**Acadia University**

**K.C. Irving Environmental Science Centre and Harriet Irving Botanical Gardens**

# EASTERN WHITE CEDAR PROJECT

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## NOVA SCOTIA SPECIES AT RISK CONSERVATION FUND

## FINAL REPORT

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## Executive Summary

Eastern White Cedar (*Thuja occidentalis*) is listed as a Vulnerable native species in Nova Scotia (http://novascotia.ca/natr/wildlife/biodiversity/species-list.asp).The K.C. Irving Environmental Science Centre and Harriet Irving Botanical Gardens has completed this research as part of its ongoing commitment to developing and supporting conservation and recovery strategies for threatened native species of the Acadian Forest Region. To ensure appropriate planting material is available for future conservation and restoration initiatives with this species, seed and branches were collected in fall 2013 and branches only in spring 2014 from five sample trees in each of five distinct habitat locations in Nova Scotia. Germination and cutting trials were completed to provide plants from both seed and branch sources. In addition, preliminary tissue culture trials were completed with shoot sample material from one of the sites, in order to determine the potential for also utilizing this approach as a tool in conservation strategies. Following cutting and germination trials and subsequent transition stages of propagated material under controlled phytotron growth conditions at the K.C. Irving Environmental Science Centre, a population of 468 potted plants has been established. This accessioned ex situ collection represents all sample sites and trees, with 80 % consisting of at least 5 plants. This material is now ready for planting on the Harriet Irving Botanical Gardens Woodland Trails at Acadia University.

## Collections

Seed and branch samples were collected in five sites representing distinct cedar habitats in Nova Scotia, including Rockland (RL), Kings County, Hectanooga (HN) and Cedarwood Lake (CL), Digby County, West Paradise (WP), Annapolis County and Thompson Station (TS), Cumberland County. A map link for the selected sites was provided in the interim report ( November 2013). Cones and branches were sampled from each of five mature trees at each site. The first series of cone and branch collections were completed from September to November 2013 (included two collections from the RL site). A second series of collections (branches only) were completed at all sites from March to April 2014. Sample material was stored at 4 C prior to processing for cutting and germination trials. Voucher specimens were collected and processed for the first and second series of collections as described in the interim report (November 2013). These have been deposited in the E.C. Smith Herbarium (ACAD), part of the Irving Biodiversity Collection at the K.C. Irving Environmental Science Centre.

## Cutting Trials

All samples were brought to the K.C. Irving Environmental Science Centre and stored in a walk-in cooler at 4 C. Terminal branch samples were prepared for cutting trials by trimming 8-10 cm heel cuttings and dipping the basal ends for 10 sec in 1000 ppm IBA ( Indole-butyric acid) dissolved in 50% isopropyl alcohol. Cuttings were inserted directly in a perlite-peat-sand bed with overheard misting for 10 sec at 20 minute intervals. Bottom heat was provided with heating cables under the substrate. Adjustments were made to the protocol following the initial results from trial 1 ( with branch samples collected in fall 2013). In trial 2 (with branch samples collected in spring 2014), both primary (terminal portions of branches) and secondary (subtending lateral portions of branches) cuttings from three of the sites (HN,CW,WP) were treated.

**Cutting Trial 1:**

Terminal cuttings from branch samples collected in fall 2013 showed variable rooting frequencies among the collection sites. Overall rooting frequencies for all five trees from each site were as follows : HN (4.0%), WP (36.0%), TS (0%), CW (60.0%), RL (32.0%). Individual trees within sites also showed variable results, with ranges as high as 0-100% observed among cutting samples from the five trees per site. As a result, several trees and sites were not represented in the rooted cutting population from trial 1.

**Cutting Trial 2:**

A second collection was completed from trees in the same sites during March to April 2014 in order to determine if cuttings rooted at higher frequencies during this collection period. In addition to five primary cuttings per sample, secondary cuttings from lateral branches were also tested among material collected from the HN, CW and WP sites. The additional testing was included in order to determine if secondary cuttings would form roots with similar frequencies to primary cuttings, and possibly to provide an augmentative source of rooted cuttings from the same amount of sample material. Rooting among cuttings was evident within three months (Fig 1). Higher rooting frequencies were generally observed in trial 2 compared with trial 1, among cuttings from four of the five sites. Results from the HN site appeared most consistent in trial 2, with rooting frequencies of 80 to 100 % and 73.9 to 86.4 % among the primary and secondary cuttings, respectively. In contrast, rooting results from the CW site ranged from 0 to 80 % for primary cuttings and 11.4 to 69.2 % for secondary cuttings. Within cutting samples from sites TS and RL (where secondary cuttings were not tested), three of the trees were not represented at all due to low rooting frequencies among the primary cuttings. In general, the same trend of variability was found among cuttings in trial 2 as with trial 1, with respect to site and tree origin. Results from cutting trial 2 are summarized in Table 1.

