Global Change Biology (2008) 14, 1–12, doi: 10.1111/j.1365-2486.2008.01577.x

Influence of simulated snow cover on the cold tolerance and freezing injury of yellow-cedar seedlings

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Abstract

It has been hypothesized that yellow-cedar [*Chamaecyparis nootkatensis* (D. Don) Spach] decline may result from root freezing injury following climate change-induced reductions in protective snow cover. To test this hypothesis, we measured the freezing tolerance and injury expression of yellow-cedar seedlings in three treatments that differed in the insulative protection they provided to soils during winter and spring: (1) full exposure to ambient temperatures (exposed treatment), (2) continuous protection from ambient temperatures via addition of perlite over pots (full protection), and (3) perlite protection only during winter and exposure to ambient temperatures during spring (partial protection). Foliage from all treatments was cold tolerant enough to prevent foliar freezing injury throughout the study period. However, on all sample dates, roots of seedlings from all treatments were only tolerant to about −5 °C – a level considerably warmer than the reported maximum cold tolerance for the species and well above the soil temperature recorded in the exposed treatment. As a result of this limited root cold tolerance, visibly uninjured roots of seedlings from the exposed treatment had significantly higher relative electrolyte leakage (REL) throughout the winter and early spring than seedlings in soil protection treatments. Seedlings from the exposed treatment also had significantly higher foliar REL values and greater visual foliar injury than seedlings from the other treatments starting in early spring. For both roots and foliage, REL measurements consistently detected tissue damage before visual injury was evident. Patterns of injury from both REL and visual injury assessments showed the same pattern: damage began with freezing injury to roots and subsequently became evident as foliar browning after spring temperatures increased. All seedlings in the exposed treatment eventually had 100% fine root damage and died. This progression of initial root damage followed by foliar browning and mortality after the onset of warming conditions is consistent with reports of yellow-cedar decline symptom development in the field.

Keywords: cold tolerance, decline, foliage, freezing injury, roots, soil temperature, yellow-cedar

Received 11 July 2007; revised version received 19 October 2007 and accepted 24 October 2007

Introduction

Yellow-cedar [*Chamaecyparis nootkatensis* (D. Don) Spach][1] is an ecologically, economically, and culturally important tree species that has experienced dramatic mortality in Southeast Alaska and nearby British Columbia for about 100 years (Hennon & Shaw, 1997; Hennon *et al.*, 2005). The extensive mortality, referred to as yellow-cedar decline, has now been documented on over 200 000 hectares (Snyder, 2005) and is not associated with fungi (Hennon, 1990; Hennon *et al.*, 1990b), insects (Shaw *et al.*, 1985), nematodes (Hennon

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[1]The taxonomic status of yellow-cedar is in question with the discovery of a tree species that has close phylogenetic affinity in northern Vietnam, *Xanthocyparis vietnamensis* Farjon & Hiep (Farjon *et al.*, 2002). Yellow-cedar may join the Vietnamese tree in this newly established genus as *Xanthocyparis nootkatensis* Farjon & Hiep. Whether this name, or the older *Callitropsis nootkatensis* (D. Don) Örest. (Little *et al.*, 2004), is adopted will be determined at the next International Botanical Congress in 2011 (Mill & Farjon, 2006).

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et al., 1986), viruses or phytoplasmas (Hennon & McWilliams, 1999) as primary agents of tree death. Because no biotic causal agent has been identified, it has been hypothesized that one or more abiotic factors have instigated the decline. Any plausible explanation of yellow-cedar decline must account for at least three lines of evidence associated with the current decline: (1) a documented change in climate or some other environmental factor synchronous with the onset of decline (around 1900), (2) the unique vulnerability of yellow-cedar relative to other tree species, and (3) greater levels of mortality in exposed sites due to soil saturation at lower elevations (Hennon *et al.*, 1990a). One hypothesis that does account for these factors is the proposition that climate change interacting with soil/site conditions has resulted in elevated rates of freezing injury that initiate tree mortality and stand decline (Hennon & Shaw, 1994, 1997; Hennon *et al.*, 2006).

The onset of yellow-cedar decline coincided with the beginning of a period of climatic warming after the Little Ice Age (ca. 1850), and the distribution of decline parallels milder winter temperature isoclines in the region (Hennon & Shaw, 1994, 1997). One consequence of milder winter temperatures that could influence yellow-cedar health is a change in the pattern of insulative snow cover. Because ambient winter temperatures often hover around freezing, and precipitation levels are commonly heavy, even small alterations in temperature can lead to precipitation dominated by rainfall, and subsequent reductions in the depth and duration of snowpack. Reductions in protective snow cover could increase possibilities for soil freezing, which would be especially damaging to roots where the depth of rooting is limited by soil water saturation (Hennon & Shaw, 1994, 1997). Beier *et al.* (2008) has documented a trend toward warmer February and March temperatures during the 1900s and less snow since the 1950s in the region experiencing cedar decline. This is consistent with large-scale observations of northern latitude warming trends (IPCC, 2007). The tight associations of dying yellow-cedar forests in areas of low snowfall and live cedar forests in areas of persistent snow (Hennon *et al.*, 2006) are consistent with the possibility that low snow cover increases opportunities for soil freezing and subsequent root injury that initiates tree mortality.

Another consequence of warmer winter temperatures that could result in increased freezing injury is the possibility of precocious dehardening that would increase the risk of freezing injury when more typical low temperatures occur (Strimbeck *et al.*, 1995; Leinonen *et al.*, 1997). Evidence suggests that yellow-cedar is more vulnerable to direct freezing injury than some sympatric species (Silim & Lavender, 1994; Schaberg *et al.*, 2005), and may be particularly prone to temperature-dependent dehardening (Puttonen & Arnott, 1994; Hawkins *et al.*, 2001) – although this may not be the case relative to western redcedar (*Thuja plicata* Donn ex D. Don) (Grossnickle & Russell, 2006). Our past research indicated that the foliage of mature yellow-cedar trees in Southeast Alaska is sufficiently cold tolerant in fall and winter to survive ambient cold exposure, but that this foliage may be uniquely vulnerable to premature dehardening and freezing injury in the spring (Schaberg *et al.*, 2005). Because published reports indicated that patterns of root cold tolerance generally follow seasonal patterns of foliar cold tolerance (Sakai & Larcher, 1987), we hypothesized that yellow-cedar roots would be uniquely vulnerable to freezing injury in the spring, especially if a lack of spring snow cover allowed for premature soil warming that induced root dehardening. This hypothesis, combined with new information on the association of yellow-cedar decline with a lack of late winter and early spring snowpack (Hennon *et al.*, 2006), helped form the basis for the study described here.

