A Stock Quality Assessment Procedure for Characterizing Nursery-Grown Seedlings'

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Abstract.--Western hemlock and western red cedar seedlings were grown in a container greenhouse system under four different nursery cultural treatments. A stock quality assessment procedure was developed to characterize a seedling's drought avoidance (i.e. needle and root surface area, needle resistance, root growth capacity), drought tolerance (i.e. osmotic potential) and cold tolerance (i.e. frost hardiness, low temperature root growth capacity) capabilities developed through the various nursery cultural treatments. Results showed western hemlock seedlings in the short-day treatments and western red cedar seedlings in the moisture stress treatments had the best stock performance potential characteristics.

INTRODUCTION

Reforestation success depends largely on matching proper seedling stocktypes with field site conditions. To achieve reforestation success, foresters must be able to characterize seedling performance potential with expected field site environmental conditions (Sutton 1988). Thus, a predictive stock quality assessment procedure needs to simulate expected field site conditions to determine what morphological parameters and physiological characteristics are important for successful seedling establishment.

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Stock quality assessment over the last decade has evolved to include a wide array of both morphological and physiological measurement procedures (see reviews by Sutton 1979, Chavasse 1980, Jaramillo 1980, Schmidt-Vogt 1981, Glerum 1988). Ritchie (1984) organized these assessment procedures into two areas called material attributes (i.e. direct measurements: nutrition, morphology, water relations, bud dormancy) and performance attributes (i.e. whole seedling response: root growth capacity, frost hardiness, stress resistance) and they were the focus of a workshop held at Oregon State University in 1984 (Duryea 1985a). Duryea (1985b) indicated that stock quality assessment procedures have come along way. However, further refining of measurement procedures is required. Specifically, a stock quality assessment program should consider physiological processes critical to seedling field performance, seedlings must be assessed under environmental conditions defined as critical to limiting field growth and survival and there must be a battery of tests to assess morphological and physiological factors important in predicting field performance success.

Within this study, western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) and western red cedar (*Thuya plicata* Donn)

seedlings, grown under four nursery cultural regimes, were examined for their capability to tolerate or avoid environmental conditions expected to influence establishment on a reforestation site. Based on stress tolerance/avoidance concepts of Levitt (1972), stock performance potential tests determined seedling's physiological and morphological response to optimal and suboptimal temperature and moisture conditions. Also measured were morphological parameters important in conferring desired stress avoidance characteristics. Only partial stock performance potential test results are presented in this paper, with further results to be reported in detail elsewhere. The actual effectiveness of a stock quality

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assessment procedure for predicting field

MATERIALS AND METHODS

Seedling Development

Western hemlock (Tsuga heterophylla (Raf.) Sarg.) seed (British Columbia Forest Service (BCFS Registered Seedlot no.3906; Lat. 48° 55" N, Long. 123° 55" W; elevation 340m) was stratified at 1° C for 4 weeks before sowing. Western red cedar (Thuya plicata Donn) seed (BCFS Registered Seedlot no. 7853; Lat.48° 50" N, Long. 124° 00" W; elevation 525m) was soaked in tap water for thirty-six hours prior to sowing. Both species were sown on March 2, 1987 in BC/CFS 313A styroblocks in a 3:1 mixture of peat and vermiculite with dolomite lime added to adjust the pH to 5.0 with coarse sand as a seed cover.

Seedlings were grown at the Pacific Forestry Centre, Victoria, B.C. (Lat. 48° 28" N). The greenhouse environment was maintained at a day/night temperature of 21/18° C, 50 percent relative humidity and natural light supplemented at night with high pressure sodium vapor lamps (i.e. 6pmol $\rm s^{-1}\ m^{-2})$

to provide a sixteen hour photoperiod. Seedlings were watered and fertilized (i.e. 20-20-20 NPK with micronutrients) twice weekly (500 mg L^{-1}) and biweekly with the heptahydrate form of ferrous sulphate (155 mg L^{-1}).