## Tissue culture

Shoot portions (0.5 – 1.0 cm) were removed from branches collected at the RL site only. The shoot sections were sterilized using a 30 sec exposure to 70% isopropyl alcohol, rinsed in DI water and exposed to 20% bleach and 1 ml of Tween 80 (surfactant) for 10 minutes. Following the bleach treatment, shoot portions were rinsed four times in autoclaved deionized water. After final excision of stem tissue, explants were placed in vials with a basal culture medium to support growth and development. Microbial contamination and shoot necrosis were evident among cultures, which required treatment of antimicrobial agents and modification to the culture media components. Viable non contaminated stock cultures were eventually established for all five tree sources from the RL site, and maintained effectively for several months (Fig 7). New shoot growth and incidences of spontaneous rooting (10.3 %) were observed among the cultures (Fig 8). These initial results indicated the feasibility of utilizing tissue culture as another tool for potential application in the preservation and propagation of Eastern White Cedar.

## Seed Germination Trials

Cones collected from each site in fall 2013 were placed into storage containers at 4 C within the K.C. Irving Environmental Science Centre Seed Bank. Seeds were manually extracted, cleaned and placed into envelopes, both for germination trials and possible reserve for longer term storage. Seeds allocated for germination trials were initially soaked for 1 hour under running tap water, transferred to Petri plates containing filter paper moistened with deionized water and placed under stratification conditions at 4 C for 2 months. This method proved ineffective due to drying of the filter paper and high rates of fungal contamination. Seeds were subsequently surface sterilized following imbibition by treating with 20% bleach and 1ml of Tween 80 for 20 minutes, followed by 4 rinses in autoclaved deionized water. Seeds were then placed on sterilized agar gel substrate in Petri plates and wrapped with Parafilm. Plates were stratified for 2 months at 4 C, and subsequently placed under CW fluorescent lights (8 hour photoperiod) and temperatures of 21-25 C. Germination was monitored over a two month period (Fig. 4). Results from the main germination trial are summarized in Table 2. Variability in response was observed among seed from the five sites. RL derived seed proved most responsive under the conditions tested with germination rates ranging from 6.6 to 27.4 %, while seed from the CW, WP and TS sites having at least one sample with 0 % germination.

## Potted Material for Planting

### Rooted cuttings

All viable rooted cuttings from trials 1 and 2 were initially potted into 4" containers and maintained under controlled growing conditions at the K.C. Irving Environmental Science Centre phytotron (Fig. 2). Viable plants were then transferred to 1 gal pots in the spring of 2014 (Fig. 3) and moved outdoors in the fall of 2014 to a transitional holding area (within the experimental garden beds at the K.C. Irving Environmental Science Centre) for overwintering (Fig. 9). Following an overwintering period, assessments of plant survival were completed in May 2015. Overall pot stage mortality for rooted cuttings from each of the five sites was < 20%. A summary of viable cutting derived plants from each site is provided in Table 3. Potted material will be held outdoors a second winter in a protected area and planted along the Harriet Irving Botanical Gardens Woodland Trails in spring 2016.

### Seedlings

Seedlings from the germination trials were transplanted into trays (Fig. 5) and grown under similar controlled conditions as the rooted cuttings. Seedlings showed slower growth rates and higher mortality than rooted cuttings under these conditions. Viable seedlings were transferred to 4 inch pots and kept in the phytotron controlled conditions till August 2015 (Fig 6). Plants were later transferred to 1 gal pots and also moved outdoors to the experimental garden in fall 2015 (Fig. 10), prior to planting in the Harriet Irving Botanical Gardens Woodland Trails. Overall tray and pot stage seedling mortality rates for all seed samples in each of the five sites ranged from 40 to 100 %. Numbers of remaining viable seedling derived plants and individual post potting survival frequencies are provided in Table 3. Potted seedling material will be overwintered and planted along with the cutting derived material in spring 2016.

