Nursery Culture Effects on Western Redcedar Seedling Growth, Defense, and Stress Resistance

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Authorization to Submit Thesis

This thesis of Kenneth Wayne Pete, submitted for the degree of Master of Science with a Major in Environmental Science and titled "Nursery Culture Effects on Western Redcedar Seedling Growth, Defense, and Stress Resistance," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

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Abstract

Western redcedar (*Thuja plicata* Donn ex. D. Don.) is a conifer with commercial, ecological, and cultural importance in the Pacific Northwest. Reforestation efforts using western redcedar often fall short due to losses as a result of ungulate browse and frost-induced mortality. This thesis aims to (1) assess the influence of fertilizer rate and photoperiod manipulation on western redcedar seedling growth and development in both the nursery and field, (2) examine the relative field growth of sheltered and unsheltered western redcedar seedlings, (3) determine whether levels of terpenes vary with nursery cultural practices, and (4) assess whether these cultural practices in turn affect seedling palatability. Objectives were met by taking morphological measurements of both greenhouse and outplanted seedlings, assessing seedling cold hardiness at a series of test temperatures, creating a field site with both sheltered and unsheltered seedlings, capturing and measuring volatile organic compounds using gas chromatography, and exposing seedlings to captive deer. Seedlings treated with the high fertilizer rate were smaller in the nursery but larger by the end of the first field season compared to those treated with the low rate. They were also more hardy at both -7 and -14°C. Those grown under a shortened day length were smaller in the nursery and remained shorter by the end of the first field season compared to those not treated with blackout but were more hardy at -7°C. Protecting seedlings from browse in the field with the use of tree shelters, particularly in combination with a high rate of fertilizer, significantly increased seedling height and diameter. It remains unclear whether foliar terpene levels or seedling palatability are affected by fertilizer rate or blackout due to insufficient sample sizes. This research will aid in western redcedar reforestation efforts.

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Introduction

Western redcedar (*Thuja plicata* Donn ex. D. Don.) is a species of ecological, cultural, and commercial importance in the Pacific Northwest. The range of the species extends along the Pacific Coast from Southeastern Alaska to Northern California and also includes an isolated interior range of populations in Idaho and Montana (Minore 1990). The species accounts for 2.5% of the total softwood volume in Western United States, with an estimated volume of 203 million cubic meters (Oswalt et al. 2014). Trees are long-lived and play a key role in coastal and interior forest ecosystems throughout the range (e.g., Aubry and Raley 2002). Wood is prized for its low density, decay resistance, and straight grain (Gonzalez 2004). For generations, Native Americans have used many parts of the tree for a variety of purposes including housing, transportation, clothing, tools for fishing and hunting, medicines, and for ceremonial purposes (Stewart 1984; Turner et al. 2000; Garibaldi and Turner 2004).

Regeneration of western redcedar is often poor due to a combination of competition, lack of shade, and damage due to ungulate browse (Adams and Mahoney 1991). The species is also relatively intolerant to cold (Minore 1979). The use of nursery-grown seedlings may increase reforestation success because of accelerated early seedling growth as a result of nutrient loading, increased overall size, and the ability to grade seedling quality (Stoeckeler and Jones 1957).

Cold hardiness is an organism's ability to tolerate cold temperatures. For conifers, there are two common tests used to measure cold hardiness: (1) the whole-plant freeze tolerance test, which examines the entire seedling (Ritchie 1984), and (2) the freeze induced electrolyte leakage (FIEL) test, which uses tissue samples (Burr et al. 1990). Nursery practices such as fertilizer application and shortened photoperiods have been shown to affect not only seedling morphology but also cold hardiness development (Colombo et al. 2001).

Western redcedar is also particularly prone to browse damage, especially in the winter when preferred food sources are unavailable. There are many methods to mitigate browse-damage, ranging from perimeter fencing or individual tree shelters to fertilization to stimulate rapid seedling growth, allowing plants to grow above the line of browse. Like many plants, western redcedar produces secondary metabolites as a form of chemical defense (Vourc'h et al. 2001). These chemicals, known as terpenoids, are natural browse-deterrents. In particular, monoterpenes such as thujone have been shown to deter ungulates due to the obstruction of fermentation in the ruminants' stomachs (Welch and Pederson 1981).

Given that reforestation efforts using western redcedar often fall short due to losses as a result of ungulate browse and frost-induced mortality, the objectives of this study are to (1) assess the influence of fertilizer rate and photoperiod manipulation on western redcedar seedling growth and development in both the nursery and field, (2) examine the relative field growth of sheltered and unsheltered western redcedar seedlings, (3) determine whether levels of terpenes vary with nursery cultural practices, and (4) assess whether these cultural practices in turn affect seedling palatability.

Materials and Methods

Sowing

Western redcedar (*Thuja plicata* Donn *ex* D. Don.) seeds were sown at University of Idaho's Center for Forest Nursery and Seedling Research in Moscow, ID (46.72°N, 116.96°W) on 16 May 2014. Seeds were obtained from Quality Seed Collections Ltd. (Kamloops, BC, Canada; Seedlot #48319, Elevation: 1000m, Crop Year: 2005). Seeds were sown in Copperblock containers (Beaver Plastics Ltd, Acheson, AB, Canada) in a mix of peat, vermiculite, and bark (45, 45, and 10% by volume, respectively) (Sun Gro Horticulture, Agawam, MA) and cultured at the nursery for 37 weeks. Containers were 15.2 cm deep with a top diameter of 4.3 cm for a total volume of 164 ml, and were lined with a patented copper coating to promote lateral root growth. On 13 June 2014, seedlings were moved to USDA Forest Service, Rocky Mountain Research Station in Moscow, ID (46.72°N, 117.00°W) where they remained for the duration of the study. The study followed a 2×2 factorial design, with 2 fertilizer treatments (high - 200 ppm N and low - 100 ppm N) × 2 blackout treatments (with and without blackout), each with five replicates of two Copperblocks each.

Fertilizer Treatments

Seedlings were fertilized with Peters Excel® 21-5-20 NPK Multi Purpose No Boron formulation (Everris NA Inc., Dublin, OH) at rates of either 200 ppm N (high) or 100 ppm N (low). Fertilizer was applied via subirrigation, with irrigation frequency determined by using the operational (manager technique) for gravimetric water content (GWC) (McDonald 1984; Dumroese et al. 2015). Seedlings were irrigated and fertilized when container GWC declined to 80% (± 2.5%) of the container weight at field capacity. Target fertilizer rates were achieved using 160 ml of concentrated fertilizer per 16 L of H_2O for 200 ppm N and 80 ml of concentrated fertilizer per 16 L of H_20 for 100 ppm N. The mixture was then added to a 27-gallon plastic storage tote, where each block soaked until fully saturated (approximately 10 minutes). Additionally, magnesium sulfate (Magriculture®; 9.8% magnesium and 12.9% sulfur; Giles Chemical, Waynesville, NC) was provided at a rate of 40 ppm every time the seedlings were irrigated. Water pH was adjusted to 5.5 via phosphoric acid.

Blackout Treatments

Photoperiod was manipulated using black and white, 6 mm-thick polyethylene blackout cloth (Hummert International, Topeka, KS) that completely obstructed direct and indirect solar radiation. The treatment began after 20 weeks of seedling growth. Blackout was initiated at 1700 h, terminated at 0900 h the following day, and lasted two weeks.

Greenhouse Measurements

First-year growth was assessed on 5 randomly selected seedlings per treatment replicate (n = 100 seedlings) on 16 February 2015. For each seedling, height was measured from the media surface to the tip of the terminal leader, root-collar diameter (RCD) was measured on the main stem at the media surface, and root and shoot volumes were measured using the water displacement method

(Burdett 1979). Upon completion of fresh material measurements, seedlings were oven-dried for 48 h at 60°C to determine root and shoot dry mass.

