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Contrasting root and shoot cold tolerance of a conifer with indeterminate growth

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Abstract

Yellow-cedar (*Callitropsis nootkatensis* (D.Don) D.P. Little), a conifer in the Cupressaceae with indeterminate growth, is undergoing severe decline and mortality in southeast Alaska and the coast of northern and central British Columbia. This decline is attributed to cold damage to roots associated with climate warming and reduced snowpack. The cold tolerance of indeterminate conifer species is little studied, and less is known about root cold tolerance, as most studies focus on shoots. We compared the seasonal cycle of cold hardiness in roots and shoots of yellow-cedar seedlings from high and low elevation populations over one year. Freezing tolerance of shoots followed a typical seasonal cycle with low levels of cold tolerance observed from April to October and moderate levels of cold tolerance observed in mid-winter. Differences in shoot cold tolerance among populations were consistent with the latitude and elevation of origin. In contrast, at the same freezing temperatures, roots of all seedlings had consistent, high levels of cold damage throughout the year.

Key words

Yellow-cypress, *Callitropsis nootkatensis*, *Chamaecyparis nootkatensis*, root cold hardiness, shoot cold hardiness, seasonal cycle

Introduction

To survive sub-zero winter temperatures in cool temperate and boreal regions, conifers and other trees undergo genetic and physiological changes that allow them to avoid or tolerate freezing. A wealth of literature exists on genetic and environmental influences on cold tolerance of conifers; however, the bulk of this literature focuses on shoot cold tolerance. Relatively few studies of cold hardiness include roots. This represents a gap in our understanding of tree response to cold, because viable, healthy roots able to conduct water and nutrients are critical to the resumption of active shoot development and growth in spring. Another under-studied aspect of conifer cold tolerance is the annual cycle of hardening and dehardening in species with indeterminate growth. These indeterminate species do not form overwintering buds, nor enter a true dormant phase (Puttonen and Arnott 1994, Hawkins et al. 2001). Most studies of cold tolerance in conifers are conducted with members of the Pinaceae with determinate growth, and much less work has been conducted with indeterminate species in the Cupressaceae or Podocarpaceae. In contrast to determinate species, cold hardening in indeterminate conifers is initiated primarily by low temperatures, rather than a shortening photoperiod (Silim and Lavender 1994).

The cold tolerance of yellow-cedar (*Callitropsis nootkatensis* (D.Don) D.P.Little) is of particular interest since the species has indeterminate growth, is found in cool to cold environments from Alaska to California, and is negatively affected by cold damage to roots (Hennon et al. 2012). Yellow-cedar is known by many names, including yellow cedar/cypress, Nootka cedar/cypress and Alaska (yellow) cedar/cypress, and its taxonomic placement has been debated for decades with formal names including *Chamaecyparis nootkatensis* (D.Don) Spach, *Cupressus nootkatensis* D. Don, and *Xanthocyparis nootkatensis* (D.Don) Farjon & D.K.Harder (Little et al. 2004, Mill and Farjon, 2006, Adams et al. 2009, Mao et al. 2010). Yellow-cedar has a large latitudinal range along the Pacific Northwest coast - from Prince William Sound in south central Alaska to the Siskiyou Mountains of northern California (Harris 1990). Apart from a few isolated, inland stands, the species is generally found within 200 km of the Pacific coast (Harris 1990, Buma et al. 2017). Yellow-cedar typically grows at higher elevations, between 600 - 2300 m in southern B.C. and 1200 – 2200 m in the southern part of its range, but north of 51 °N latitude, it may be found to sea level (Harris 1990). Yellow-cedar is currently undergoing severe decline and mortality throughout its entire range but the highest mortality rates occur in southeast

Alaska and along the coast of northern and central British Columbia (Buma et al., 2017). Since the 1880s, approximately 243,000 hectares in southeast Alaska (Mulvey 2021) and 47,000 hectares in northern B.C. have been affected by yellow-cedar decline (Westfall and Ebata 2009). Research on this phenomenon began in the 1980s and the first hypothesized causes of mortality were biotic pests such as fungi, insects, nematodes, viruses and mycoplasmas, or bears, but all of these possible causative agents were ruled out during the 1990s (Hennon et al. 2006). In the early 2000s, researchers began to evaluate abiotic factors, particularly soil chemistry, in areas with high yellow-cedar mortality, but they found no relationship between element toxicity, depletion, or pH imbalance and tree death (D'Amore and Hennon 2006, Hennon et al. 2006). Abiotic environmental factors, mainly soil and air temperatures, were found to best correlate with yellow-cedar decline. This led to the currently accepted hypothesis that yellow-cedar decline is occurring due to a changing climate (Wooton and Klinkenberg 2011, Hennon et al. 2012, Buma et al. 2017, Comeau et al. 2019).