Table 1 Summary of rooting frequencies for cutting trial 2 (winter 2014 collections)

| Cutting Source | Tree | # PC | # SC | # Rooted PC (%) | # Rooted SC (%) |
|---|---|---|---|---|---|
| HN | 1 | 5 | 46 | 4 (80.0) | 34 (73.9) |
| | 2 | 5 | 26 | 5 (100) | 23 (88.5) |
| | 3 | 5 | 44 | 4 (80.0) | 38 (86.4) |
| | 4 | 5 | 43 | 5 (100) | 38 (88.4) |
| | 5 | 5 | 21 | 4 (80.0) | 21 (85.7) |
| CW | 1 | 5 | 45 | 2 (40.0) | 27 (60.0) |
| | 2 | 5 | 42 | 4 (80.0) | 23 (54.8) |
| | 3 | 5 | 41 | 4 (80.0) | 9 (22.0) |
| | 4 | 5 | 26 | 3 (60.0) | 18 (69.2) |
| | 5 | 5 | 44 | 0 (0) | 5 (11.4) |
| WP | 1 | 5 | 34 | 5 (100) | 15 (44.1) |
| | 2 | 5 | 37 | 2 (40.0) | 5 (13.5) |
| | 3 | 5 | 42 | 4 (80.0) | 29 (69.0) |
| | 4 | 5 | 46 | 2 (40.0) | 27 (58.7) |
| | 5 | 5 | 45 | 5 (100) | 36 (80.0) |
| TS | 1 | 5 | - | 1 (20.0) | - |
| | 2 | 5 | - | 0 (0) | - |
| | 3 | 5 | - | 0 (0) | - |
| | 4 | 5 | - | 1 (20.0) | - |
| | 5 | 5 | - | 2 (40.0) | - |
| RL | 1 | 5 | - | 4 (80.0) | - |
| | 2 | 5 | - | 3 (60.0) | - |
| | 3 | 5 | - | 3 (60.0) | - |
| | 4 | 5 | - | 3 (60.0) | - |
| | 5 | 5 | - | 0 (0) | - |

Table 2 Summary of germination trials from seeds collected in fall 2013

| Seed Source | Tree | # Seed | # Germinants | % Germination |
|---|---|---|---|---|
| HN | 1 | 232 | 3 | 1.3 |
| | 2 | 307 | 4 | 1.3 |
| | 3 | 173 | 1 | 0.6 |
| | 4 | 390 | 11 | 2.8 |
| | 5 | 101 | 1 | 1.0 |
| CW | 1 | 218 | 0 | 0 |
| | 2 | 280 | 6 | 2.1 |
| | 3 | 118 | 1 | 0.8 |
| | 4 | 112 | 0 | 0 |
| | 5 | 120 | 1 | 0.8 |
| WP | 1 | 68 | 0 | 0 |
| | 2 | 165 | 5 | 3.0 |
| | 3 | 115 | 1 | 0.9 |
| | 4 | 228 | 10 | 4.4 |
| | 5 | 90 | 0 | 0 |
| TS | 1 | 29 | 0 | 0 |
| | 2 | 80 | 7 | 8.8 |
| | 3 | 74 | 1 | 1.4 |
| | 4 | 56 | 5 | 8.9 |
| | 5 | 100 | 27 | 27.0 |
| RL | 1 | 61 | 4 | 6.6 |
| | 2 | 260 | 26 | 10.0 |
| | 3 | 82 | 7 | 8.5 |
| | 4 | 135 | 37 | 27.4 |
| | 5 | 100 | 17 | 17.0 |

Table 3 Summary of viable potted material from cutting trials (as of May 2015) and germination trial (as of October 2015)