Outplanting and Field Measurements

Seedlings were outplanted approximately 34 km northeast of Orofino, ID (46.54°N, 115.90°W) on Potlatch Corporation's Mad Bear PCT site on 21 May 2015. The site was harvested in 2014 using an excavator, and leftover slash was piled and burned. The soil belongs to the Jaype-Revling complex, characterized by deep, well-drained, volcanic ash over alluvium and/or lacustrine deposits (Soil Survey Staff, NRCS 2016). To measure soil moisture, temperature and relative humidity, Em50 data loggers with EC-TM soil moisture and temperature sensors and EHT environmental relative humidity and temperature sensors (Decagon Devices, Pullman, WA) were installed at three different locations within the plantation. Sensors were installed at the surface to measure air temperature and relative humidity as well as at 15.3 cm and 20.3 cm below the surface to measure soil temperature and volumetric water content.

For each treatment replicate, 30 seedlings were hand-planted using a spearhead spade shovel. Overburden was scraped away to ensure each seedling was planted in nutrient bearing soil layers. Within each row, seedlings were spaced at 0.6 m with rows 0.9 m apart. To assess chemical versus mechanical defense, rigid seedling protectors (10 cm diameter × 91 cm height; Forestry Suppliers, Inc., Jackson, MS), hereafter referred to as tree shelters, were installed on every other seedling in each row.

Initial seedling height (vertical distance from ground-line to tip of terminal leader) and RCD (diameter of main stem at ground-line) were measured 9 days after outplanting (30 May 2015). Seedling survival and morphology measurements (on surviving seedlings) were taken again 13 Nov 2015.

Cold Hardiness

Cold hardiness sampling was conducted on 8 February 2015, on seedlings grown in the greenhouse for 37 weeks. For each treatment replicate, 1 randomly selected seedling was destructively sampled to assess cold hardiness via the freeze-induced electrolyte leakage (FIEL) method (Ritchie and Shula 1984). Needles were cut into 1 cm segments, making sure to cut segments at both ends to ensure equal diffusion rates of electrolytes. Needle segments were transferred into 10 ml vials. Each vial contained 5 cut segments, along with 2.5 ml of deionized (DI) water and a single grain of sand. Using a ScienTemp Lo-Cold programmable freezer (Scientemp Corp., Adran, MI), samples were exposed to six test temperatures (-7, -14, -21, -28, -35, and -40°C) and to the control temperature of 2°C. The freezer was set to include an 84 min ramp and a 60 min hold period for each test temperature. Once all vials had been removed and thawed, 7.5 ml of DI water was added to each. Vials were agitated on an orbital shaker (250 rpm) for 1.5 - 2 h to promote steady diffusion before initial measurement. Electrical conductivity (EC_1) of each vial solution was measured with an EC/TDS meter (SevenEasy conductivity meter; Mettler Toledo, Columbus, OH). After initial measurements were taken, vials and their contents were placed in an autoclave (Maket Forge Sterilmatic, Vernon Hills, IL) at 121°C for 20 min to achieve total cell death. Vials were equilibrated to room temperature and re-measured for total EC (EC_T). For each sample, an Index of Damage (I_d) was calculated using the following formula:

$$I_d = EC_1/EC_T$$

Volatile Organic Compounds

Volatile terpene analysis was conducted on 5 randomly selected seedlings from each treatment replicate at two different times. The first sampling was done on seedlings obtained from cold storage 15 August 2015 and allowed a two-day thawing period. Those same seedlings were transplanted into standard one-gallon round pots and grown in the RMRS greenhouse for five weeks before the second sampling. Seedlings were housed in Teflon® chambers developed by Welch Fluorocarbon, Inc. (Dover, New Hampshire) with PVC support braces. Supplemental LED lighting developed by Philips (GreenPower DR/W LED 120-110V, Philips, TX) was used to increase photosynthetic activity. Lab temperatures remained a constant 20°C during sampling. We used a sampling pump (CleanAir Engineering Inc., Palatine, IL) equipped with an exhaust and intake valve, which supplied chambers with filtered air. To collect volatile organic compounds (VOC), we used ORBO™ 1103 Porapak™-Q (50/80), 150/75 mg (Sigma-Aldrich, St. Louis, MO) tubes inserted into an intake port on each chamber. The sampling period lasted 6 h per treatment replicate.

Gas chromatography mass spectrometry data were acquired using a Hewlett-Packard (Agilent Technologies, Santa Clara, CA) 5973 Mass Select Detector with a 6890 Gas Chromatograph at the University of Idaho Mass Spectrometry Core Facility (Moscow, ID). A one microliter sample was injected with an Automated Liquid Sampler into the inlet set at 180°C. The inlet was programmed for split injection with a split ratio of 20:1. The helium carrier gas flow was set to 5 psi. The column used was a Varian Factor Four™ (P/N CP 8944) VF-5ms (Agilent Technologies, Santa Clara, CA). It is a 30 m fused silica capillary column with 0.25 mm ID and a 0.25 μm film. An initial oven temperature of 60°C was held for 2 min after injection and then ramped at 10°C min^{-1} for 7 min to 130°C and then 30°C min^{-1} for the next 5 min to 280°C and held for 3 min. The interface to the mass spectrometer was held at 250°C. Data were acquired in scan mode in the mass range of 34 to 700 Da at 2.24 scans sec^{-1} starting 2 min after injection. Electron impact ionization at 70 eV was used for ionization. The ion source was set to 220°C. Analytes were identified by searching the Wiley Registry of Mass Spectral Data (DOI: 10.1002/9780470175217).

Controlled Deer Herbivory Study

On 21 and 22 December 2015, seedlings were exposed to captive deer. For the feeding trial, I studied the behavior of 5 female white-tailed deer (*Odocoileus virginianus* Zimmerman) located at Washington State University's Wild Ungulate Facility (Pullman, WA, USA; 46.73°N 117.13°W).

Deer were housed in pens measuring 2 m × 4 m, with a single deer in each pen. Animals were placed in the pen 24 h prior to feeding trials to minimize stress during the trials. During this holding time, animals were fed wild-grown western redcedar collected from Moscow Mountain (Latah County, ID; 46.81°N, 116.95°W).

In separate trials, each deer was exposed to two different sized seedlings (initial container seedlings that had been removed from cold storage and 4 L transplants) from all four treatments (low fertilizer without blackout, low fertilizer with blackout, high fertilizer without blackout, and high fertilizer with blackout). The smaller seedlings were removed from cold storage one day prior to the feeding trials. Pots of the larger seedlings were placed in the openings of a cement cinder block to prevent the deer from knocking over the material. Three smaller seedlings were fastened to wood planks using rubber stoppers and held in place by concrete cinder blocks. For each seedling size, placement of the four treatments was randomized among corners within the pens to minimize bias. Deer also had access to their pelletized food and to water. The deer were exposed to the western redcedar for 1 h or until seedlings were entirely consumed. The deer were assessed based on whether or not each seedling presented was browsed. The deer were monitored in person and video recorded using GoPro Hero+ (GoPro, San Mateo, CA) action cameras.

Statistical Analyses

All data were analyzed using SAS version 13.1 Software (SAS, Inc., Cary, NC). Differences in morphological variables among treatments were identified using PROC GLIMMIX using an alpha level of 0.05. Raw data were averaged within replicate for all variables except the field morphology metrics (for which raw data were analyzed without first being averaged within each replicate). Type III tests of fixed effects were used to examine interactions and main effects.

Results

Greenhouse

Fertilizer rate significantly impacted western redcedar seedling height and root-collar diameter (RCD) in the nursery, but not root volume (RV), shoot volume (SV), root dry mass (RDM), or shoot dry mass (SDM; Table 1). Interestingly, seedlings were 8% taller and 6% thicker when grown with the low fertilizer rate compared to the high rate. Blackout treatment affected morphology such that seedlings not treated with blackout were 19% taller, 9% thicker, and had 13% greater RDM, 24% greater SDM, and 20% greater SV compared to seedlings treated with blackout. Seedling RV did not differ significantly between blackout treatments.