Seedlings were grown under the above greenhouse regime until July 20, 1987 when mean seedling shoot height had reached 15.8 and 16.3 cm for western hemlock and western red cedar, respectively. At this point four dormancy development treatments were applied to one fourth of the seedling population for each species. The dormancy treatments were as follows:

- Long-day wet (LDW); seedlings continued to receive the above greenhouse regime until the end of August.
- Long-day dry (LDD); seedlings had the extended photoperiod as in the above stated greenhouse regime, but on July 20, 1987 the moisture stress treatment was initiated.
- Short-day dry (SDD); seedlings, on July 20, 1987, had the moisture stress treatment initiated and had photoperiod reduced to eight hours on August 1,1987.
- 4. Short-day wet (SDW); seedlings continued to receive the above stated watering and fertilization regime until the end of August (as in 1) but had photoperiod reduced to eight hours on August 1, 1987.

All dormancy induction treatments were concluded on August 29, 1987, after which a regular watering and natural daylength regime was resumed. Fertilizer (10-51-16 NPK with micronutrients) was applied (500 mg L⁻¹) weekly until November and biweekly thereafter. Temperatures (day/night) were set at 20/10°C until 15 September, 17/8°C until 10 October, 15/5°C until 15 October, 13/4°C until 11 November, 10/3°C until 18 November and 8/0°C until seedlings were put into cold storage (2°C) on 11 January 1988.

In the moisture stress treatment, styroblocks were allowed to dry down to approximately 2.85 kg below their saturated weight before rewatering, plus fertilizing, to saturation and repeating the drying cycle. Throughout the six week period seedlings were subjected to six drying cycles. Seedling water status was monitored with predawn and noon xylem pressure potential readings during the moisture stress treatments. Xylem pressure potential readings were taken with a pressure chamber on six replicates from each treatment following procedures described by Ritchie and Hinckley (1975). Average predawn and noon xylem pressure readings for each species at the end of drying cycles were -0.3 and -0.7 MPa for western hemlock and -0.4 and -1.0 MPa for western red cedar, respectively. Though readings appeared to indicate little seedling water stress, many western hemlock

seedling shoots were wilted by the afternoon of the last day of each drying cycle. Thus, western hemlock shoot wilt was used as the indicator to end a drying cycle.

Statistical design of the greenhouse layout was a modified Latin Square. The four dormancy induction treatments were randomly assigned to four positions on the greenhouse benches. The two species were randomly assigned to opposite sides of each treatment block position. Over the course of the experiment styroblocks within a dormancy treatment were rotated every six weeks. Analysis at the end of the greenhouse operations showed no effect of bench location.

Seedling shoot height was nondestructively assessed (n=25) biweekly during the growing season, weekly during the dormancy treatment period and biweekly until October 23,1987.

Stock Performance Potential Tests

During January and February 1988 western hemlock and western red cedar seedlings in all treatments were assessed for physiological and morphological characteristics. Below is a brief description of the various stock performance potential tests.

Needle and root surface area

Surface area measurements were used to determine the needle transpiration area to root absorption area produced by all species/treatment combinations. Twentyfive seedlings from each species/treatment combination were dissected into workable shoot and root sections and processed through a Li-3100 (Li-Cor Inc.) area meter. Analysis of variance and Tukey's mean separation test were used to determine treatment differences within a species (Steele and Torrie 1980).

Root growth capacity

Standard soil/pot test.--Seedlings from each species/treatment combination were placed in pots (8 replicates with 3 seedlings per pot) containing a 3:1 mixture of peat and vermiculite plus dolimite lime (2Kg m⁻³). Pots were placed in a completely randomized design within environmentally controlled (i.e. $22/10^{\circ}C$ day/night temperature, 55% relative humidity, 16 hr photoperiod at 200 mu mol s⁻¹ m⁻²) growth rooms. Seedlings were grown for seven days, after which root development was assessed using Burdett's (1979) semiquantitative scale.

Hydroponi_c t_est.--Root growth capacity was also assessed for all species/treatment combinations in a hydroponic system. Seedling root systems were placed in a darkened aerated aquarium at a water temperature of 5° or 22°C and then seedlings were grown in a controlled environment growth room (i.e. 22°C air temperature, relative humidity 50%, 16 hr photoperiod at 650 mu mol s^{-1} M-2) for fourteen days. Root development was assessed in all species/treatment combinations (n=10) after fourteen days. The root classification system used for RGC testing was modified from the classification scheme outlined by Burdett (1979). The root classification categories are as follows: 0 = no roots, 1 = new roots but none over .5 cm, 2 = 1 to 3 new roots over 1 cm, 3 =4 to 10 new roots over 1 cm, 4 = 11 to 30 new roots over 1 cm, 5 = 31 to 50 new roots over 1 cm, 6 = 51 to 75 new roots over 1 cm, 7 = 76 to 100 new roots over 1 cm and, 8 = > 100 new roots over 1 cm. Analysis of variance and Tukey's mean separation test were used to determine treatment differences within a species.