| Cutting Source | Tree | Cutting Trial #1 | Cutting Trial #2 | Germination Trial | Total |
|---|---|---|---|---|---|
| HN | 1 | 1 | 33 | 0 | 34 |
| | 2 | 0 | 26 | 0 | 26 |
| | 3 | 0 | 39 | 0 | 39 |
| | 4 | 0 | 38 | 4 | 42 |
| | 5 | 0 | 18 | 0 | 18 |
| CW | 1 | 0 | 29 | - | 29 |
| | 2 | 4 | 20 | 0 | 24 |
| | 3 | 2 | 14 | 0 | 16 |
| | 4 | 5 | 17 | - | 22 |
| | 5 | 2 | 5 | 0 | 7 |
| WP | 1 | 2 | 17 | - | 19 |
| | 2 | 0 | 4 | 0 | 4 |
| | 3 | 2 | 33 | 0 | 35 |
| | 4 | 2 | 27 | 0 | 29 |
| | 5 | 3 | 39 | - | 42 |
| TS | 1 | 0 | 1 | - | 1 |
| | 2 | 0 | 0 | 2 | 2 |
| | 3 | 0 | 1 | 1 | 2 |
| | 4 | 0 | 2 | 2 | 4 |
| | 5 | 0 | 0 | 19 | 19 |
| RL | 1 | 3 | 4 | 1 | 8 |
| | 2 | 3 | 1 | 8 | 12 |
| | 3 | 4 | 3 | 1 | 8 |
| | 4 | 0 | 3 | 9 | 12 |
| | 5 | 3 | 3 | 8 | 14 |

## Strategies and Recommendations

Propagation trials involving cone and branch collections of Eastern White Cedar trees from five tree specimens among five sites in Nova Scotia have provided a representative total population of 468 seed and cutting derived plants. This population is now available for planting in the Harriet Irving Botanical Gardens Woodland Trails, and will provide a source of accessioned ex situ material to support education, conservation and restoration initiatives for this species. Verification of planting, including design with respect to site and tree source, will be provided in spring 2016.

Although a substantial number of plants were derived from the propagation trials in this study, the effectiveness of both seed and cutting based methods showed limitations in germination and rooting frequencies and survival of plants. These issues may require further attention in order to more fully implement propagation methods as part of the development of effective ongoing ex situ strategies for supporting conservation and restoration activities.

Propagation from cuttings proved more effective than seed in terms of total number of plants produced and site/tree samples represented. Low germination rates limited the number of available seedlings for planting, as well as higher mortality after planting. There was also more time required to germinate and grow seedlings to a similar size as the rooted cuttings. This aspect delayed the project timeline.

The issue of low germination rates also needs to be considered further as it impacts the effectiveness of possible future development of an ex situ seed bank collection for this species. Additional trials are recommended to study seed viability and germination conditions more extensively. This research could be done in consultation and collaboration with the National Forest Tree Seed Centre in Fredericton, NB, which currently holds samples of Eastern White Cedar in its seed bank.

Results from cutting trial 2 indicated that secondary shoot cuttings generally rooted as well as primary shoot cuttings, and thus could be utilized to augment rooted cutting populations. There appeared to be no discernable difference between rooted cuttings of primary and secondary shoot origin, in relation to morphological aspects such as branching and plagiotropism. Thus it appears secondary shoot cuttings can be utilized to ensure sufficient numbers of plants are obtained from branch samples of individual trees. However, increasing numbers of rooted cuttings per branch sample will not address the potential issue of ensuring sufficient genetic diversity, if cuttings are utilized to a greater degree than seed for establishing ex situ plants for possible application in conservation and restoration activities. Therefore, a greater number of trees may need to be sampled in order to provide a sufficient genetic mosaic to represent natural populations of Eastern White Cedar.

In both cutting and seed propagation trials, intra- and inter-site variability in response was observed under the conditions used for testing rooting and germination capacities. This is an important aspect that needs to be considered when developing an ex situ strategy for effective preservation of biodiversity and provision of sufficient planting stock for possible future restoration activity. The TS site may be of particular concern at present. It was noted that the collected branch material appeared to be of low vigor, with relatively small new growth increments compared with branches from other sites. Rooting frequencies were also lowest among primary cuttings tested from this site. Habitat and health status of trees in this site should be further investigated.

Initial results from tissue culture trials suggested the potential utilization of this approach as an augmentative conservation strategy for longer term maintenance of shoot material arising from branch samples of Eastern White Cedar. However, microbial contamination and progressive necrosis may need to be further addressed in future studies. This approach may also offer an alternative means of propagation from seed samples with low germination rates. Further research should focus on testing the propagation of seedlings arising from germination trials. Procedures for more effective surface sterilization of seed and culture on various enriched media to control microbial contamination may need development to further enhance initiation, stabilization and multiplication efficiencies.