Field Site

Air and soil data were collected hourly from 24 June through 13 November 2015 from three data loggers installed throughout the plantation. During this time, air temperature ranged from -4.0°C to 43.3°C, with large daily fluctuations (Figure 1) and relative humidity ranged from 0.15 to 1.0 (Figure 2). Much less variation was observed for soil temperature and moisture (Figures 3 through 6). At 15.2 cm below the surface, soil temperature ranged from 4.8°C to 22.1°C and volumetric water content ranged from 0.318 to 0.639 m^3/m^3. At 20.3 cm below the surface, soil temperature ranged from 4.4°C to 22.7°C and volumetric water content ranged from 0.101 to 0.647 m^3/m^3.

Outplanted Seedling Development

Although seedlings given the low fertilizer rate were initially 5% taller and 4% thicker compared to those given the high fertilizer rate, this trend reversed after one field growing season (Table 2). Seedlings given the high fertilizer rate were 10% taller when protected from browse (with no difference in final seedling height between fertilizer treatments for unprotected seedlings) and 8% thicker than seedlings given the low fertilizer rate by the end of the first field growing season. Further, protected seedlings were 54% taller than unprotected seedlings when given the high fertilizer rate and at the low fertilizer rate, protected seedlings were 42% taller than unprotected seedlings by the end of the first field growing season. Neither height nor RCD of seedlings protected from browse via tree shelters differed initially from that of unprotected seedlings, but by the end of the field growing season, seedlings were > 40% taller and 7% thicker when protected versus not protected from browse. Blackout was not a significant effect for initial or final RCD but was significant for initial and final seedling height such that seedlings treated with blackout in the nursery were initially 1% shorter and remained 6% shorter by the end of the first field growing season compared with seedlings not treated with blackout, regardless of fertilizer rate or protection from browse.

Cold Hardiness

Cold hardiness of western redcedar seedlings was affected by fertilizer rate and blackout treatment (Table 3). At -7°C, I_d was 9% lower among western redcedar seedlings treated with the high fertilizer rate compared to the low rate, regardless of blackout treatment. I_d was also 9% lower at -7°C among seedlings treated with blackout compared to those not treated with blackout, regardless of fertilizer treatment. At -14°C, the main effects of fertilizer and blackout significantly interacted such that I_d was significantly higher among seedlings given the low fertilizer rate and not treated with blackout compared to seedlings given the high fertilizer rate and either not treated with blackout (57% higher) or treated with blackout (47% higher). I_d of seedlings given the low rate of fertilizer and treated with blackout did not differ significantly from any other treatment level. Neither of the main effects, nor their interaction, were significant at test temperatures ≤ -21°C.

Volatile Organic Compound Analysis

The major peaks observed from the gas chromatography mass spectrometry (e.g., Figure 7) were those of pinene and sabinene, while thujone, the target terpene, was rarely observed. Levels of pinene could not be assessed and levels sabinene did not differ significantly between the fertilizer treatments or the blackout treatments (Table 4).

Controlled Deer Herbivory Study

Almost every seedling presented to four of the five individual deer was browsed, regardless of treatment (Figure 8 and Appendix 1). The exception to this was a single 4 L seedling that had received the high rate of fertilizer and the blackout treatment, which was not browsed by individual PP. On the other hand, individual LC browsed very few of the seedlings presented (only 3 of the 8 seedlings were browsed by this individual).

Discussion

Morphology

Nursery cultural practices, namely fertilizer rate and shortened day length, affect western redcedar seeding growth and morphology. Seedlings treated with the high fertilizer rate were smaller in the nursery but larger by the end of the first field season compared to those treated with the low rate. Those grown under a shortened day length were smaller in the nursery and remained shorter by the end of the first field season compared to those not treated with blackout. Greatest seedling heights were achieved with a combined use of tree shelters and the high fertilizer rate, with seedlings reaching an average height of 44 cm after a single growing season in the field. While this height is still well below the defined free-to-grow metric of 1.2 m for deer browse (Vila et al. 2003), seedlings planted into tree shelters grew an average of 19 cm over the 24-week growing season while those planted without the use of shelters grew an average of only 6 cm over this same time period. Further, when combined with the high fertilizer rate, sheltered seedlings grew an average of 4 cm more than sheltered seedlings given low fertilizer rates over the season. The use of tree shelters may be a viable means to overcome browse damage in western redcedar reforestation efforts.

Although seedling morphology differed statistically in the nursery, differences were so small that they were visually undetectable. By the end of the field growing season, however, statistical differences among treatments became noticeable.

Cold Hardiness

Initiation and maintenance of cold hardiness in conifers is complex (Wisniewski et al. 2003) and controlled by a combination of environmental factors including day length, temperature, and water availability (Bigras and Colombo 2013). For western redcedar, cold hardiness development is regulated primarily by low temperatures (Silim and Lavender 1994). Grossnickle (1992) found seasonal shifts in cold hardiness of western redcedar, with lethal temperatures (LT_{50}) ranging from -4°C in October to -20°C in February, and attributes this to changes in tissue water content.

Nursery cultural practices can influence cold hardiness of conifers (Colombo et al. 2001). For example, manipulating day length (i.e., shortening day length via blackout treatment) has been shown to accelerate hardening in both Sitka spruce (Hawkins et al. 1996) and Douglas-fir seedlings subjected to test temperatures below –18 °C (Jacobs et al. 2008). Further, fertilizer application can affect hardening as has been shown for red spruce (De Hays et al. 1989), Scots pine (Rikala and Repo 1997), red pine (Islam et al. 2009), and black spruce seedlings (Bigras et al. 1996).

The results of my research show that fertilizer application and photoperiod manipulation influenced the relative cold hardiness of western redcedar seedlings, with greater hardiness at -7 °C and -14 °C (temperatures relevant to those experienced during a typical growing season in the region; Figure 1), when given the high fertilizer rate and hardened with shortened day length through the use of blackout treatment. This is particularly useful information given the increasing climate variability (e.g., Gray and Hamann 2013; Coops and Waring 2011) and likely consequential effects on the distribution of this long-lived, keystone species.

Terpenes

The combination of highly variable levels of terpenes and low sample sizes resulted in large standard errors, suggesting that future research should employ large samples sizes in similar investigations to ensure an accurate estimate of the mean within each treatment level. Although levels of terpenes did not differ significantly among treatments, trends suggest that levels of sabinene were higher among seedlings subjected to the low fertilizer rate compared to the high rate. This finding contradicts the expectation that fertilization would increase foliar nitrogen, which would in turn increase foliar terpene levels; however, it corresponds to previous findings that nitrogen fertilization suppressed monoterpene production in grand fir (Muzika et al. 1989) and Douglas-fir seedlings (Litvak et al. 2002). Yet, Burney and Jacobs (2011) found that foliar monoterpene concentrations for western redcedar increased at higher fertilization rates, and correspondingly likelihood of browse was greater for non-fertilized than fertilized seedlings.

It would be interesting to analyze terpene levels following browse damage to determine whether production is a response to browse damage. Previous research suggests that for western redcedar, browse results in decreased foliar monoterpene concentrations (Litvak and Monson 1998; Vourc'h et al. 2001; Vourc'h et al. 2002; Litvak et al. 2002; Vourc'h et al. 2003; Burney and Jacobs 2012).