Anthropogenic climate change is now widely acknowledged as a contributor to global forest decline. Between 1997 and 2012 in the United States, 'decline-type mortality events', which are often due to the crossing of an environmental threshold, disturbed more forested area than fire and harvesting, combined (Cohen et al. 2016). Within yellow-cedar's range, global warming is causing reduced snowpack and a shortening of the winter season, leaving plant tissue at a higher risk of freezing injury. Early loss of snowpack exposes soil and shallow roots to fluctuating air temperatures, whereas sufficient snowpack cover insulates the soil and protects the roots (Schaberg et al. 2011, Ambroise et al. 2020). While an overall trend of warming winters is being observed, this results in potentially injurious conditions for yellow-cedar due to reduced snowfall and early loss of snowpack, combined with frequent occurrence of severe thaw-freeze events (Beier et al. 2008). A number of studies have reported that yellow-cedar mortality is higher in open-canopy forests at low elevations with typically wet soils, or on shallow soils over bedrock (Hennon et al. 1990, Schaberg et al. 2005, Wooton and Klinkenberg 2011). Wet soils or shallow bedrock result in shallow root systems. In exposed soils under open canopies, these shallow roots may be subject to early dehardening in the spring, risking freezing injury if a cold snap occurs. Recent studies on yellow-cedar decline on the islands of Haida Gwaii, B.C., observed especially severe freezing damage to roots on sites with wet soils and open canopy conditions (Comeau et al., 2019). As climate change results in this loss of mature, seed-

producing trees of a particular species, restructuring of forest communities over large regions may be observed (Bisbing et al. 2019).

To combat yellow-cedar decline, root cold hardiness could be incorporated as a selection trait in tree improvement programs for the species. To that end, it would be desirable to know the annual cycle of root cold tolerance and whether variation exists in this trait so that genetic testing programs could be more focussed. While many studies focus on cold tolerance of yellow-cedar shoots, less is known about root cold tolerance. Our objective was to follow the seasonal cycle of cold hardening and dehardening in four populations of yellow-cedar from high and low elevations over one year to observe the relative level and depth of cold hardening in roots and shoots of this indeterminate species.

Materials and Methods

Four populations of yellow-cedar were selected to test variation in freezing tolerance among populations from contrasting regions. Two of the groups were wildstand provenances: John Day (JD) seeds were collected from old growth yellow-cedar in Oregon at 44.317 °N, 119.082 °W and an elevation of 1375 m (-6.3 °C winter mean minimum temperature), and Coquihalla (Coq) seeds were collected from old growth yellow-cedar in southern B.C. at 49.617 °N, 121.033 °W and an elevation of 1140 m (-8.4 °C winter mean minimum temperature). The other two groups were first generation progeny of controlled crosses. Family 329 was a cross between a female yellow-cedar from Buck Lake, CA (41.81 °N, 123.69 °W, elevation 1360 m) and a male yellow-cedar from John Day, OR (44.32 °N, 119.08 °W, elevation 1375 m) (-0.8 °C and -6.2 °C winter mean minimum temperatures, respectively). Family 372 was a cross between a female yellow-cedar from Dago Creek, B.C. (49.87 °N, 117.92 °W, elevation 1700 m) and a male yellow-cedar from Evans Lake, B.C. (49.85 °N, 117.7 °W, elevation 1690 m) (-10.4 °C and -10.1 °C winter mean minimum temperatures, respectively). Winter mean minimum temperatures were calculated by ClimateWNA from 1961-1990 climate normals (Wang et al. 2016). The crosses were achieved by grafting scions collected from the parent trees and performing controlled breeding to produce seed.