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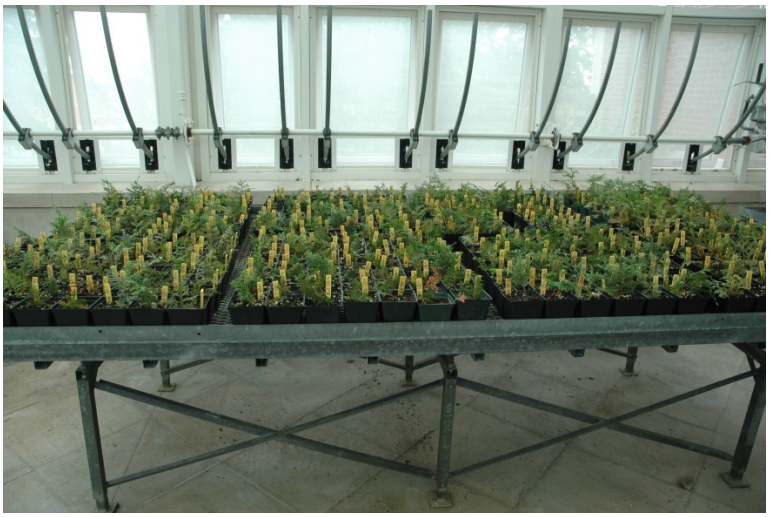
FIGURES 1-3 Rooted cuttings in propagation bed, planted material in 4 inch pots for initial growth stage under phytotron, conditions, and later growth stage in 1 gal pots



FIGURES 4-6 Seedlings from germination trials; transition from agar plates to propagation trays to 4 inch pots