Feeding Trials

Previous studies have used captive animals to examine ungulate foraging behavior and browse palatability (Schwartz et al. 1980; Gillingham and Burnell 1989; Edlich and Stotler 2012; Wang et al. 2015; Rea et al. 2017), but to my knowledge this is the first study to use captive deer in a western redcedar feeding trial. With the exception of one particular individual, almost every seedling presented was browsed in this controlled feeding trial, regardless of treatment. Previous studies use a variety of measures to quantify foraging behavior including total biomass consumed, feeding frequency and duration, and sniffing frequency and duration. Future research to better quantify levels of browse among treatments and link to differences in terpene levels would help us better understand the interplay among foliar chemistry and palatability and what effect (if any) nursery culture has on terpene levels. Another option for using captive deer would be to create a small-scale field site enclosed by deer-proof fencing as has been done in other studies (e.g., Rea et al. 2017). The site could have multiple forage types, including western redcedar with predetermined terpene levels, and be used as a preference study.

Conclusions

This study shows that fertilizer application and shortened day lengths can affect western redcedar seedling growth and development. I found that tree shelters remain the most effective way to protect seedlings from ungulate browse. They are, however, costly and require extra labor for installation and removal. With the development of finer scales to measure the degree of browse, the use of controlled deer may be a useful tool for analyzing forage selection.

Although not all of the objectives were met in this study, I was able to clarify some questions regarding western redcedar. This study shows that altering fertilizer application and implementing short day treatments can affect western redcedar seedling growth and development. This carried into physiological measurements such as cold hardiness. I was able to produce a seedling with increased cold tolerance by changing fertilizer levels. This is a useful tool for those looking to outplant on a cold site or sites susceptible to early frosts. Nursery protocols can be based on environmental conditions of designated field site.

This study also confirmed that tree shelters remain the most effective way to protect western redcedar seedlings from ungulate browse. They are costly and require extra labor for installation and removal, but they may prove to be financially feasible over time. The process of outplanting seedlings is costly considering site preparation, plant material, and the labor cost for planting the seedlings. With the high likelihood of some mortality from ungulate browse in the field, any surviving seedlings must offset the costs of the dead seedlings. Additional research on tree shelters can be done to find ideal height, material, and application methods.

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Table 1: Effects of fertilizer rate and blackout treatment on first year nursery growth of *Thuja plicata* (western redcedar). Means (± SE) are presented. RCD = root collar diameter. Within each column, significant differences (at $\alpha = 0.05$) are indicated between fertilizer rates using uppercase letters and between blackout treatments using lowercase letters; n = 100; significant F ratios and p values are presented in bold.

Treatment		Height (cm)	RCD (mm)	Root Volume (cm^3)	Shoot Volume (cm^3)	Root Dry Mass (g)	Shoot Dry Mass (g)
Fertilizer[1] (F)	Low	25.7 ± 0.6 A	2.45 ± 0.06 A	2.87 ± 0.11	9.27 ± 0.24	0.43 ± 0.02	2.23 ± 0.10
	High	23.9 ± 0.6 B	2.32 ± 0.06 B	2.74 ± 0.11	8.75 ± 0.24	0.40 ± 0.02	2.11 ± 0.10
Blackout[2] (B)	No	26.9 ± 0.6 a	2.48 ± 0.06 a	2.85 ± 0.11	9.84 ± 0.24 a	0.44 ± 0.02 a	2.40 ± 0.10 a
	Yes	22.7 ± 0.6 b	2.28 ± 0.06 b	2.76 ± 0.11	8.18 ± 0.24 b	0.39 ± 0.02 b	1.94 ± 0.10 b

Source	df	F (p value)	F (p value)	F (p value)	F (p value)	F (p value)	F (p value)
Fertilizer (F)	1	**15.00**	**6.81**	0.91	1.85	3.11	2.45
		(0.0002)	**(0.0106)**	(0.3420)	(0.1770)	(0.0811)	(0.1212)
Blackout (B)	1	**82.40**	**15.17**	0.40	**18.67**	**5.26**	**34.12**
		(<0.0001)	**(0.0002)**	(0.5272)	**(<0.0001)**	**(0.0241)**	**(<0.0001)**
F*B	1	0.04	0.77	0.17	2.32	0.37	1.46
		(0.8506)	(0.3823)	(0.6800)	(0.1310)	(0.5450)	(0.2305)

[1] - low = 100 ppm N and high = 200 ppm N; [2] - yes = complete obstruction of solar radiation after 20 weeks of seedling growth from 1700 h through 0900 h the following day for a period of two weeks

Table 2: Effects of fertilizer rate, blackout treatment, and seedling protection on first year field growth of *Thuja plicata* (western redcedar). Means (± SE) are presented. RCD = root collar diameter. Within each column, significant differences (at α = 0.05) are indicated between fertilizer rates using uppercase letters, between blackout treatments using lowercase letters, and between protection treatments using symbols, unless main effects interacted; n = 600; significant F ratios and p values are presented in bold.

Treatment		Initial Height (cm)	Final Height (cm)	Initial RCD (mm)	Final RCD (mm)
Fertilizer (F)	Low	23.19 ± 0.31 A	34.03 ± 0.66	2.56 ± 0.04 A	5.35 ± 0.16 B
	High	22.07 ± 0.31 B	36.20 ± 0.66	2.45 ± 0.04 B	5.80 ± 0.16 A
Blackout (B)	No	23.71 ± 0.31 a	36.08 ± 0.66 a	2.48 ± 0.04	5.57 ± 0.16
	Yes	23.54 ± 0.31 b	34.15 ± 0.66 b	2.53 ± 0.04	5.57 ± 0.16
Protection (P)	No	22.52 ± 0.31	28.31 ± 0.66	2.51 ± 0.04	5.38 ± 0.16 *
	Yes	22.74 ± 0.31	41.93 ± 0.65	2.49 ± 0.04	5.76 ± 0.16 ^
Source	df	F (p value)	F (p value)	F (p value)	F (p value)
Fertilizer[1] (F)	1	**20.63 (<0.0001)**	9.17 (0.0026)	**9.36 (0.0023)**	**24.55 (<0.0001)**
Blackout[2] (B)	1	**77.45 (<0.0001)**	**7.28 (0.0072)**	2.16 (0.1414)	0.00 (0.9899)
Protection[3] (P)	1	0.79 (0.3736)	362.60 (<0.0001)	0.37 (0.5436)	**18.00 (<0.0001)**
F*B	1	0.00 (0.9752)	0.56 (0.4530)	0.19 (0.6648)	0.01 (0.9253)
F*P	1	0.15 (0.6983)	**5.77 (0.0166)**	0.15 (0.7030)	0.17 (0.6820)
B*P	1	0.40 (0.5265)	0.30 (0.5829)	0.35 (0.5529)	0.00 (0.9815)
F*B*P	1	2.56 (0.1099)	0.21 (0.6470)	0.06 (0.8125)	0.06 (0.8005)

[1] - low = 100 ppm N and high = 200 ppm N; [2] - yes = complete obstruction of solar radiation after 20 weeks of seedling growth from 1700 h through 0900 h the following day for a period of two weeks; [3] - yes = outplanted seedlings protected from ungulate browse via rigid seedling protectors.

Table 3: Effects of fertilizer rate and blackout treatment on cold hardiness of *Thuja plicata* (western redcedar) at different test temperatures. Mean I_d (± SE) are presented. Within each column, significant differences (at $\alpha = 0.05$) are indicated between fertilizer rates using uppercase letters and between blackout treatments using lowercase letters, unless the main effects interacted; n = 20; significant F ratios and p values are presented in bold.