Frost hardiness

Frost hardiness was assessed for all species/treatment combinations at -9,-12,-15 and -18°C test temperatures. Frost hardiness assessment was conducted by the B.C. Ministry of Forest and Lands, Surrey nursery. The method used was standard provincial procedure for the seedling browning test (Simpson M.o.F.L. personal communication). Seedlings from each species/treatment combination (n=40) were divided into two groups and assessed in a replicated experiment at the above mentioned temperatures. Analysis of variance and Tukey's mean separation test were used to determine treatment differences within a species.

Osmotic potential

Pressure-volume analysis was used to determine osmotic potential at saturation and turgor loss point for all species/treatment combinations. Six replicates for each species/treatment combination were used for determination of pressure-volume curves with techniques described by Hinckley et al. (1980). Osmotic potentials were then determined by using a software program (Schulte and Hinckley 1985). Analysis of variance and Tukey's mean separation test were used to determine treatment differences within a species.

Needle resistance

Needle resistance was used to determine cuticular development for all species/treatment combinations. Seedlings were potted in a sand culture system, placed in the controlled environment room (described in the root growth capacity section), well watered for five days and then allowed to slowly dry down. Seedlings were assessed for needle resistance during the time they were well watered and after continuously monitored base xylem pressure potentials had reached -1.5 MPa.

Needle resistance was measured in a foliage cuvette with a porometer (Li6250, Li-Cor Inc.). For each measurement period, readings were taken on ten randomly selected seedlings from each species/treatment combination. Readings were taken one to three hours after the lights went off in the evening. At the end of the experiment, sample branches were removed and needle surface area was determined with a Li3100 area meter. Analysis of variance and Tukey's mean separation test were used to determine treatment differences within a species.

RESULTS

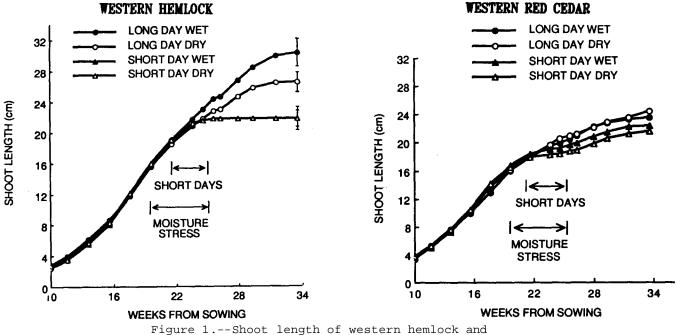
Growing season height growth

Western hemlock seedlings in the short-day treatments ceased shoot elongation by the end of the dormancy induction treatment, August 28, 1987 (Fig. 1). Seedlings in the long-day treatments continued shoot extension until early October. Short-day treatments had greater affect on the phenology of shoot growth than moisture stress treatments.

Western red cedar seedlings showed dormancy induction treatments to have no effect on shoot phenology or growth rate (Fig. 1). Short-day and moisture stress treatments did depress the rate of shoot growth, but not to any significant degree.

Needle and root surface area

Western hemlock seedlings in the LDW treatment had significantly more needle surface area than all other treatments (Fig. 2). Seedlings in the SDD and SDW treatments had the lowest and second lowest needle surface area, respectively. Root surface area was similar between all treatments.



.gure 1.--Shoot length of western hemlock and western red cedar seedlings subjected to the dormancy induction treatments: a) long-day dry, b) long-day wet, c) short-day dry or d) shortday wet. Treatments (shown by arrows) were applied from July 20, 1987 until August 29, 1987. Vertical lines indicate ± 1 SE.

