50 m altitude. In addition, we (jim) was able to join helicopter-based visits to 100-300 m elevation sites over three days in the Prince Rupert and Work channel areas. In total, twigs, including naturally rooted twigs at soil level, were collected from ~150 trees/genotypes at various sites (collected 20-100m apart at each site to avoid clones and half siblings). We also took GPS coordinates and altitude data for most plants collected. At SFU, we built a high-humidity greenhouse with automatic misting system and used it 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, a total of three seedlings (20-30 cm height) were observed. All other observed smaller plants/bushes appear to be clones from underground runner shoots, especially at boggy sites. 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.

Collection and screening for freezing resistance:

- Rooted plants from ~ 50 trees

- 34 rooted genotypes cold hardening in 4C cold room for freezing tolerance tests at University of Victoria in April
- Cuttings with calli (first step towards rooting) from another ~40 trees, with a subset that have roots.
- In-vitro axenic shoot cultures of all trees with living foliage. This is a back-up to avoid losing genotypes due to rot in the traditional soil-based rooting.
- Freezing tolerance data from roots and shoots from three families of trees
- A set of ~ 300 genes that are expressed at higher levels in freezing tolerant shoots relative to freezing sensitive roots in seedlings 24 hours after removal from cold storage.
- 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.

Methods summary

- Plant culture and root freezing tolerance assays
- Twigs are brought back to SFU in black garbage bags and kept in cold room until use.
- Multiple (30-50) cuttings per tree dipped in rooting hormone with fungicide and placed in sunshine mix 4 in Styrofoam seedling blocks. Blocks are placed in a 2x10m greenhouse within SFU large greenhouse with periodical automated misting and heating from below as per established procedures. Air temperature is regulated to 15-18^{o}C. Ligh intensity is approximately 500 micro-Einstein (□E) at cutting level with 16-hour day. Rooted plants are transferred to larger pots, fertilized and placed at high intensity (~1000 □E) for root and shoot growth. Plants with roots at pot lining are moved to 4C cold room for cold hardening. to 15-20C by heating and ventilation. Light is ~500 □E at cutting level with 16-hour day. Fine roots and foliage are collected and separated into 300 mg portions and subjected for 24h to 4^{o}C (control), -8^{o}C, and -12^{o}C, in calibrated freezer with 3-5 biological replicates. Cellular damage from freezing of roots and foliage is assessed by electrolyte leakage assays using the equipment and expertise of Barbara Hawkins. 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 is assessed by measuring the electrical conductivity of a solution containing the frozen tissues. Relative electrolyte leakage curves are obtained across the range of tested temperatures and the inflection point on the curve is used to determine cold tolerance as described by Hawkins et al. 1994 and 2001.
- <u>In-vitro shoot cultures as a back-up alternative to rooting in soil to avoid losing genotypes to rot</u>
- In-vitro axenic cultures are established as per protocol for adult hard-to-root *Cupressus sempervirens*. Shoot tips are washed in 0.1% Tween 20 solution on shaker, surface sterilized in 40% bleach for 5 minutes, washed in sterile water five times, blotted dry on sterile filter paper, and cut at the base to remove bleach damaged tissue. Shoots are placed in test tubes containing Woody Plant Medium (WPM) supplemented with 0.8% agar, 0.1 mg/L benzyl adenine and 0.01 mg/L naphthyl acetic acid to stimulate cell division and shoot growth. Media also contains 100mg/L timentin and 0.5% plant protection medium to suppress microbial growth. Shoots are sub-cultured monthly until a length of 5-8 cm is reached, and thereafter placed hormone-free WPM supplemented with 0.01% activated charcoal to induce roots. Rooted plants are transferred to soil and gradually hardened to lower humidity before transfer to the greenhouse.
- <u>Identification of genes with potential role in in yellow-cedar freezing tolerance</u>
- Cold-hardened frozen (-2C) yellow cedar and Western hemlock seedlings (Western Forest Products) are placed in growth chamber with 16 hour light, 15C for 24 hours before collection of tissues for freezing tolerance assays and RNA extraction. RNA is purified as per standard CTAB procedure, quantified and used for RNAseq library construction (Lexogen, full-length mRNA V2 kit) and sequenced by Genome Quebec at the NovaSeq 4000 machine by paired end 100 base option. Sequence reads are trimmed for adapters and low-quality score, assembled on the Trinity platform, and used for relative gene expression quantification using the edgeR software package. Genes with higher expression in the freezing resistant foliage and with annotations related to freezing and abiotic stress tolerance are identified by gene ontology (GO) term analysis. Candidate genes are manually curated by comparison to related and functionally characterized genes with roles in cold and related drought tolerance pathways.

Key outcomes for BC Parks

Although the above description of ecological findings should be viewed as preliminary based on a total time of 10 days spent in the GBR, primary at low altitude and limited to the Douglas channel, Terrace, Prince Rupert and Work channel areas, the study nevertheless suggest several working hypotheses around the distribution and survival of yellow-cedar, a tree species that in Canada is primarily found in the GBR:

- Existing maps of yellow-cedar distribution overestimates 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 that these areas do not provide protection to this species. Higher altitude areas were not assessed.
- All areas 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.

Relevance to BC Parks management

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 a larger and 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. It is currently unknown where trees with higher root freezing resistance can be found. Higher altitude sites are colder, but roots are typically covered by insulating snow. Low altitude sites to the South of current decline may, on the other hand, have been selected for a longer time to the adverse thaw-freeze conditions that results in decline. We intend to explore these hypotheses in the second year of the project.
2. 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.
3. The probability of finding yellow-cedar trees increase at higher altitude up to the tree line. However, these sites are also difficult to reach and require either the use of helicopter, or targeted multi-day hikes in potentially dangerous environments.
4. 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. We have therefore begun using in-vitro axenic cultures as a safer, albeit labor-intensive, back-up option.
5. At the highest temperature amenable to reproducible freezing (-8^{o}C, Barbara Hawkins lab), the roots of all tested genotypes freeze to death. Two not mutually exclusive explanations are (a) YC roots do not acquire meaningful freezing resistance (b) freezing resistance exists in the 0 to -8^{o}C range. We are building a freezing apparatus based on circulation of anti-freeze rather than current air-based freezing in freezer to test the -2 to -8 range.
6. The project needs longer-term funding and dedicated graduate students to succeed. I am working on both aspects and have an MSc candidate working on the project.

Conclusions

It is too early to say if natural genetic variants with higher than average freezing resistance exists as only a few half-sib families have been tested to date, all being highly sensitive to minus 8C, and as the first larger batch of trees will be tested in April. The second year of funding should allow us to obtain answers to this question.

References and links

BC Ministry of Environment and climate change strategy press release, slated for March 21, 2020.

The project has also been presented to fellow scientists at formal and informal talks, however not yet published on-line.

Contact info

Dr. Jim Mattsson, Department of Biological Sciences at SFU, jmattsso@sfu.ca, 778 782-4291/4594