Treatment		-7 °C	-14 °C	-21 °C	-28 °C	-35 °C	-40 °C
Fertilizer[1] (F)	Low	0.12 ± 0.004 A	0.20 ± 0.01	0.42 ± 0.02	0.51 ± 0.02	0.53 ± 0.02	0.53 ± 0.01
	High	0.11 ± 0.004 B	0.15 ± 0.01	0.40 ± 0.02	0.54 ± 0.02	0.54 ± 0.02	0.56 ± 0.01
Blackout[2] (B)	No	0.12 ± 0.004 a	0.18 ± 0.01	0.40 ± 0.02	0.51 ± 0.02	0.55 ± 0.02	0.56 ± 0.01
	Yes	0.11 ± 0.004 b	0.16 ± 0.01	0.42 ± 0.02	0.54 ± 0.02	0.52 ± 0.02	0.53 ± 0.01
Source	**df**	**F (p value)**	**F (p value)**	**F (p value)**	**F (p value)**	**F (p value)**	**F (p value)**
Fertilizer (F)	1	**5.16 (0.0373)**	11.26 (0.0040)	0.45 (0.5123)	0.68 (0.4222)	0.06 (0.8157)	1.29 (0.2733)
Blackout (B)	1	**5.16 (0.0373)**	1.46 (0.2446)	0.54 (0.4736)	1.00 (0.3321)	0.61 (0.4475)	3.23 (0.0913)
F*B	1	0.42 (0.5286)	**4.61 (0.0474)**	1.39 (0.2550)	0.50 (0.4903)	0.14 (0.7145)	0.08 (0.7803)

[1] - low = 100 ppm N and high = 200 ppm N; [2] - yes = complete obstruction of solar radiation after 20 weeks of seedling growth from 1700 h through 0900 h the following day for a period of two weeks

Table 4: Effects of fertilizer rate and blackout treatment on levels of pinene and sabinene in one-year-old container- grown *Thuja plicata* (western redcedar) seedlings. Means (± SE) are presented; n = 20; significant F ratios and p values are presented in bold.

Treatment		Pinene (ppb)	Sabinene (ppb)
Fertilizer[1] (F)	Low	.	10.24 ± 3.45
	High	3.68 ± 0.84	3.33 ± 2.43
Blackout[2] (B)	No	.	9.99 ± 3.02
	Yes	3.67 ± 0.86	3.57 ± 2.97
Source	**df**	**F (p value)**	**F (p value)**
Fertilizer (F)	1	0.07 (0.8054)	2.42 (0.1951)
Blackout (B)	1	0.08 (0.7803)	2.05 (0.2252)
F*B	1	.	1.99 (0.2315)

[1] - low = 100 ppm N and high = 200 ppm N; [2] - yes = complete obstruction of solar radiation after 20 weeks of seedling growth from 1700 h through 0900 h the following day for a period of two weeks; . = non-estimable.

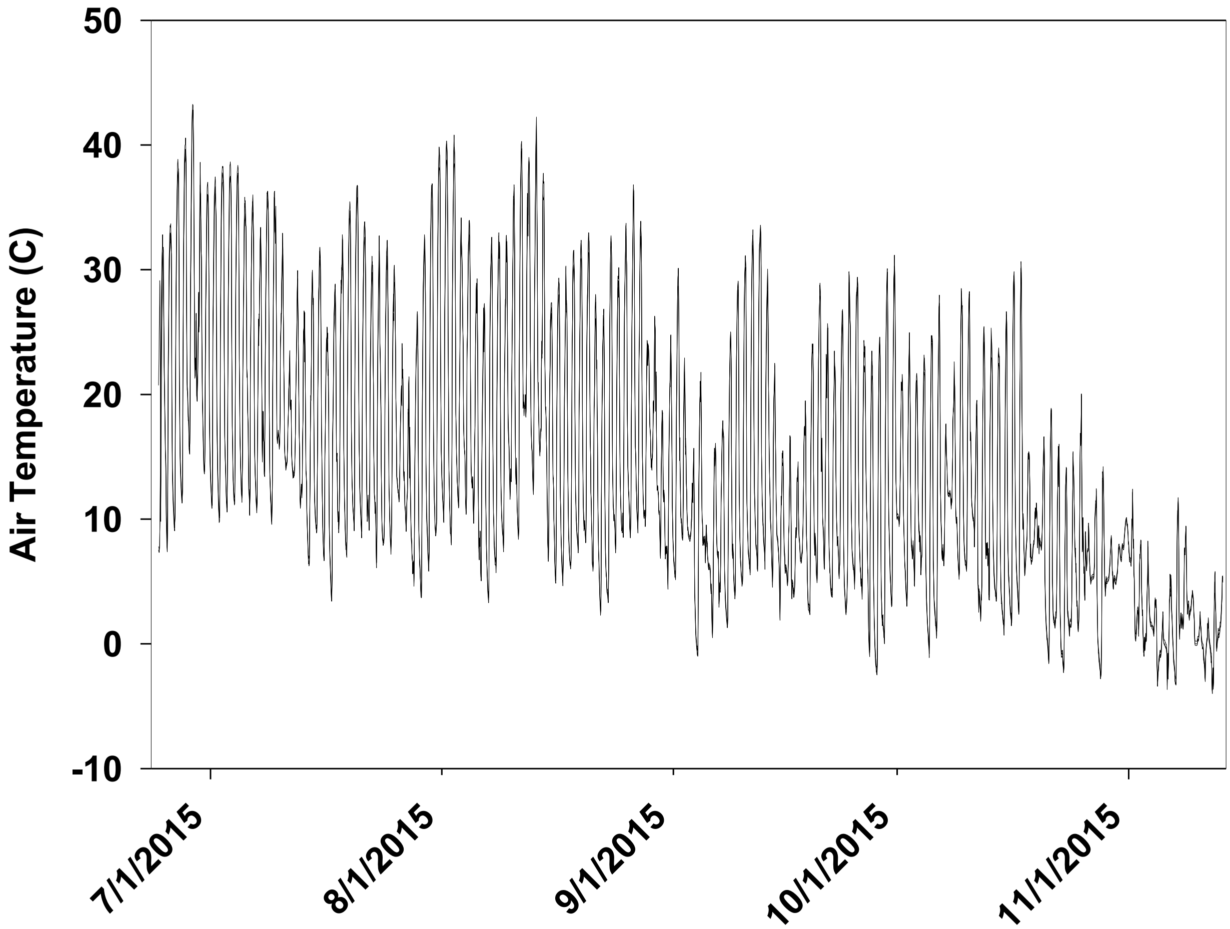

Figure 1: Hourly air temperature (°C) from 24 June 2015 through 13 November 2015 as recorded on three Em50 data loggers at the field site - Potlatch Corporation's Mad Bear PCT site in Orofino, ID (46.54°N, 115.90°W).