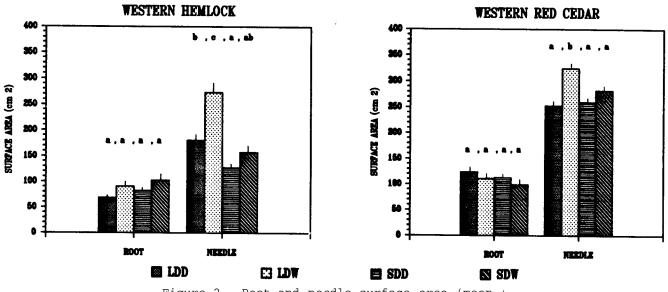


Figure 2.--Root and needle surface area (mean + SE) of western hemlock and western red cedar seedlings from the dormancy induction treatments: a) long-day dry (LDD),b) longday wet (LDW),c) short-day dry (SDD) or d) short-day wet (SDW).Significant treatment differences, for roots or needles, determined by Tukey's mean separation test (p=.05) are shown by different letters.

Western red cedar seedlings in the LDW treatment had the highest needle surface area (Fig. 2). Root surface area was similar between all treatments.

Root growth capacity

Hydroponic root growth capacity (RGC) data for western hemlock at 22° C shows the SDD treatment produced more new roots than all other treatments while the SDW treatment produced the least number of roots compared to other treatments (Fig. 3A). In the soil/pot system LDD treatment had a significantly lower RGC (Fig. 3B). At 5° C new root growth was greater in the short day treatments (Fig. 3A).

Western red cedar hydroponic RGC data at 22° C shows new root growth was greatest in the LDW treatment and least in the sDW treatment (Fig. 3A). There were no treatment differences for seedlings tested in the soil/pot system (Fig. 3B). At 5° C new root growth was low in all treatments (Fig. 3A).

Frost hardiness

Western hemlock seedlings in the LDW treatment had the greatest amount of needle damage at all measured temperatures (Fig. 4). Seedlings in the

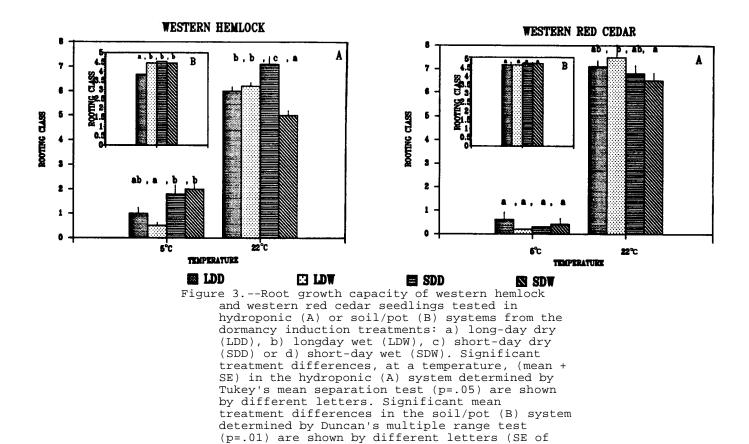
LDD treatment showed the second highest percent of needle damage at lower temperatures (i.e. -15 and -18° C). Seedlings in the SDW treatment had the least amount of needle damage at lower temperatures.

Western red cedar seedlings in the LDW treatment had the greatest amount of needle damage at all temperatures (Fig. 4). At lower temperatures (i.e. -15 and -18° C), seedlings in the SDW treatment had needle damage comparable to the LDW treatment. At -18° C seedlings in the LDD treatment had the least amount of needle damage.

Osmotic potential

Western hemlock seedlings in the SDW treatment had the most negative osmotic potential, at saturated and turgor loss point, of all treatments (Fig. 5). Seedlings in the SDD treatment had a more negative osmotic potential at turgor loss point compared to LDD and LDW,but not as negative as SDW. The LDD treatment showed the least negative saturated and turgor loss point osmotic potentials of all treatments.

Western red cedar seedlings showed no statistically significant difference between treatments at both saturated and turgor loss point (Fig. 5). However,



pop.+/-.16 for western hemlock and +/.14 for

western red cedar).