We propose that a combination of predisposing factors (e.g. a reduction in insulative snowpack with climate warming, soil drainage that influences the depth of rooting, soil warming that may result in precocious dehardening) and instigating factors (e.g. periodic low-temperature events) may interact to increase the likelihood of decline-inducing freezing injury – especially to vulnerable root tissues. To test if a lack of insulative protection of soils resulted in premature root dehardening, freezing injury, and plant decline, we manipulated the insulation around the pots of yellow-cedar seedlings by either burying pots in perlite to simulate snow cover, or exposing pots to ambient temperature fluctuations. Some pots were exposed to ambient temperatures throughout the winter and spring, whereas other pots were exposed to ambient fluxes only during the spring – this allowed us to distinguish between winter and spring damage to root systems. Measurements were made on a monthly basis from February through May to evaluate the timing of any treatment-induced influences on physiology and health.

Materials and methods

Seedling establishment and treatments

Yellow-cedar seedlings were grown in a medium of sand and perlite mixture (3 : 1) in plastic pots (22 cm tall, 22 cm diameter) for 1.5 years before the study and throughout the experiment. These seedlings were 4 years old, approximately 30 cm in height, and were grown from seed collected in 1993 at 180 m elevation on Mitkof Island in the central portion of Southeast Alaska at N56°31′, W132°46′. The experiment was con-

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ducted in Juneau, AK (N58°22′, W134°37′), at an elevation of 5 m above sea level during the 2004–2005 winter and 2005 spring. The site had a south–southeast aspect with full exposure to sunlight throughout the day.

A randomized complete block experimental design was used for this experiment. Before treatment initiation, 144 yellow-cedar seedlings were randomly separated into six experimental blocks. Perlite was added to some seedling groups within blocks to produce treatments that provided insulation over pots and simulated snow cover, which suppresses temperature fluctuation in soils and presumably protects yellow-cedar roots from acute freezing injury. Perlite has been used in other experiments to insulate roots from winter freezing injury (e.g. DeHayes *et al.*, 1999; Schaberg *et al.*, 2000). Within each block, eight seedlings were randomly assigned to each of three simulated snow cover treatments: (1) exposed (no protection from added perlite), (2) partial protection (perlite covered pots for only the winter months – December 2004 through most of March 2005), and (3) full protection (perlite covered pots for the entire experiment). Seedlings within each treatment were placed in eight-seedling plots and were arranged 25 cm apart to allow for airflow between plots. The seedlings in the partial and full protection treatments were arranged in the same manner, but perlite was added between pots and to a depth of 10 cm above the rooting medium. Perlite was removed from the partial treatment on March 30, 2005. Throughout the experiment, natural snow was cleared by hand from all treatments, so that the seedlings and their pots remained fully exposed to the assigned treatment. Treatments and air- and soil-temperature measurements were initiated on December 3, 2004. All seedlings had been grown in an exposed condition before this date.

On each of the four sample collection dates in 2005 (27 February, 27 March, 24 April, and 22 May), two randomly selected seedlings per treatment from each of the six blocks were removed for cold tolerance, membrane integrity, and visual injury assessments. A total of 12 seedlings per treatment were assessed on each sample date. These dates were chosen to bracket the most likely time period of potential dehardening in yellow-cedar observed in our previous field experiment (Schaberg *et al.*, 2005). Following visual injury assessments of foliage and roots, whole seedlings were packed in damp paper towels, wrapped in plastic bags, and shipped overnight to the University of Vermont analytical laboratory for cold tolerance and membrane integrity assessments.

Cold tolerance

Cold tolerance was assessed by measuring the electrolyte leakage of foliar and root tissues following controlled exposure to a series of progressively lower test temperatures. The distal segments of primary shoots were sampled to assay foliar cold tolerance levels (Hawkins *et al.*, 2001; Schaberg *et al.*, 2005), and fine roots (i.e. <2 mm in diameter) were used to estimate cold tolerance levels for roots (McKay, 1998; Folk *et al.*, 1999). Root systems were washed in cold tap water to remove potting media, and only light-colored roots that showed no visible damage were assessed for cold tolerance. Following initial preparations, foliage and root tissue from each seedling were rinsed in iced distilled water to remove surface ions and then chopped into 5 mm sections to produce one bulked sample per plant and tissue type. Per sampling date, this process resulted in one bulked sample per seedling and tissue type (i.e. root and foliage) for two seedlings from each of the six blocks and three treatments. Subsamples of bulked tissue were measured volumetrically (approximately 0.3 mL or 0.1–0.2 g) into 64-cell styrene trays for freezing tests, in which each sample tray was exposed to a different test temperature. Freezing stress was imposed using the methods of Strimbeck *et al.* (1995) and Schaberg *et al.* (2000). For roots, test temperatures ranged from 5 to −20 °C in −5 °C increments, with an additional test temperature at −30 °C. For foliage in February and March, test temperatures ranged from −3 to −51 °C in −6 °C increments, with additional test temperatures at −60, −70, and −80 °C. For foliage in April and May, test temperatures ranged from 5 to −40 °C in −5 °C increments. The rate of freezing was $-6\,^{\circ}\mathrm{C\,h^{-1}}$ for all tests. Freezer temperature was held at least 20 min at each test temperature, after which one replicate tray was removed from the freezer, placed in a precooled styrene foam container, and transferred to a separate freezer at −5 °C. After trays equilibrated to −5 °C, they were transferred in foam containers to a refrigerator at 4 °C and held until thawed. A mild detergent solution (3.5 mL of 0.1% v/v Triton X-100 – deionized water) at 4 °C was added to each cell, and sample trays were held in a high-humidity cabinet and shaken at room temperature for 8 h. Initial conductivity of the effusate was measured using a multielectrode instrument (Wavefront Technology, Ann Arbor, MI, USA), then samples were dried for at least 48 h at 50 °C to kill the tissue, soaked in fresh detergent solution for 24 h, and then the final conductivity was measured. Relative electrolyte leakage (REL), a measure of cell injury calculated as the proportion of initial to final conductivity, was used to calculate T_m, the temperature at the mid-point of a sigmoid curve fit to REL data for all test temperatures. T_m values were calculated via nonlinear curve-fitting (JMP, SAS

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Institute, Cary, NC, USA) using the following equation (Anderson *et al.*, 1988):

$$\mathrm{REL} = Y_{\min} + \frac{Y_{\max} - Y_{\min}}{1 + e^{k(T_m - T)}},$$

where $Y_{\min}$ and $Y_{\max}$ are the values of REL for uninjured and completely freeze-stressed tissue, respectively, k describes the steepness of the REL response to freezing stress, and T is the temperature in °C.