Seed was stratified in the fall of 2017 for approximately five months. In February 2018, seed was germinated and planted in 125 mL containers in the Cowichan Lake Research Station greenhouse (48.818 °N, 124.126 °W) following standard cultural practices (Russell et al. 1990).

In May 2019, the seedlings were planted in larger (336 mL) containers and maintained in an unheated greenhouse. In mid-September 2019, the seedlings were moved to an outdoor, unshaded, fenced compound where they were exposed to occasional sub-zero temperatures each month from October 2019 to April 2020, with a minimum air temperature of -10.5 °C in January 2020 (Environment Canada Historical Weather Data, Lake Cowichan, B.C.) .

Freezing tests were conducted at four- to five-week intervals every month from August 2019 – August 2020 at the University of Victoria. Eight seedlings per population were tested each month until March 2020, and then due to restrictions on research imposed by the COVID-19 pandemic, four seedlings per population were tested. JD seedlings were not available for the final August 2020 test. On each test date, seedlings were removed from their containers and approximately 10 fine roots (< 2 mm diameter) from each seedling were immediately chopped into 5 mm segments and 10 segments were placed into each of four vials, containing 0.2 mL of water. The non-lignified leader on the seedling stem was stripped of its scale-like leaves, chopped into 5 mm segments, and 5 segments were placed into each of 4 vials, containing 0.2 mL of water. Samples were kept in the refrigerator at 4 °C overnight. There were 64 samples (4 provenances x 8 trees x 2 organs) from August 2019 – March 2020 and 32 samples (4 populations x 4 trees x 2 organs) from April -August 2020 per temperature (control, and test temperatures of -8 °C, -13 °C, and -18 °C) for a total of 256 or 128 samples, respectively. Yellow-cedar roots were expected to have low freezing tolerance based on results of earlier studies (e.g. Puttonen and Arnott 1994, Schaberg et al. 2008). From preliminary trials, a -8 °C test temperature was chosen as the warmest freezing treatment that would reliably induce freezing of root and shoot tissues, and a ten degree spread in test temperatures was expected to induce a range of damage in both shoots and roots throughout the year.

Control sample vials were kept in the refrigerator and the rest were frozen in a programmable freezer at a cooling rate of 5°C per hour. After reaching -8°C, the samples were held at this temperature for an hour, after which all sample vials for that test temperature were removed and placed in the refrigerator to thaw. The cooling and freezing cycle continued for the -13°C and -18°C test temperatures. After thawing overnight, 10 mL distilled water was added to every vial, including the control samples, and the vials were shaken for 24 h. Then, the first conductivity measures were made ($\mu\text{S}/\text{m}$) (Jenway 4020 Conductivity Meter). After measurement, all samples were heat-killed in an oven at 100°C for 2 h, shaken at room

temperature overnight, and then measured for maximum electrolyte leakage. The index of injury was calculated from the conductivity measurements of the test and control vials, after treatment and heat kill, respectively (Flint et al. 1967).

Index of injury data were analyzed by month with analysis of variance (PROC GLM, SAS Institute Inc. 1989). The main effects in the model were as follows: the population (4 levels), organ (2 levels) test temperature (3 levels), replicate (8 or 4), plus the respective interactions. Means were compared by least significant difference tests (LSD) and differences were considered statistically significant if $P \leq 0.05$.

Results

Freezing tolerance of yellow-cedar shoots followed a typical seasonal cycle with high levels of freezing damage observed from April to October and moderate levels of freezing tolerance observed in mid-winter, from December to February (Figure 1A). In contrast, roots of yellow-cedar seedlings had consistent, high levels of freezing damage throughout the year. Mean index of injury of roots, averaged over the three freezing temperatures, ranged between 85 and 100% in all months of the year, indicating very low levels of freezing tolerance, even in winter (Figure 1B). In every month except August 2019, roots sustained significantly more freezing damage than shoots (Table 1, Figure 1). Higher mean index of injury values in shoots in August 2019 compared to August 2020 (Figure 1) was likely due to the greater freezing sensitivity of greenhouse-grown seedlings (2019) compared to those growing outdoors (2020).