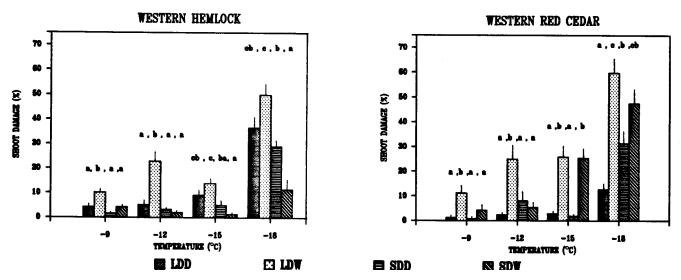


Figure 4.--Frost hardiness (mean + SE)of western hemlock and western red cedar seedlings from dormancy induction treatments[:] a) long-day dry (LDD), b) long-day wet (LDW), c) shortday dry (SDD) or d) short-day wet (SDW). Significant treatment differences, at a temperature, determined by Tukey's mean separation test (p=.05) are shown by different letters. the moisture stress treatment did cause a slightly more negative turgor loss point osmotic potential.

Needle resistance

Needle resistance of well watered western hemlock seedlings showed no difference between treatments (Fig. 6). Under water stress conditions needle resistance was highest in the LDW and LDD treatments. Seedlings in the SDW treatment had the lowest needle resistance values, while SDD was slightly higher, under water stress conditions.

Western red cedar seedlings, under well watered conditions, showed no difference in needle resistance between treatments (Fig. 6). Under water stress conditions seedlings in the SDD treatment had the greatest level of needle resistance. Seedlings in the SDW treatment had the second highest level of needle resistance under water stress conditions, while seedlings in the LDD and LDW treatments had the lowest levels of needle resistance.

DISCUSSION

Western hemlock seedling development in the nursery showed shortdays, applied in early August, stopped shoot elongation, while moisture stress did not. These results substantiate conclusions from a similar experiment conducted in 1986 and reported at this meeting (Arnottet.al. 1988). Western red cedar seedlings seasonal shoot height did not show any response to nursery cultural treatments. Apparently the cultural treatments were not severe enough or were not the proper environmental cues to change shoot height development.

A balanced root/shoot ratio, or more accurately the absorbing surface to transpiring surface ratio, is important in reducing the development of high seedling water deficits caused when absorption lags behind transpiration (Kramer and Kozlowski 1979). This reasoning was used for defining root and needle areas as one of the stock performance potential tests. Results showed short-day and moisture stress treatments reduced needle surface area in both species. Western hemlock seedlings in the LDD, SDW and SDD treatment had progressively less needle area, respectively, while all moisture stress and short-day treatment combinations in western red cedar reduced needle area equally. Other research has shown that short-days (review of literature Arnott and Mitchell 1982), moisture stress (Kramer 1969) or both (D'Aoust and Cameron 1982, Macey and Arnott 1986) can alter seedling shoot morphology.

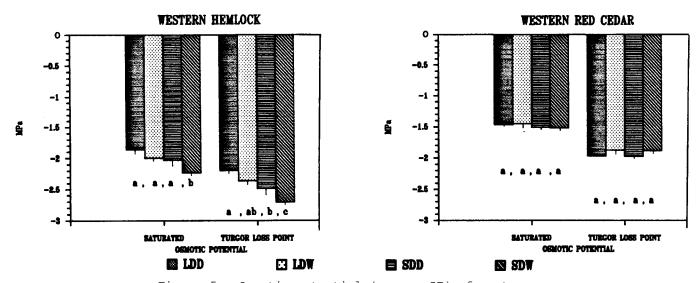
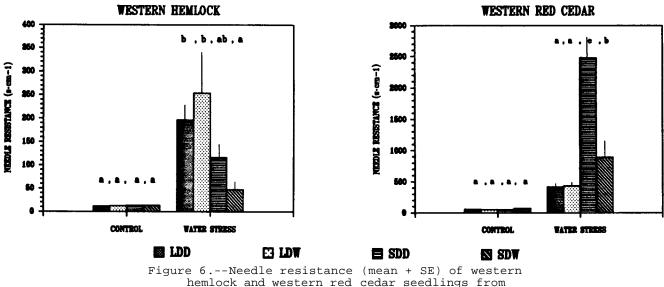


Figure 5.--Osmotic potential (mean + SE) of western hemlock and western red cedar seedlings from dormancy induction treatments: a) long-day dry (LDD), b) long-day wet (LDW), c) short-day dry (SDD) or d) short-day wet (SDW). Significant treatment differences, at saturated or turgor loss point, determined by Tukey's mean separation test (p=.05) are shown by different letters.



hemlock and western red cedar seedlings from dormancy induction treatments: a) long-day dry (LDD), b) long-day wet (LDW), c) short-day dry (SDD) or d) short-day wet (SDW). Significant treatment differences, within control or water stress, determined by Tukey's mean separation test (p=.05) are shown by different letters.