Injury assessment

Membrane integrity. REL from plant cells is routinely used as a measure of membrane stability, and has been employed to detect changes in membrane integrity associated with inherent differences in cell viability (van Bilsen & Hoekstra, 1993), as well as imposed stresses including dehydration (Vasquez-Tello *et al.*, 1990; Kuhns *et al.*, 1993), freezing (DeHayes & Williams, 1989; Strimbeck *et al.*, 1995), high temperatures (Ruter, 1996), osmotic stress (Zwiazek & Blake, 1991), changes in mineral nutrition (David *et al.*, 1994; Branquinho *et al.*, 1997), and acid mist treatment (DeHayes *et al.*, 1999; Schaberg *et al.*, 2001). Measurements of REL provide a sensitive indicator of tissue damage that is often detectable before the expression of visible injury (Dlugokecka & Kacperska-Palacz, 1978; Zwiazek & Blake, 1991; Percival, 2004). We used foliar and root REL data from tissues not exposed to experimental freezing stress (i.e. for the highest test temperature used in cold tolerance tests) to estimate baseline membrane integrity and incipient field injury. As per sampling criteria for cold tolerance assessments, visibly injured tissues were excluded when foliage and roots were sampled for REL measurements. Thus, REL data provide an estimate of damage to visibly uninjured tissues.

Visible injury. The crowns and roots of seedlings were assessed for discoloration and other gross damage symptoms when seedlings were harvested before cold tolerance assessments. Samples of fine roots (<2 mm diameter) were examined using a dissecting microscope (10 × magnification) for browning in the cortex and stele tissues by slicing these tissues with a razor blade during the second (two perlite and two exposed seedlings), third (eight perlite-covered and eight exposed seedlings), and fourth (all seedlings – 12 seedlings from each treatment) sampling periods. For each of these seedlings, 30 fine roots were examined and results were recorded as % dead (mortality).

Temperature and weather data

Logging devices were used to record hourly air temperature and soil temperature in each of the treatments in three of the six blocks to document conditions experienced by the protected and exposed seedlings. Air temperature was recorded with Water Temperature Pro Hobo devices (Onset Computer Corp., Bourne, MA, USA) mounted on wood stakes 30 cm above the rooting medium of the pots in the middle of nine treatments. Soil temperature was recorded in the same treatments using outdoor Hobo U12 loggers (Onset Computer Corp.). The range, accuracy, and response times for air and soil temperature devices, respectively, were as follows: −40 to 70 °C, + 0.2 °C at 25 °C, and 12 min in air moving at 2 m s^{-1}; −40 to 50 °C, + 0.25 °C at 20 °C, and 3 min in air moving at 2 m s^{-1} and 1 min in water. Each of these soil loggers had four probes, which were placed individually at the mid-point of the rooting media of one seedling that was to be removed during each of the four sampling intervals. Probes were placed at half depth in the rooting medium and half the distance from the center to the east side of the pots in the same treatments as for air temperature. Data from a single logger recording hourly air temperature was used to specify temperatures that the seedlings had experienced earlier in the winter, before the establishment of the treatments. Weather data (Western Regional Climate Center) from the Juneau airport weather station ['Juneau AP, Alaska (504100)'], located 2 km from the experiment, were used to indicate mean daily minima and maxima values during winter and spring over the previous 30 years.

Statistical analyses

Analyses of variance were used to test for differences in T_m and REL attributable to soil treatment and block effects for roots and foliar tissues sampled at each of the four sampling dates. Specific differences among factor means were determined using the Tukey HSD test. Differences were considered statistically significant if $P \leq 0.05$.

Results and discussion

Patterns of cold tolerance

Foliage examined in this study was cold tolerant enough (−27.16 ± 0.43 °C in February and −24.57 ± 1.42 °C in March for all treatments combined) to avoid direct injury from ambient temperatures throughout the winter (Fig. 1a). These findings were consistent with our past findings for mature yellow-cedar trees (Schaberg

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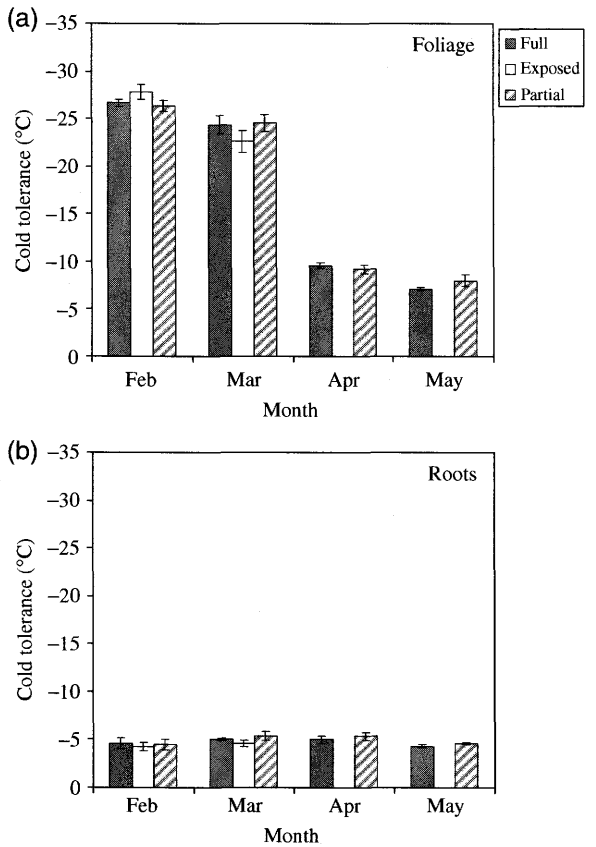

Fig. 1 The influence of soil protection treatments on mean (±SE) cold tolerance measured as T_m for the (a) foliage and (b) roots of yellow-cedar seedlings over 4 months in winter/spring 2005. No significant differences in cold tolerance attributable to treatment were found. No cold tolerance estimates exist for seedlings from the exposed treatment in April and May, because extensive tissue damage precluded the accurate calculation of T_m values.

et al., 2005). Although foliage dramatically dehardened between March and April (losing on an average over 15 °C of cold tolerance in 1 month), residual hardiness (−9.42 ± 0.25 °C in April and −7.56 ± 0.33 °C in May for all treatments) was sufficient to protect foliage from the ambient low temperatures experienced during the year of this experiment (Fig. 2a). More importantly, there were no detectable treatment effects on patterns of cold hardiness or dehardening of foliage for any period of the study. Differential exposure of rooting media to ambient temperatures had no influence on foliar cold tolerance. No significant block effects were found for this or any other measurement parameter.