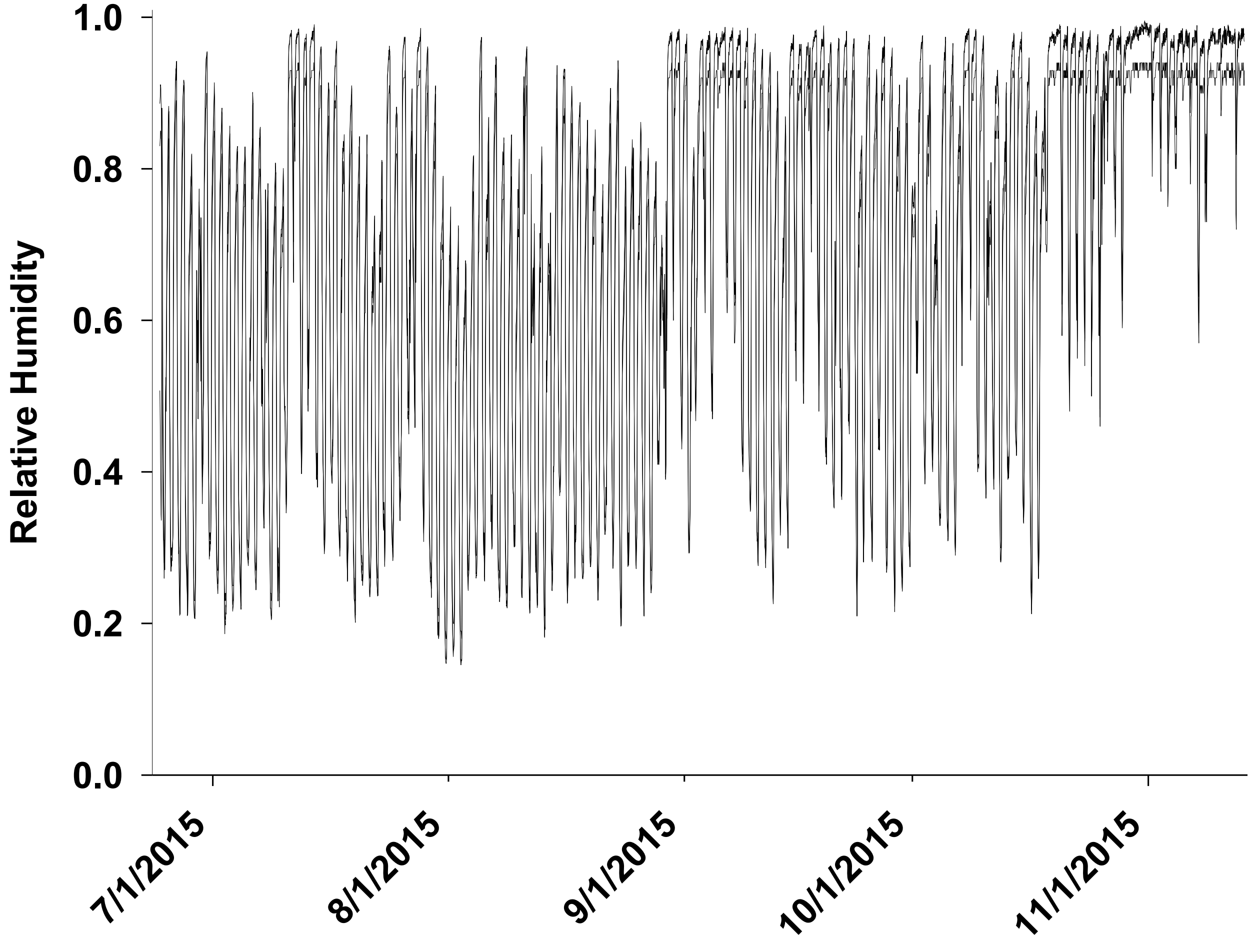

Figure 2: Hourly relative humidity from 24 June 2015 through 13 November 2015 as recorded on three Em50 data loggers at the field site - Potlatch Corporation’s Mad Bear PCT site in Orofino, ID (46.54°N, 115.90°W).

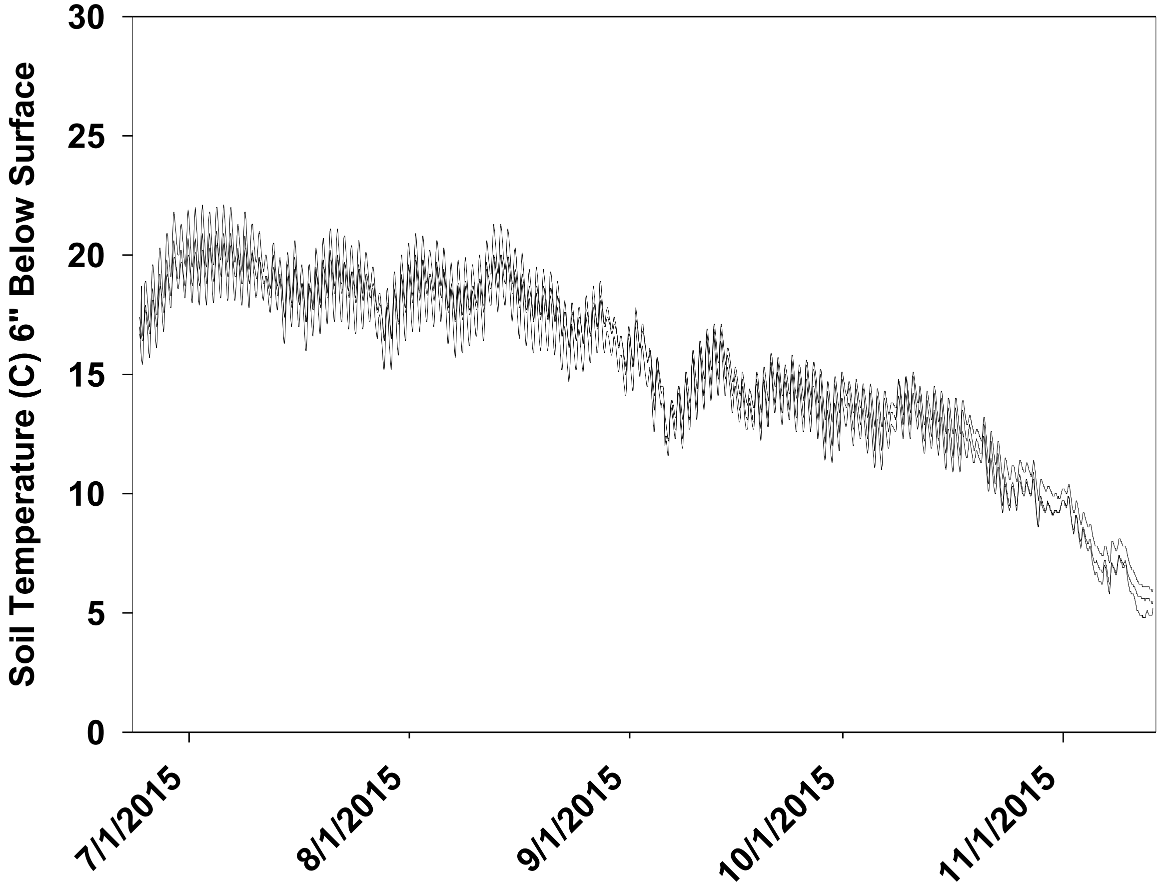

Figure 3: Hourly temperature (°C) at 15.2 cm below soil surface from 24 June 2015 through 13 November 2015 as recorded on three Em50 data loggers at the field site - Potlatch Corporation's Mad Bear PCT site in Orofino, ID (46.54°N, 115.90°W).