Root area in both species was similar in all nursery cultural treatments. This is contrary to the widely held belief that nursery cultural practices which stop shoot growth will result in a transfer of that seedling growth potential partially into root growth as well as into caliper and bud development (Ledig et al. 1970, Tinus and McDonald 1979). Upon examination of unreported morphological data, nursery cultural treatments did not alter caliper development in either species, but bud development in western hemlock was improved in the SDD and SDW treatments. Other researchers have also found that arresting shoot growth with nursery cultural practices did not result in reallocation of seedling growth potential into other areas of measurable seedling morphology (Heide 1974, Burdett and Yamamoto 1986, Arnott et al. 1988).

A quality seedling needs to have as high a root/shoot ratio as possible to ensure optimum field survival (Thompson 1985). Thus, short-day and moisture stress treatments in both species will improve the root/shoot ratio by reducing needle surface area development but not through enhanced root area development.

Results from root growth capacity (RGC) tests differed depending upon testing procedure. As expected, under optimal root temperature conditions the

seven day soil/pot system produced fewer roots than the fourteen day hydroponic system in comparable species/treatment combinations. This difference between the two testing procedures was due to study length. Seedlings of both species tested at 22°C in the hydroponic or soil/pot system showed statistically significant treatment differences in RGC class. However, it is questionable whether this difference is biologically important because all RGC classifications were 5 or greater in the hydroponic system and 4 or greater in the soil/pot system. Outplanting studies comparing RGC with field survival have shown that above an RGC value of 1 to 3, field survival is usually greater than 80 percent (Burdett et al. 1983, Dunsworth 1986, Burdett 1987). Thus, seedlings from all species/treatment combinations produced in this study have the potential for good field survival.

However, it must be asked whether this testing method is a true representation of edaphic conditions a seedling encounters during early season planting. Seedlings are normally planted in late winter or early spring when soil temperatures are just above 5°C. An RGC test which examines root responses at low root temperatures might provide a stress tolerance test that more effectively predicts nursery cultural treatments influence on early root growth in field planted seedlings. The low temperature test showed western hemlock seedlings in the SDW and SDD treatments produce RGC class values which have predicted good field survival in Pacific Northwest coastal conifers (Dunsworth 1986) compared to other treatments, while western red cedar seedlings did not show any treatment differences. Further work needs to be undertaken to determine whether a low temperature RGC testing procedure would provide useful information on seedling performance as it relates to field site conditions.

Frost hardiness testing was conducted on seedlings during the late winter to determine the level of frost tolerance provided by the nursery cultural treatments. Findings indicate that seedlings of both species in the LDW treatment had the least frost hardiness. Other researchers have also found that nonacclimatized seedlings will develop inadequate frost hardiness (Christersson 1978, D'Aoust and Cameron 1982, Colombo et al. 1982). Western hemlock seedlings developed greater frost hardiness in the short-day treatments, while western red cedar developed greater frost hardiness in the moisture stress treatments. Research has shown short-day treatments can improve frost hardiness (Timmis and Worall 1975, Christersson 1978, D'Aoust and Cameron 1982, Colombo et al. 1982), while moisture stress treatments can improve frost hardiness in some studies (i.e.Douglas-fir, Tanaka and Timmis 1974, Blake et al. 1979) but not in others (i.e. black spruce, D'Aoust and Cameron 1982). This difference in species frost hardiness response to cultural treatments needs to be considered when developing a nursery growing regime.