In contrast to foliage, roots of seedlings from all treatments expressed only limited levels of cold hardiness (hovering around −5 °C) that were statistically indistinguishable from one another throughout the study period (Fig. 1b). Past research has indicated that the roots of yellow-cedar stecklings (i.e. rooted cuttings) can reach a maximum cold hardiness of about −12 °C, whereas cold tolerance levels are closer to −6 °C before cold acclimation and after deacclimation (Puttonen & Arnott, 1994). The roots of some conifer seedlings such as lodgepole pine [*Pinus contorta* D. Don] and mountain hemlock [*Tsuga mertensiana* (Bong.) Carr.] reach maximum cold tolerance levels that are fairly limited (e.g. approximately −8 °C; Coleman *et al.*, 1992). However, the roots of most conifer species appear to reach greater depths of cold hardiness. For example, Bigras & Calmé (1994) reported that the maximum cold tolerance of the roots of black spruce [*Picea mariana* (Mill.) B.S.P.] seedlings was about −12 °C, and Coleman *et al.* (1992) found that the roots of Pacific silver fir [*Abies amabilits* (Dougl.) Forbes] and subalpine fir [*Abies lasiocarpa* (Hook.) Nutt.] seedlings reached similar hardiness levels. Sutinen *et al.* (1998) noted that the roots of mature Scots pine (*Pinus sylvestris* L.) were tolerant to −20 °C, a level of hardiness also noted for seedlings of this species (Lindström & Stattin, 1994), whereas the roots of Norway spruce [*Picea abies* (L.) Karst.] seedlings can survive exposures to −25 °C (Lindström & Stattin, 1994).

The roots of the yellow-cedar seedlings in our experiment either never adequately hardened in the fall, or they dehardened below the species' reported −12 °C average maximum hardiness level to about −5 °C by the time of our first measurement in February. Although gross seasonal patterns of cold tolerance in foliage and roots may generally parallel one another (Sakai & Larcher, 1987), other evidence indicates that, whereas roots achieve maximum hardiness levels by late fall (e.g. November or December), they can lose as much as 10 °C cold tolerance by mid-winter (e.g. February; Sutinen *et al.*, 1998). Rapid reduction in root cold tolerance can be provoked by thaw conditions (Bigras & D'Aoust, 1993; Lindström & Stattin, 1994). Air temperatures during our experiment persisted above 30-year mean maxima throughout November before treatments began in early December (Fig. 2a) – potentially resulting in treatment-independent dehardening. However, significant reductions in root cold tolerance can also occur in the presence of continual subfreezing temperatures (Lindström & Stattin, 1994) such as those that predominated after treatment application (Fig. 2a).

Yellow-cedar is a high-elevation tree throughout most of its range (Harris, 1990). Perhaps an ability to deharden its roots is an adaptation to living in areas of heavy snow accumulation. Deep cold hardiness would be of great importance in fall before snowfall is typically adequate enough to protect roots from ambient tem-

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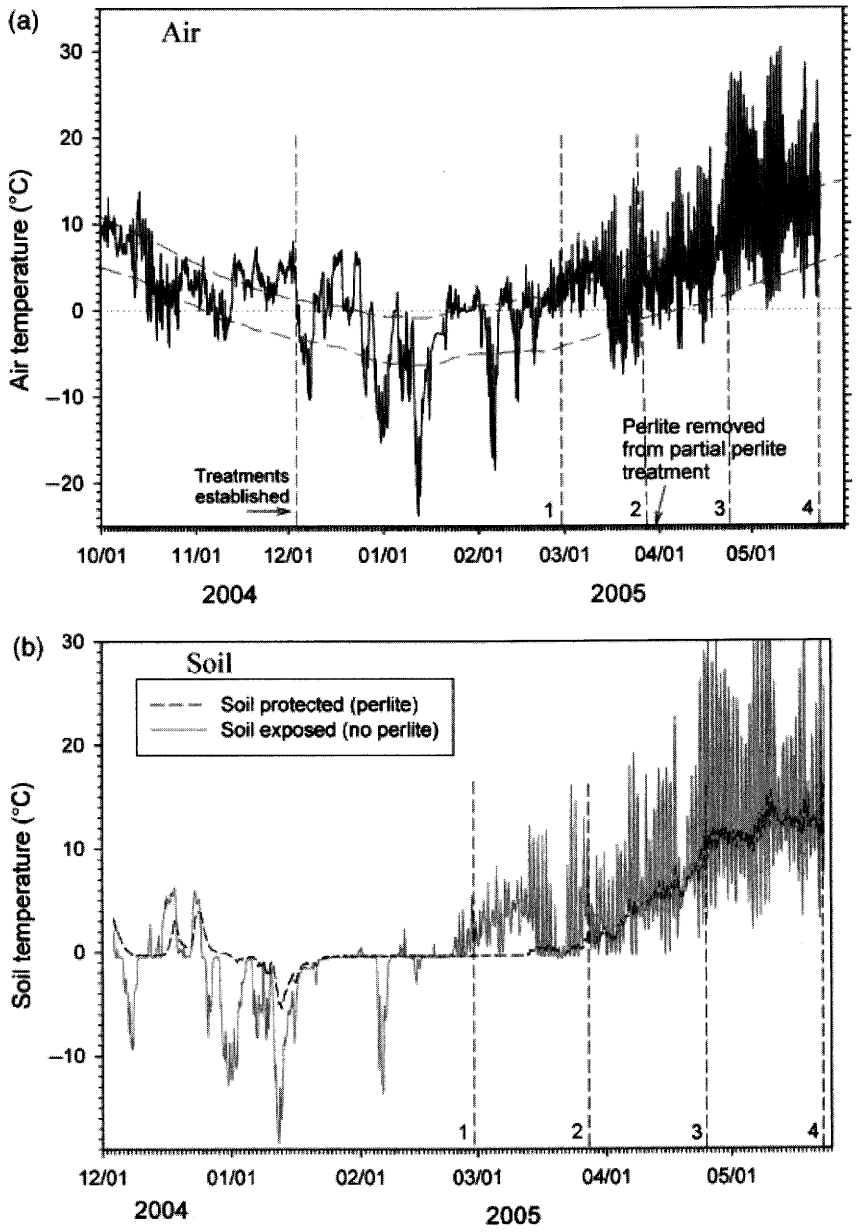

Fig. 2 Air (a) and soil (b) temperatures for the study period. (a) Hourly air temperature from October 1, 2004, through May 31, 2005 (solid line), and mean minima and maxima values for the previous 30 years (dashed lines Western Climate Center). Hourly temperatures are the mean of nine logging devices after the treatments were established and one device prior to treatments. Note the distinct thaw–freeze cycles. The onset of treatments and sample dates for foliar and root testing are given. (b) Mean hourly soil temperature from potted yellow-cedar seedlings from December 1, 2004, through May 2005. Temperature data from the partial perlite treatment combined with perlite treatment before 30 March, but then combined with the exposed treatment after perlite was removed on this date.

perature lows. However, because of a high probability of snowpack through late winter and early spring, there may have been little adaptive advantage for the species to maintain high root hardiness levels when it was unlikely to be needed because snow typically protected roots from freezing injury.