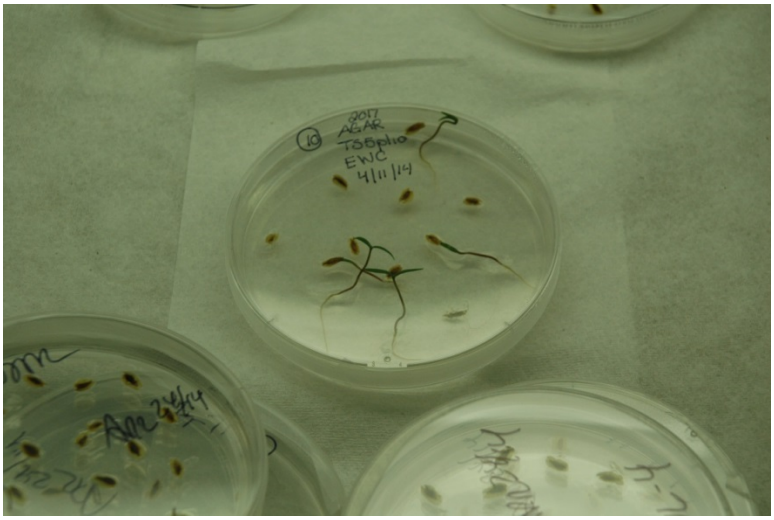



FIGURE 7 Tissue culture population of shoot explants from RL site branch samples

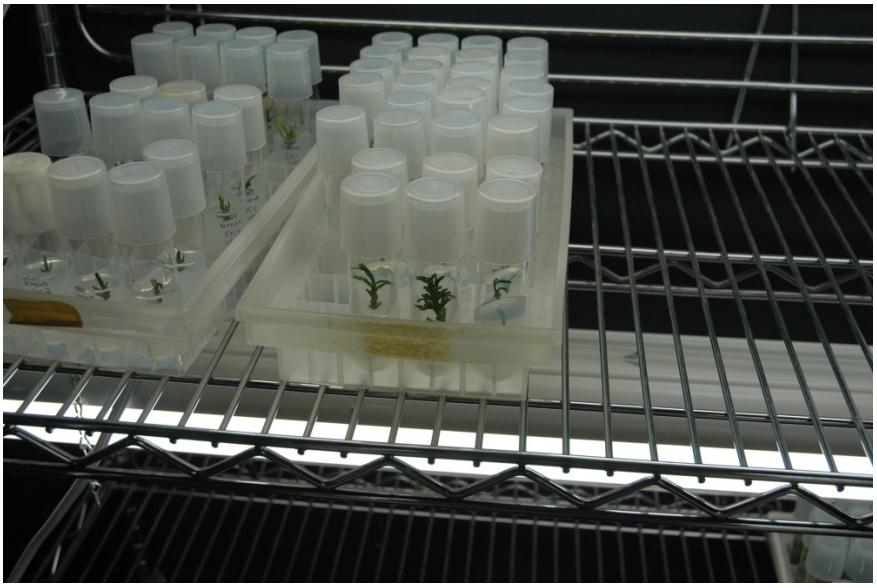


FIGURE 8 Individual shoot culture showing root formation

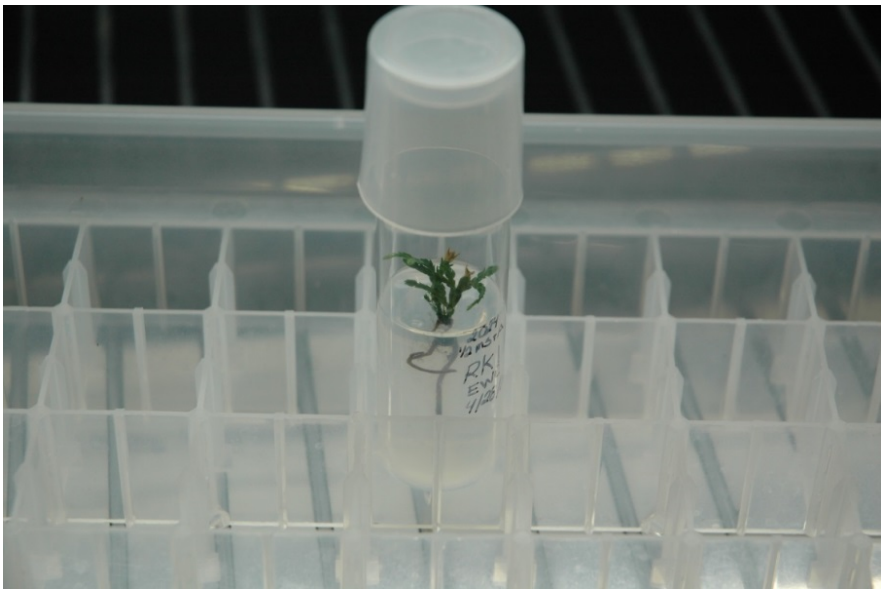


FIGURE 9 Population of potted rooted cuttings (fall 2014) in outdoor holding area prior to planting

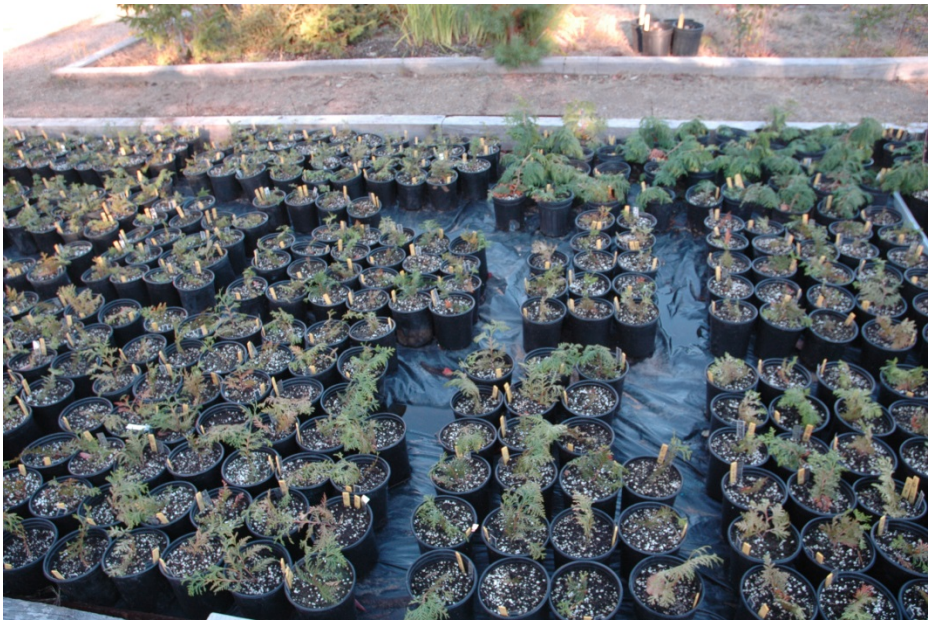


FIGURE 10 Population of potted seedlings (fall 2015) in outdoor holding area prior to planting