Interestingly, the combination of short-day and moisture stress was not as effective in conferring frost hardiness as just the short-day treatment in western hemlock and the moisture stress treatment in western red cedar. Work with black spruce has shown this same response (D'Aoust and Cameron 1982). This lack of synergism between short-day and moisture stress to improve frost hardiness indicates that the combined influence creates an environment too stressful for full frost hardiness development.

Osmotic adjustment in western hemlock seedlings was greatest in the SDW followed by the SDD treatment. Western hemlocks' osmotic adjustment in the shortday treatment is an interesting phenomenon. Dickson and Nelson (1982) working with cottonwood found short-day treatments used to induce dormancy increased the sugar levels in leaves. Sugars and organic acids have been shown to cause osmotic adjustment in a number of species (Osonubi and Davies 1978, Sharp and Davies 1979). Thus, the short-day treatment in western hemlock could have promoted increased sugar and organic acid production resulting in increased osmotic adjustment.

Western red cedar seedlings showed only a slight osmotic adjustment in the LDD and SDD treatments. Previously reported work with conifer seedlings has shown greater osmotic adjustment in response to moisture stress (Kandiko et al. 1980, Seiler and Johnson 1985, Bongarten and Teskey 1986, Grossnickle 1988). For western red cedar the problem seemed to be that the drying cycles were not long enough to develop sufficient seedling water stress for greater osmotic adjustment to occur. Further work needs to be conducted to develop moisture stress treatments that will provide maximum osmotic adjustment in western red cedar with minimum impact on other desirable seedling attributes.

Needle resistance is a combination of stomatal, mesophyll and cuticular resistances (Hinckley et al. 1978). As long as the stomata are partially open they are the primary factor influencing needle water loss. However, if the stomata are forced to close (e.g. via seedling water stress and/or darkness) the subsequent measurement of needle resistance would represent the cuticular resistance of the needles. This was the working hypothesis developed for the needle resistance stock performance potential test.

Western hemlock under water stress conditions (i.e. -1.5MPa) resulted in the LDW and LDD treatments having the greatest level of needle resistance. At first glance this would seem to be contrary to the working hypothesis. However, if needle resistance data is examined in conjunction with the osmotic potential data, it shows that needle resistance measurements were taken on seedlings that had never reached the turgor loss point (i.e. range from -2.2 to -2.7 MPa). Thus, these needle resistance measurements were taken at a medium level of water stress for western hemlock. In this condition the SDW and SDD treatments responded to the moderate water stress by keeping their stomata open slightly during the dark phase. Studies have shown that conifers

stomata, under low to moderate moisture stress, can remain open during the dark (Running 1976, Blake and Ferrell 1977).

Western red cedar seedlings showed high needle resistance in the SDD and SDW treatments at the -1.5 MPa measurement time. Western red cedar seedlings at this measurement time had daytime seedling water stress exceed their turgor loss point (i.e. range from -1.8 to -1.95 MPa) which resulted in stomatal closure in all treatments. Thus, when stomata were closed, the cuticular resistance was highest in the SDD and SDW treatments. Seedling water stress will produce seedlings with thicker more cutinized needles (Rook 1972), while short-day treatment in combination with low temperature have been found to reduce seedling transpiration rates (Christersson 1972). Short-day and moisture stress treatments seem to reduce needle water loss in western red cedar.

CONCLUSION

The intent of this stock quality assessment procedure was to develop a testing system that would characterize drought tolerance and avoidance plus frost hardiness and cold tolerance in western hemlock and western red cedar seedlings. This first approximation shows that there is merit to this approach because it provided a good overall picture of how seedlings, treated with different nursery cultural regimes, will respond to potentially deleterious field conditions. Further refinements of the needle resistance and low temperature RGC tests are required. Once refinements are incorporated, foresters will be able to determine seedling performance potential as it relates to both optimal and deleterious field site environmental conditions.

The findings reported show daylength and moisture stress nursery cultural treatments, as applied in this study, can influence the physiological and morphological characteristics of western hemlock, but only some morphological and physiological characteristics of western red cedar seedlings. Western hemlock seedlings in the SDW and SDD treatments and western red cedar seedlings in the LDD and SDD treatments had the best overall stock performance potential grade from the stock quality assessment procedure developed in this study. Further work is required to develop nursery cultural treatments that properly modify the seedlings physiological and morphological characteristics desired for improving establishment on reforestation sites.

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