Populations typically exhibited significant differences in cold hardiness (Table 1). In most cases, the Population x Organ interaction was not significant, indicating that a similar ranking of populations was observed for both root and shoot hardiness (Table 1). In the winter months of December, January and February, the B.C. populations, Coquihalla and 372 were, on average, more hardy than the U.S. populations – particularly for roots (Figure 1).

Freezing temperature had a significant effect on index of injury in all months except June (Table 1), when similar, high levels of damage were observed at all test temperatures in roots. In all months other than June, and for June shoots, damage increased with lower test temperatures (Figure 2). The Temperature x Organ interaction was significant in all months (Table 1) because shoots typically showed a wide range in damage between the -8 °C and -18 °C test temperatures

(Figure 2A), while roots showed relatively high levels of damage at all test temperatures (Figure 2B).

Discussion

Plant roots are generally known to be less cold tolerant than shoots and leaves (Ambroise et al. 2020). Several hypotheses have been proposed to explain this difference, including the high water content of roots facilitating intracellular ice formation, and the insulating effect of soil reducing root exposure to low temperatures that promote hardening (Ambroise et al. 2020). This trend of low root cold tolerance has also been observed in conifers (Coleman et al. 1992, Ryyppö et al. 1998), but few cool temperate or boreal conifer species show such minimal root hardening as is observed in yellow-cedar (Bigras et al. 2001). Coleman et al. (1992) observed temperatures resulting in 50% index of injury (LT_{50}) that ranged from -3 °C in September to -13 °C in December in *Abies lasiocarpa*, and from -3.5 °C in September to -7.5 °C over the same period in *Tsuga mertensiana*, both species from the same habitats as yellow-cedar. LT_{50} could not be estimated in our study because root damage was so great, even at -8 °C, that the extrapolation to 50% index of injury was above 0 °C (data not shown). Schaberg et al. (2011) compared root cold hardiness of mature trees of five sympatric conifers, including yellow-cedar, over the fall, winter and spring in Ketchikan, Alaska, and found that yellow-cedar had the least root cold tolerance of the five species.

Root cold tolerance of yellow-cedar has been measured in other studies (Puttonen and Arnott, 1994; Schaberg et al. 2008) but these studies did not assess cold tolerance throughout the year. Puttonen and Arnott (1994) showed that LT_{50} of roots of yellow-cedar rooted cuttings declined from a high of -3 °C to -12 °C over a 7.4 week acclimation period. Curiously, the -12 °C LT_{50} minimum was achieved in a long-day, warm temperature treatment as well as the hardening treatments. It is possible that adventitious roots formed on cuttings may achieve greater hardiness than seedling roots, or the rooted cuttings may have experienced drought, which can increase cold tolerance (Bansal et al. 2015). Schaberg et al. (2008) observed low levels of cold tolerance in roots of four-year-old yellow-cedar seedlings, with and without simulated snow cover, in February, March, April and May in Juneau, AK. In all months and all treatments, yellow-cedar roots were only cold tolerant to -5 °C. In the field, fine roots of mature yellow-cedar trees achieved the greatest cold tolerance of -6.2 °C in January (Schaberg et al. 2011). These results

match those of our study and suggest that root cold tolerance of seedlings and mature trees is comparable. The combined results of these earlier studies and our work suggest that future studies of root cold tolerance in yellow-cedar should include relatively warm, but still subfreezing temperatures (e.g. -5 °C) in testing.

The serious yellow-cedar decline observed across more than 243,000 hectares in Alaska (Mulvey 2021) and 47,000 hectares in British Columbia (Westfall and Ebata 2009) has been attributed to loss of winter snowpack increasing the likelihood of soil freezing and subsequent injury to fine roots of yellow-cedar that are shallow and not cold tolerant (Schaberg et al. 2011, Hennon et al. 2012). In areas affected by yellow-cedar decline, minimum soil temperatures of -8 °C and -13 °C have been observed at 7.5 cm depth in snow-free soils, accompanying minimum air temperatures of -9 °C and -14 °C, respectively, whereas snow-covered soils did not freeze (D'Amore and Hennon 2006, Hennon et al. 2010, respectively). Our study supports the hypothesis that yellow-cedar decline is related to freezing injury of shallow roots and offers only faint hope for selection of cold-tolerant populations, as we observed no significant differences in root cold hardiness of contrasting populations from California and British Columbia. Even cold hardiness of shoots of yellow-cedar shows less variation among populations that is observed in determinate conifer species, as seen in this study and others (Hawkins et al. 1994, Davradou and Hawkins 1998). Both quantitative and molecular genetic studies show yellow-cedar to be a generalist species with high phenotypic plasticity and low levels of local adaptation (Hennon et al. 2016). Variation among yellow-cedar populations does exist, however (reviewed in Hennon et al. 2016), therefore we suggest that selection programs for cold tolerance should focus on surviving individuals in areas exhibiting yellow-cedar decline and mortality.