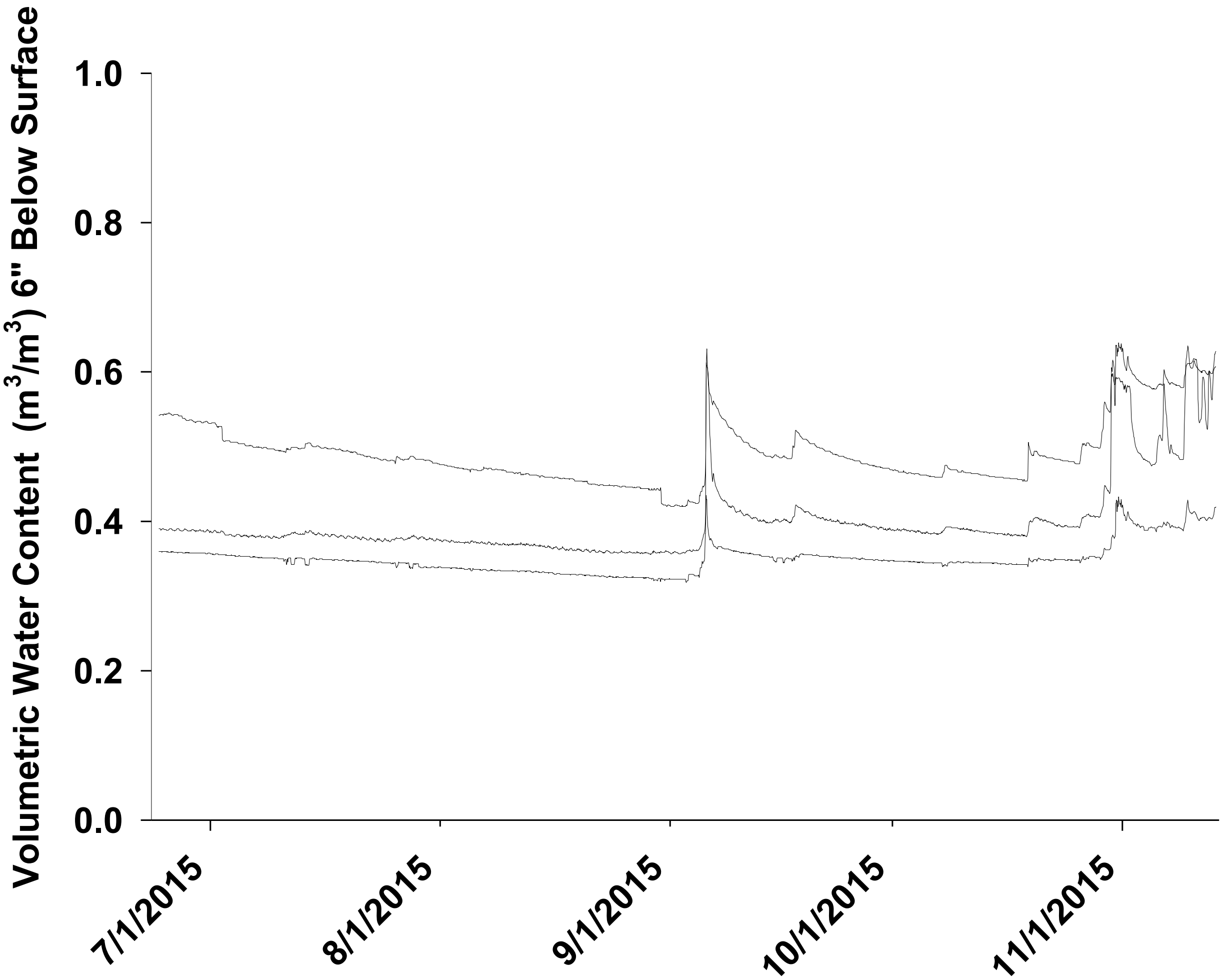

Figure 4: Hourly volumetric water content (m3/m3) at 15.2 cm below soil surface from 24 June 2015 through 13 November 2015 as recorded on three Em50 data loggers at the field site - Potlatch Corporation's Mad Bear PCT site in Orofino, ID (46.54°N, 115.90°W).

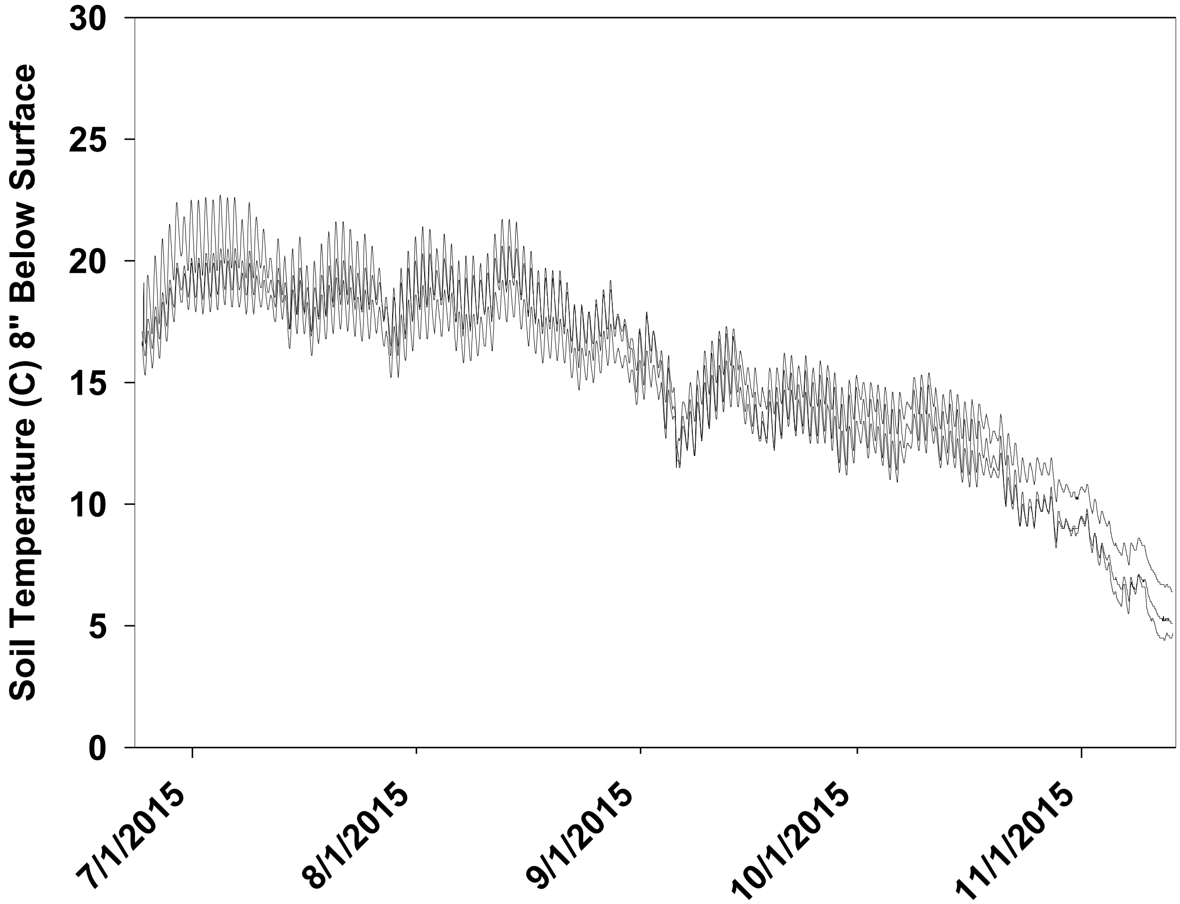

Figure 5: Hourly temperature (°C) at 20.3 cm below soil surface from 24 June 2015 through 13 November 2015 as recorded on three Em50 data loggers at the field site - Potlatch Corporation's Mad Bear PCT site in Orofino, ID (46.54°N, 115.90°W).