Patterns of injury expression

We measured significantly greater baseline electrolyte leakage (i.e. for tissues not exposed to experimental freezing tests) from roots of seedlings exposed to lower soil temperatures (exposed treatment) relative to seed-

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lings in treatments where roots were buffered from ambient temperature extremes (partial and full protective treatments) (Fig. 3b). Although roots can exhibit high REL levels even when undamaged (e.g. Bigras & Calmé, 1994), greater electrolyte leakage for roots in the exposed treatments relative to roots in the other treatments is a direct measure of elevated membrane disruption and damage within otherwise visibly uninjured root tissue from this treatment.

Electrolyte leakage data indicated that root damage was first evident in February, was more statistically distinguishable by March, and persisted through April. In May, differences in root electrolyte leakage among seedlings in the various treatments were no longer significant, largely because leakage levels of seedlings in the partial and full protective treatments increased considerably relative to levels measured during previous months. Increased leakiness of roots of seedlings in these treatments likely reflects normal seasonal changes in REL seen for undamaged roots in other species (e.g. McKay, 1998). Higher levels of electrolyte leakage for roots during the growing season have been attributed to seasonal changes in membrane (protein and lipid) composition (McKay, 1998).

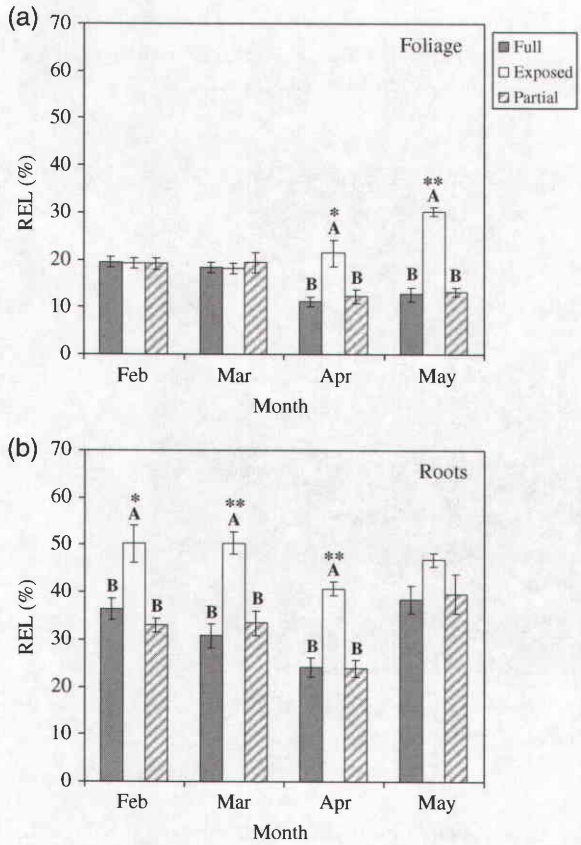

Fig. 3 The influence of soil protection treatments on mean (±SE) relative electrolyte leakage (REL) of tissues before experimental freezing tests for (a) foliage and (b) roots of yellow-cedar seedlings over 4 months in winter/spring 2005. Significance values for comparisons within sample date are as follows: $^{*}P \leq 0.05$ and $^{**}P \leq 0.01$ (ANOVA). Means with the same letter are not significantly different based on the Tukey HSD test.

In comparison with electrolyte leakage measures of visually uninjured tissue, visible injury of roots and foliage appeared delayed. The roots and foliage of seedlings appeared healthy and there was no evidence of injury at the first sampling on 27 February. Roots from all exposed seedlings appeared to be damaged at the second sample date (27 March), but foliage on these seedlings remained green (Fig. 4). Most fine roots observed from these exposed seedlings had brownish cortex tissue, but the stele tissue was often still white. On 27 March, both the roots and foliage from the two perlite treatments appeared healthy, with <10% dead fine roots (Fig. 4). At the third sampling on 24 April, foliage had begun to die and extensive root damage was noted in all exposed seedlings. Mortality to fine roots was much higher in exposed seedlings (81%) than in the perlite or partial perlite treatments (9% and 5%, respectively). New white root tips were apparent on all seedlings in both perlite treatments (healthy roots); most were 2–5 mm long with some up to 10 mm.

More severe foliar symptoms developed in all exposed seedlings around 25 April with the onset of warmer days. The crowns of some seedlings turned orange-brown, and others remained somewhat green but were dry and brittle to the touch. Every seedling in the exposed treatment had foliar symptoms, indicating that it was dead or nearly dead on the final sample date, yet all seedlings in the protective treatments remained green (Fig. 5). All fine roots (100%) from exposed seedlings were dead, while only 11% and 13% of fine roots on the perlite and partial perlite seedlings were dead at this time.

Because seedlings in the full and partial protective treatments showed no signs of damage, we are confident that root damage occurred after 3 December when perlite was added to protect the roots of seedlings in these treatments – perhaps during the extended cold periods (−5 °C and lower) in late December and mid-January (Fig. 2a). Furthermore, because seedlings in the partial protective treatment never exhibited signs of injury, it is evident that damage was not induced after the root systems of seedlings had the protection of perlite removed on 30 March. Thus, damage must have occurred as a result of one or more low-temperature events between 3 December and 30 March (Fig. 2a).

There were perhaps eight cold events in January and February in which soil temperatures in pots for the exposed treatment dropped below −5 °C, four of which

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Fig. 4 Differences in visible injury for fine roots between protected seeding (left) showing healthy roots and healthy foliage and exposed seedling (right) showing damaged roots and apparently healthy foliage. Photograph taken on March 27, 2005.