Given that global change will likely lead to thinning snowpacks, silvicultural practises should identify and avoid high-risk sites. Wooton and Klinkenberg (2011) completed a landscape-level analysis of yellow-cedar decline on the British Columbia coast and found that elevation was the most significant difference between declining and healthy populations, but also observed the importance of slope, aspect, and the effect of soil drainage on yellow-cedar health. A greater proportion of populations in decline occur on gentle slopes while healthy populations are more frequently found on steeper slopes (24°-40°) (Wooton & Klinkenberg, 2011). Soil saturation could be a factor on the flatter areas, creating stands with open canopies and shallow roots (Hennon et al., 2016). Declining stands often have southwest or west aspects (Wooton &

Klinkenberg, 2011), which may experience warm afternoon temperatures causing snow melt and exposing roots. Yellow-cedar forests are not resilient to this climate change-induced mortality, and shifts in community composition and stand dynamics can be expected (Bisbing et al. 2019). Forest management options to preserve the species include planting yellow-cedar at higher elevations or on deeper soils where, even without snowpack, fine roots are protected (Hennon et al. 2016).

Healthy roots are vital to plant survival as they provide water, nutrients and stability to the plant. While the indeterminate growth pattern of yellow-cedar growth results in low shoot cold tolerance compared to the cold tolerance of sympatric, determinate conifer species (Schaberg et al. 2005), the cold sensitivity of roots may be the greatest threat to the health of yellow-cedar.

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Table 1. Significance (P values) of the main effects and their interactions in the ANOVA of index of injury values from monthly controlled freezing tests in 2019 and 2020 with shoots and roots (Organ) of yellow-cedar seedlings from four populations (Pop) frozen to three test temperatures (Temp).

	2019						2020							
Main effects and interactions	df	Aug12	Sep16	Oct7	Nov4	Dec9	Jan6	Feb3	Mar9	Apr14	May19	Jun15	Jul13	Aug10
Temp	2	0.0001	0.0001	0.0001	0.0001	0.0002	0.0001	0.0001	0.0001	0.0001	0.001	0.92	0.0001	0.0001
Organ	1	0.76	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Pop	3	0.006	0.001	0.29	0.21	0.01	0.009	0.007	0.0004	0.04	0.007	0.32	0.51	0.94
TxO	2	0.78	0.0001	0.0001	0.0001	0.005	0.0001	0.14	0.03	0.0001	0.003	0.0004	0.0001	0.0001
TxP	6	0.60	0.09	0.71	0.94	0.83	0.89	0.93	0.95	0.37	0.85	0.56	0.58	0.82
OxP	3	0.06	0.0001	0.51	0.50	0.44	0.07	0.05	0.07	0.48	0.01	0.25	0.22	0.47
PxOxT	6	0.74	0.04	0.26	0.32	0.11	0.54	0.95	0.15	0.66	0.55	0.51	0.60	0.46

Figure 1 Mean ($\pm$ S.E.) index of injury (%) averaged over three freezing temperatures, -8, -13 and -18 °C for stems (A) and roots (B) of four populations of yellow-cedar (329 and 372 controlled crosses, and Coquihalla (COQ) and John Day (JD) wildstand seed origin) tested each month for cold hardiness over one year. Population JD was not tested in the final month.

Figure 2 Mean ($\pm$ S.E.) index of injury (%) at three freezing temperatures, -8 °C (light grey), -13 °C (dark grey) and -18 °C (black) for stems (A) and roots (B) averaged over four populations of yellow-cedar tested each month for cold hardiness over one year.