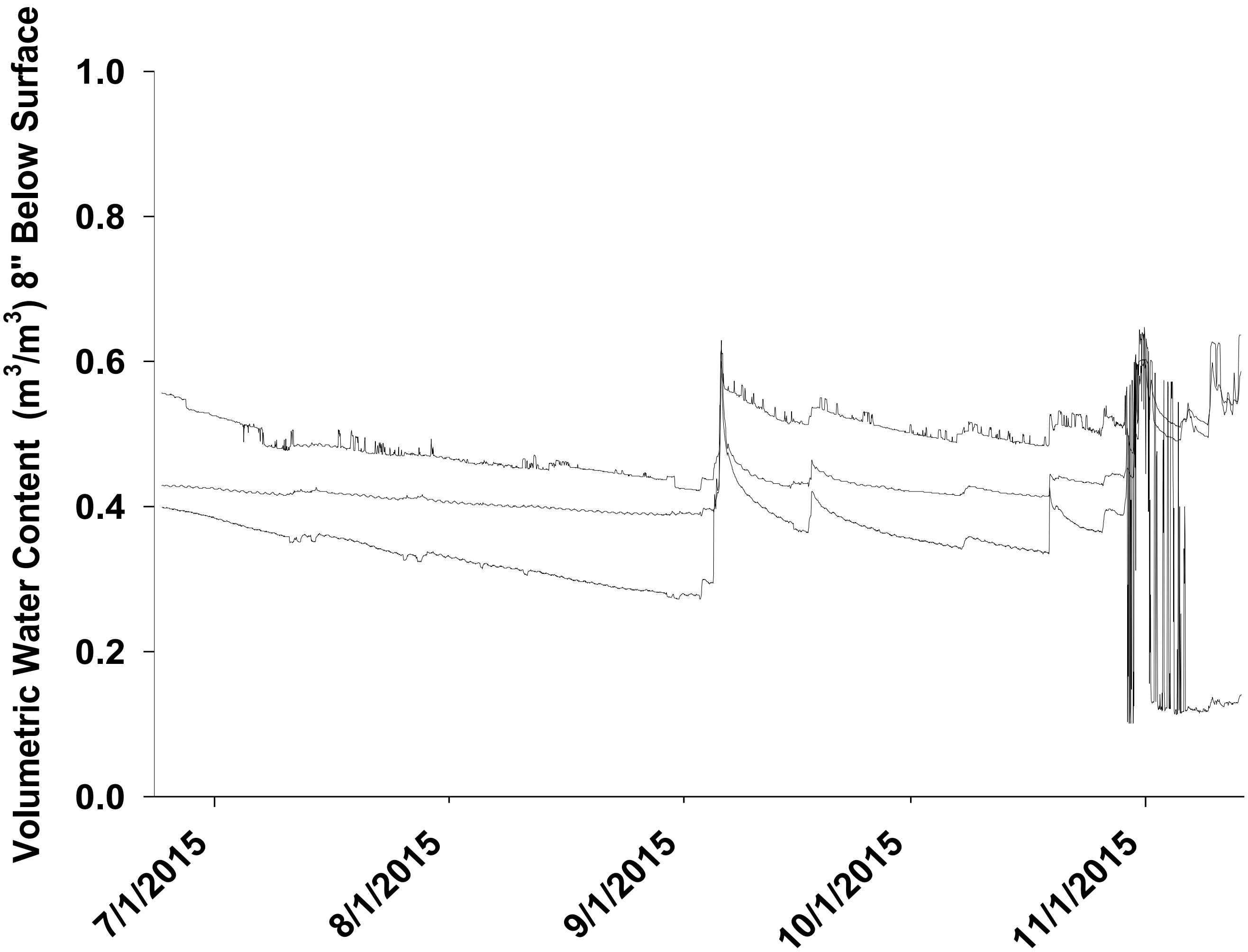

Figure 6: Hourly volumetric water content (m3/m3) at 20.3 cm below soil surface from 24 June 2015 through 13 November 2015 as recorded on three Em50 data loggers at the field site - Potlatch Corporation's Mad Bear PCT site in Orofino, ID (46.54°N, 115.90°W).

Area Percent Report -- Sorted by Retention Time

```
Information from Data File:
File        : C:\HPCHEM\1\DATA\Kenneth\KP731154.D
Operator    :
Acquired    : 31 Jul 2015   12:38 pm using AcqMethod TERPEN04
Sample Name: Sample 4
Misc Info   : Thuja volatiles in hexane
Vial Number: 4
CurrentMeth: C:\HPCHEM\1\METHODS\TERPEN04.M
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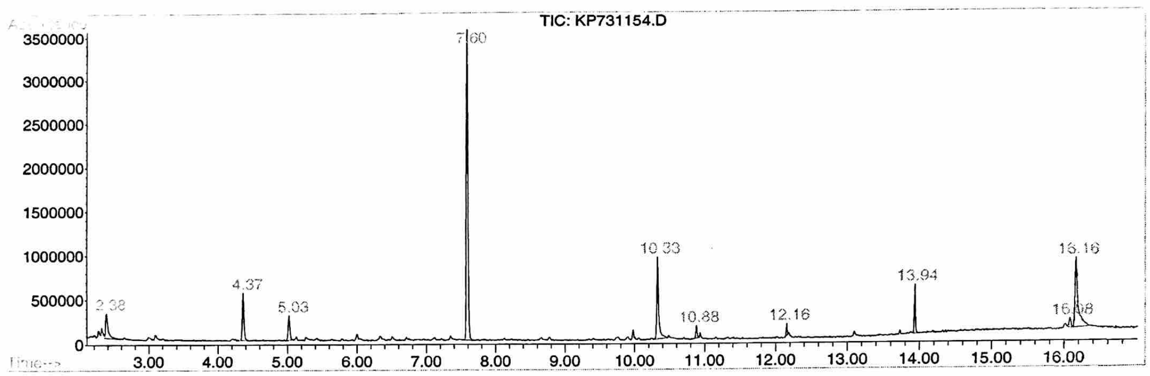

Ret Time	Signal Descr	Area	% Pk	%LPk
2.382	TIC	7359367	100.000	100.000
4.365	TIC	8748365	100.000	100.000
5.025	TIC	4706573	100.000	100.000
7.598	TIC	55713472	100.000	100.000
10.327	TIC	16701039	100.000	100.000
10.885	TIC	1618535	100.000	100.000
12.158	TIC	1094390	100.000	100.000
13.939	TIC	5754514	100.000	100.000
16.084	TIC	1936308	100.000	100.000
16.165	TIC	22117389	100.000	100.000

Figure 7: Readout highlighting *Thuja* volatiles in hexane with major and minor peaks identified.

Figure 8: Deer browsing on western redcedar seedling during feeding trial.

Appendix 1. Browse Data from the Controlled Feeding Trial

Fertilizer Rate[1]	Blackout Treatment[2]	Replicate	Seedling Type[3]	Deer[4]	Browse[5]
Low	No	1	4 liter	1	Yes
Low	No	2	4 liter	2	Yes
Low	No	3	4 liter	3	Yes
Low	No	4	4 liter	4	Yes
Low	No	5	4 liter	5	Yes
Low	Yes	1	4 liter	1	Yes
Low	Yes	2	4 liter	2	Yes
Low	Yes	3	4 liter	3	Yes
Low	Yes	4	4 liter	4	Yes
Low	Yes	5	4 liter	5	Yes
High	No	1	4 liter	1	Yes
High	No	2	4 liter	2	No
High	No	3	4 liter	3	Yes
High	No	4	4 liter	4	Yes
High	No	5	4 liter	5	Yes
High	Yes	1	4 liter	1	Yes
High	Yes	2	4 liter	2	No
High	Yes	3	4 liter	3	Yes
High	Yes	4	4 liter	4	Yes
High	Yes	5	4 liter	5	No
Low	No	1	Seedling	4	Yes
Low	No	2	Seedling	3	Yes
Low	No	3	Seedling	5	Yes
Low	No	4	Seedling	2	No
Low	No	5	Seedling	1	Yes
Low	Yes	1	Seedling	4	Yes
Low	Yes	2	Seedling	3	Yes
Low	Yes	3	Seedling	5	Yes
Low	Yes	4	Seedling	2	No
Low	Yes	5	Seedling	1	Yes
High	No	1	Seedling	4	Yes
High	No	2	Seedling	3	Yes
High	No	3	Seedling	5	Yes
High	No	4	Seedling	2	Yes
High	No	5	Seedling	1	Yes
High	Yes	1	Seedling	4	Yes
High	Yes	2	Seedling	3	Yes
High	Yes	3	Seedling	5	Yes
High	Yes	4	Seedling	2	No

High	Yes	5	Seedling	1	Yes

[1] - high - 200 ppm N and low - 100 ppm N; [2] - yes = complete obstruction of solar radiation after 20 weeks of seedling growth from 1700 h through 0900 h the following day for a period of two weeks; [3] - Seedling = initial copperblock-grown seedlings that had been removed from cold storage and 4 liter = 1-4 liter transplants; [4] - 1 = WR, 2 = LC, 3 = TR, 4 = SA, and 5 = PP; [5] - No = plant material not browsed and Yes = plant material browsed.