Fig. 5 Differences in the visible injury of foliage of yellow-cedar seedlings during the fourth sampling interval on 22 May removed from their blocks and arranged by treatments: full protection (left), partial protection (middle), exposed (right).

experienced temperatures considerably below this threshold (Fig. 2b). One event, around 11 January, was by far the coldest period, with air temperatures below −20 °C. Two of these events were preceded by warm temperatures, creating thaw–freeze episodes that can induce freezing injury (Bourque *et al.*, 2005). In other years, relatively cold periods also occurred in March (Fig. 2a). Soil temperature data show that perlite can insulate pots to a point, but that there is a breakthrough after several days of extreme cold when soil temperatures then decline. Also, soil in the perlite treatment warmed slowly in April and May. Thus, perlite functioned to buffer soil temperature, but given enough exposure to warm or cold temperatures, perlite was not a perfect surrogate for snow in this experiment.

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Although our cold tolerance measurements began after these cold events, the minimal cold tolerance levels we documented in late February suggest that roots were uniquely vulnerable to freezing injury close to the time when ambient temperature lows actually occurred. We cannot determine which of these events between December and late February (or possibly combinations of them) caused the root injury we detected.

Foliar electrolyte leakage measurements indicate that damage (greater membrane disruption and electrolyte loss) also occurred only for seedlings in the exposed treatment (Fig. 3a). Furthermore, foliar injury was first detected in April, directly following a warm period, and long after root damage was first detected by REL in February (Fig. 3). Because ambient air temperatures never reached lows required to induce direct foliar freezing injury, we propose that foliar injury was a later expression of root injury that occurred earlier in the study. In particular, extensive root freezing injury for seedlings in the exposed treatment could have reduced functional root capacity enough, so that foliage was undersupplied with water once transpirational demands increased in the spring. Thus, foliar injury could represent secondary desiccation damage that followed prior root freezing injury and was only expressed following the onset of warmer weather. Root freezing injury has been shown to decrease water uptake, reduce shoot water potentials, and result in increased foliar damage and mortality for many coniferous species (e.g. see review by Bigras & Dumais, 2005). In the current study, all seedlings in the exposed treatment eventually had 100% fine root damage, the complete browning of foliage, and eventually died.

Consistencies with field symptoms

The pattern of initial root injury followed by later foliar damage and mortality is consistent with field reports of the nature and sequence of injury that results in yellow-cedar decline (Hennon & Shaw, 1997; Hennon *et al.*, 2006). Mature trees generally die more slowly; excavation of root systems of trees in various stages of crown decline indicated that fine root death was the initial symptom that a tree was beginning to decline (Hennon *et al.*, 1990b). Foliar symptoms developed after significant portions of the root system were dead, with proximal foliage dying and then distal foliage often the last tissues alive before trees finally die. We have observed freezing injury to seedlings and saplings, which typically is expressed as death to newer distal foliage, and the older proximal foliage is often not killed. We found that limited mid-winter root cold tolerance levels resulted in high levels of root injury, first chiefly detected as increases in REL for visibly uninjured roots, and then progressing to visible root injury. It has been consistently shown that REL measurements provide a sensitive indicator of tissue damage that is often detectable before visible injury expression (Dlugokecka & Kacperska-Palacz, 1978; Zwiazek & Blake, 1991; Percival, 2004). Foliar damage, also first detected as increases in REL and then progressing to prominent visible injury, became evident after warmer temperatures prevailed and transpirationally induced desiccation was more likely. This progression from initial root injury to foliar browning and whole plant death mimic decline symptoms seen in the field, and provide the first quantitative experimental evidence that root freezing injury could be an instigating cause of yellow-cedar decline.

Although consistent with the basic pattern of injury and symptom progression in the field, the study described here, which employed uniform protection or exposure of roots grown in homogeneous rooting media with equal rooting depths, provided a highly simplified test of the type of environmental conditions that may contribute to injury in the field. Temporal and spatial variability in a variety of factors that protect roots from freezing injury (e.g. amount of insulative protection from snowpack, soil depth, and drainage that may allow roots to grow beyond the frost zone, etc.) would make injury expression in the field more diverse than that experienced in our simplified simulation. Temporal and spatial variability in temperature patterns that may predispose or incite freezing injury (e.g. the depth and duration of freezing events, the occurrence of thaws that may deharden tissues before cold snaps) would similarly complicate injury expression in the field. Differential mixes of root protection and exposures to freezing temperatures over space and time would account for the more nuanced expression of injury seen in the field (e.g. crowns generally dying rapidly as a unit, but at times declining more progressively over years; Hennon & Shaw, 1997). As in the current seedling study, rapid crown deterioration would follow near-complete root freezing injury and mortality. In contrast, slower crown loss would likely result from more limited freezing damage of roots, such as when only superficial roots were injured on trees where site conditions allowed for deeper root penetration. Thus, freezing injury could act as a chronic damaging factor when shallow roots were injured and killed every few years, slowly depleting tree carbohydrate resources and diminishing water and nutrient uptake capacities. The experimental circumstances of this study were limited (e.g. with a uniform level of root protection, and exposure to only one season's particular temperature trends) compared with the range of complexities that are possible in the field. Nonetheless, the sequence and nature of injury expres-

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sion uniquely resemble those typically associated with field reports of decline.

Although our study provides the only experimentally induced recreation of yellow-cedar decline symptomology, recognition that various site factors may help protect or exacerbate cold exposure and freezing damage may provide opportunities to further test the possibility that root freezing damage instigates yellow-cedar decline. We have developed a conceptual scenario of the interplay of factors that may contribute to yellow-cedar decline (Fig. 6). This scenario highlights various circumstances (e.g. wet soils that may limit rooting depth, greater exposure to soil freezing or temperature fluctuations when snow is absent, etc.) that may modulate the risk of root freezing injury and associated decline. We have already begun to evaluate if patterns of injury in the field reflect variations in these factors across the landscape. For example, soil temperatures below the −5 °C threshold occur in late winter and early spring at shallow depths (e.g. 7.5 cm) but are less common deeper (e.g. 15 cm); also, the presence of snow buffers against these apparently lethal soil temperatures (D'Amore & Hennon, 2006). Indeed, in one event during the 2003–2004 winter, we recorded sustained soil temperatures below −10 °C at 7.5 cm below the soil surface at field sites experiencing yellow-cedar decline, but more moderate temperatures above the −5 °C threshold at this same soil depth at higher elevations where snow was present (Hennon, 2007). The close association of the decline problem and the absence of late winter–early spring snow has been observed at spatial scales ranging from the entire region of Southeast Alaska to the small watershed (Hennon *et al.*, 2006). At the latter scale, wet cedar forests appear healthy where snow persists through March or April, presumably protecting cedar roots past the last hard freeze. In addition, landscape-scale crown injury is usually most noticeable in early to mid-summer when foliage starts to turn an orange-brown color. As with the seedlings in the current study, uniform crown discoloration for trees in the field is a possible indication that root injuries do not manifest themselves until the reduced transpirational capacity associated with root loss leads to foliar desiccation and mortality. By forming the basis for the design of controlled experiments such as the current study, and providing the context for evaluating patterns of injury expression in the field, the scenario depicted in Fig. 6 provides a useful guide for studies of the cause(s) of yellow-cedar decline. It is important that future tests of this scenario include sympatric species such as western redcedar or western hemlock [*Tsuga heterophylla* (Raf.) Sarg.], so that the unique vulnerability of yellow-cedar to injury and decline can be specifically evaluated.

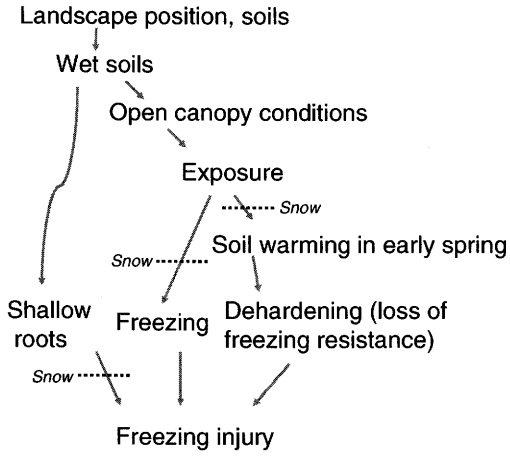

Fig. 6 Conceptual scenario leading to yellow-cedar decline and mortality. This scenario proposes that landscape features and soil properties produce poor drainage that creates open canopy forests and shallow rooting. Open canopy conditions increase the likelihood of soil temperature fluctuations that may be directly damaging to fine roots (e.g. freeze or freeze/thaw events), or may predispose roots to damage due to premature dehardening (thaws). Increased snowpack protects yellow-cedars by (1) insulating the soil so that it does not reach lethal sub-freezing temperatures, and/or (2) delaying soil warming and associated dehardening. As highlighted, snow would mitigate the direct and indirect consequences of exposure-induced soil temperature perturbations that instigate yellow-cedar decline.

Acknowledgements

We thank Melinda and Jonas Lamb for their help in sampling seedlings at the Juneau facility, and Kelly Baggett, John Bennink, Cathie Borer, De Campolungo, Tammy Coe, Josh Halman, Chris Hansen, Brett Huggett, Brynne Lazarus, Paula Murakami, and Michelle Turner in Burlington for assistance with cold tolerance experiments. Gratitude is also extended to Paula Murakami for her help with the graphics for this manuscript. We also thank Drs Barbara Hawkins, John Russell, and Alan Mitchell for their helpful suggestions regarding earlier drafts of this manuscript.

References

Anderson JA, Kenna MP, Taliaferro CM (1988) Cold hardiness of 'Midiron' and 'Tifgreen' bermudagrass. *HortScience*, **23**, 748–750.

Beier CM, Sink SE, Hennon PE, D'Amore DV, Juday GP (2008) Twentieth-century warming and the dendroclimatology of declining yellow-cedar forests in southeastern Alaska. *Canadian Journal of Forest Research*, in press.

Bigras FJ, Calmé S (1994) Viability tests for estimating root cold tolerance of black spruce seedlings. *Canadian Journal of Forest Research*, **24**, 1039–1048.

Bigras FJ, D'Aoust AL (1993) Influence of photoperiod on shoot and root cold tolerance and bud phenology of white spruce

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seedlings (*Picea glauca*). *Canadian Journal of Forest Research*, **23**, 219–228.

Bigras FJ, Dumais D (2005) Root-freezing damage in the containerized nursery: impacts on plantation sites – a review. *New Forests*, **30**, 167–184.

Bourque CP-A, Cox RM, Allen DJ, Arp PA, Meng F-R (2005) Spatial extent of winter thaw events in eastern North America: historical weather records in relation to yellow birch decline. *Global Change Biology*, **11**, 1477–1492.

Branquinho C, Brown DH, Catarino F (1997) The cellular location of Cu in lichens and its effects on membrane integrity and chlorophyll fluorescence. *Environmental Experimental Botany*, **38**, 165–179.

Coleman MD, Hinckley TM, McNaughton G, Smit BA (1992) Root cold hardiness and native distribution of subalpine conifers. *Canadian Journal of Forest Research*, **22**, 932–938.

D'Amore DV, Hennon PE (2006) Evaluation of soil saturation, soil chemistry, and early spring soil and air temperatures as risk factors in yellow-cedar decline. *Global Change Biology*, **12**, 524–545.

David PP, Nelson PV, Sanders DC (1994) A humic acid improves growth of tomato seedling in solution culture. *Journal of Plant Nutrition*, **17**, 173–184.

DeHayes DH, Schaberg PG, Hawley GJ, Strimbeck GR (1999) Acid rain impacts calcium nutrition and forest health. *BioScience*, **49**, 789–800.

DeHayes DH, Williams MW (1989) *Critical temperature: a quantitative method of assessing cold tolerance*. USDA Forest Service General Technical Report, NE-134.

Dlugokecka E, Kacperska-Palacz A (1978) Re-examination of electrical conductivity method for estimation of drought injury. *Biologia Plantarum (Prague)*, **20**, 262–267.

Farjon A, Hiep NT, Harder DK, Loc PK, Averyanov L (2002) The new genus and species in Cupressaceae (Coniferales) from northern Vietnam, *Xanthocyparis vietnamensis*. *Novon*, **12**, 179–189.

Folk RS, Grossnickle SC, Axelrood P, Trotter D (1999) Seed lot, nursery, and bud dormancy effects on root electrolyte leakage of Douglas-fir (*Pseudotsuge menziesii*) seedlings. *Canadian Journal of Forest Research*, **29**, 1269–1281.

Grossnickle SC, Russell JH (2006) Yellow-cedar and western redcedar ecophysiological response to fall, winter and early spring temperature conditions. *Annals of Forest Science*, **63**, 1–8.

Harris AS (1990) *Chamaecyparis nootkatensis* (D. Don) Spach: Alaska-cedar. In: *Silvics of North America, Vol. 1. Conifers. Agricultural Handbook 654* (eds Burns RM, Honkala BH), pp. 97–102. USDA Forest Service, Washington, DC.

Hawkins BJ, Russell JH, Arnott J (2001) Cold hardiness of yellow-cedar (*Chamaecyparis nootkatensis* (D. Don) Spach). In: *Conifer Cold Hardiness* (eds Bigras J, Colombo SJ), pp. 531–554. Kluwer Academic Publishers, Dordrecht, the Netherlands.

Hennon P, D'Amore D, Wittwer D *et al.* (2006) Climate warming, reduced snow, and freezing injury could explain the demise of yellow-cedar in Southeast Alaska, USA. *World Resource Review*, **18**, 427–450.

Hennon PE (1990) Fungi on *Chamaecyparis nootkatensis*. *Mycologia*, **82**, 59–66.

Hennon PE (2007) The vital role of snow in protecting yellow-cedar from an extensive forest decline in Alaska. In: *Proceedings of the 75th Western Snow Conference* (ed. McGurk B), pp. 77–88. San Francisco Public Utilities Commission, San Francisco, CA.

Hennon PE, D'Amore D, Zeglen S, Grainger M (2005) *Yellow-cedar decline in the North Coast district of British Columbia*. USDA Forest Service General Technical Report, RN-549.

Hennon PE, Hansen EM, Shaw CG III (1990a) Dynamics of decline and mortality of *Chamaecyparis nootkatensis* in Southeast Alaska. *Canadian Journal of Botany*, **68**, 651–662.

Hennon PE, McWilliams MG (1999) Decline symptoms do not develop with grafting from dying yellow-cedar. *Canadian Journal of Forest Research*, **29**, 1985–1988.

Hennon PE, Newcomb GB, Shaw CG, Hansen EM (1986) Nematodes associated with dying *Chamaecyparis nootkatensis* in South-Eastern Alaska. *Plant Disease*, **70**, 352.

Hennon PE, Shaw CG (1994) Did climatic warming trigger the onset and development of yellow-cedar decline in Southeast Alaska? *European Journal of Forest Pathology*, **24**, 399–418.

Hennon PE, Shaw CG (1997) The enigma of yellow-cedar decline: what is killing these long-lived, defensive trees? *Journal of Forestry*, **95**, 4–10.

Hennon PE, Shaw CG, Hansen EM (1990b) Symptoms and fungal associations of declining *Chamaecyparis nootkatensis* in Southeast Alaska. *Plant Disease*, **74**, 267–273.

IPCC (2007) Executive summary of the Intergovernmental Panel on Climate Change, February 2007, www.ipcc.com.ch

Kuhns MR, Stroup WW, Gebre GM (1993) Dehydration tolerance of five bur oak (*Quercus macrocarpa*) seed sources from Texas, Nebraska, Minnesota, and New York. *Canadian Journal of Forest Research*, **23**, 387–393.

Leinonen I, Repo T, Hanninen H (1997) Changing environmental effects on frost hardiness of Scots pine during dehardening. *Annals of Botany*, **79**, 133–138.

Lindström A, Stattin E (1994) Root freezing tolerance and vitality of Norway spruce and Scots pine seedlings: influence of storage duration, storage temperature, and prestorage root freezing. *Canadian Journal of Forest Research*, **24**, 2477–2484.

Little DP, Schwarzbach AE, Adams RP, Hsieh CF (2004) The circumscription and phylogenetic relationships of *Callitropsis* and the newly described genus *Xanthocyparis* (Cupressaceae). *American Journal of Botany*, **91**, 1872–1881.

McKay HM (1998) Root electrolyte leakage and root growth potential as indicators of spruce and larch establishment. *Silva Fennica*, **32**, 241–252.

Mill RR, Farjon A (2006) Proposal to conserve the name *Xanthocyparis* against *Callitropsis* Öerst. (Cuppressaceae). *Taxon*, **55**, 229–231.

Percival GC (2004) Evaluation of physiological tests as predictors of young tree establishment and growth. *Journal of Arboriculture*, **30**, 80–91.

Puttonen P, Arnott JT (1994) Influence of photoperiod and temperature on growth, gas exchange, and cold hardiness of yellow cypress stecklings. *Canadian Journal of Forest Research*, **24**, 1608–1616.

Ruter JM (1996) High-temperature tolerance of heritage river birch roots decreased by pot-in-pot production systems. *HortScience*, **31**, 813–814.

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Sakai A, Larcher W (1987) *Frost Survival in Plants*. Springer, New York.

Schaberg PG, DeHayes DH, Hawley GJ (2001) Anthropogenic calcium depletion: a unique threat to forest ecosystem health? *Ecosystem Health*, **7**, 214–228.

Schaberg PG, DeHayes DH, Hawley GJ, Strimbeck GR, Cumming JR, Murakami PF, Borer CH (2000) Acid mist and soil Ca and Al alter the mineral nutrition and physiology of red spruce. *Tree Physiology*, **20**, 73–85.

Schaberg PG, Hennon PE, D'Amore DV, Hawley GJ, Borer CH (2005) Seasonal differences in the cold tolerance of yellow-cedar and western hemlock trees at a site affected by yellow-cedar decline. *Canadian Journal of Forest Research*, **35**, 2065–2070.

Shaw CG, Eglitis A, Laurent TH, Hennon PE (1985) Decline and mortality of *Chamaecyparis nootkatensis* in Southeastern Alaska, a problem of long duration but unknown cause. *Plant Disease*, **69**, 13–17.

Silim SN, Lavender D (1994) Seasonal patterns and environmental regulation of frost hardiness in shoots of seedlings of *Thuja plicata*, *Chamaecyparis nootkatensis*, and *Picea glauca*. *Canadian Journal of Botany*, **72**, 309–316.

Snyder C (2005) *Forest health conditions in Alaska – 2005*. Protection Report R10-PR-5. USDA Forest Service, Forest Health Protection, Juneau, AK, 92 pp.

Strimbeck GR, Schaberg PG, DeHayes DH, Shane JS, Hawley GJ (1995) Midwinter dehardening of montane red spruce during a natural thaw. *Canadian Journal of Forest Research*, **25**, 2040–2044.

Sutinen M-L, Ritari A, Holappa T, Kujala K (1998) Seasonal changes in soil temperature and in the frost hardiness of Scots pine (*Pinus sylvestris*) roots under subarctic conditions. *Canadian Journal of Forest Research*, **28**, 946–950.

van Bilsen DGJL, Hoekstra FA (1993) Decreased membrane integrity in aging *Typha latifolia* L. pollen: accumulation of lysolipids and free fatty acids. *Plant Physiology*, **101**, 675–682.

Vasquez-Tello A, Zuily-Fodil Y, Pham Thi AT, Viera Da Silva JB (1990) Electrolyte and Pi leakages and soluble sugar content as physiological tests for screening resistance to water stress in *Phaseolus* and *Vigna* species. *Journal of Experimental Botany*, **41**, 827–832.

Zwiazek JJ, Blake TJ (1991) Early detection of membrane injury in black spruce (*Picea mariana*). *Canadian Journal of Forest Research*, **21**, 401–404.

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