Effect of Nursery Culture on Morphological and Physiological Development of Western Hemlock Seedlings[1]

J.T. Arnott, B.G. Dunsworth, and C. O'Reilly[2]

Abstract.-Western hemlock seedlings were grown in two container sizes, subjected to short days and moderate moisture stress in July, lifted at three dates during the winter and cold stored for periods of up to four months. The influence of these cultural treatments on seedling morphology and physiology was measured. Short days effectively stopped shoot growth extension; moisture stress did not. Root growth capacity and dormancy release tests indicated a preference for lifting hemlock immediately *before* planting in mid-March, or after two months of cold storage from a mid-January lifting date.

INTRODUCTION

Variation in seedling survival and growth after outplanting reflects differences in the quality of the seedlings as they leave the nursery (Ritchie 1984). Quality is defined *by* certain morphological and physiological criteria and, because it is essential to successful plantation establishment, it has been the subject of considerable research and review (Bunting 1980; Duryea 1985; Ritchie 1984; Schmidt-Vogt 1981; Sutton 1979). Advances in nursery technology and containerized stock rearing (Scarratt gi al. 1981; Tinus and McDonald 1979) provide many nurseries with the ability to grow a wide range of seedling types with different morphological and physiological characteristics. However, these seedling characteristics must be tailored for specific ecological conditions at the planting site and nursery growers must have the knowledge and experience to grow specific seedling stock types for a particular site.

A comprehensive study was made of the effects of nursery cultural regimes on the growth, development, morphology and physiology of container-grown western hemlock (Tsuga heterophylla (Raf.) Sarg.) seedlings. This paper reports the influence o f container cavity size, dormancy induction regime, time of lift, and duration of cold storage on the morphology and physiology of the seedlings. Results of other aspects of the nursery experiment will be reported elsewhere (O'Reilly et al. 1989a, 1989b) while the early growth response of these different types of seedlings after outplanting will be reported by O'Reilly et al. (1989c) at this meeting.

MATERIALS AND METHODS

Western hemlock seeds (British Columbia Forest Service, Registered Seedlot No. 3097; Lat. 48°39N, Long. 123°39W; Elevation 760 m) from Vancouver Island were stratified at 2°C for four weeks before sowing February 12, 1986 in BC/CFS styroblocks (Beaver Plastics Ltd., Edmonton, Alberta)[3] of small (PSB 313[4] abbreviated to S3) and large (PSB 415B[4] abbreviated to S4) cavity diameters. The styroblocks were placed in an experimental greenhouse at the Pacific Forestry Center, Victoria, B.C. (Lat. 48°28N) maintained at 21°/18°C (day/night), 50 % humidity and an 18-h photoperiod. Natural day length was supplemented by high pressure sodium vapour lights

1Paper presented at the combined meeting of the Western Forest Nursery Council and the Forest Nursery Association of British Columbia, Vernon, B.C. August 8-11,1988.

[2]J. T. Arnott is a Research Scientist, Pacific Forestry Center, Canadian Forestry Service, Victoria, B.C.; B. G. Dunsworth is an Ecophysiologist, MacMillan Bloedel Ltd., Nanaimo, B.C. and C. O'Reilly is a Research Associate, Biology Department, University of Victoria, Victoria, B.C.

'Mention of specific commercial products and formulations does not constitute endorsement *by* the Canadian Forestry Service.

[4]PSB 313 and PSB 415B styroblocks have respective cavity diameters of 27 and 35 mm, ribbed cavity volumes of 57 and 102 cm^3 and spatial densities of 932 and 526 cavities$\cdot M^{-2}$

that provided a photon flux density of at least 6 µmol.s^{-1}•m^{-2} (500 lx) at the seedling level. The styroblocks contained a 3:1 mixture of peat and vermiculite with 2.0 kg-M^{-3} dolomite lime (10 mesh and finer) added. The styroblocks were misted daily during germination, and fertilized with biweekly applications of 20N-20P-20K fertilizer with micronutrients (Green Valley Fertilizer, Surrey, B.C.) at 500 mg-L^{-1} and every two weeks with the heptahydrate form of ferrous sulphate at 155 mg-L^{-1}. After September 15, greenhouse temperatures were set a 18°/15°C (day/night) until September 29, 15°/10°C until October 27, 15°/5°C until November 17, and 10°/5°C, thereafter.

Dormancy Induction Treatments

The seedlings were subjected to four dormancy induction regimes; a long- or short-day photoperiod of 18 h and 8 h, respectively, under conditions of moderate moisture stress (dry) or no moisture stress conditions (wet). In this section we use the term dormancy to mean a suspension of shoot length growth without specifying the physiological state of the plant (Downs and Bevington 1981) or the stage of bud development (O'Reilly d al. 1989b). The objective of applying these dormancy induction regimes, and growing the seedlings in small and large containers, was to create a range of different morphological seedling types. Induction regimes began on July 15, 1986, five months after seeding, and ended four weeks later. Styroblocks in the moisture stress treatments were allowed to dry down to 2.8 (S3) and 3.1(S4) kg below their saturated weight before re-watering to saturation with 20N20P-20K fertilizer added. This dry-down was repeated three times during the four-week dormancy induction period. Predawn xylem water potentials of the seedlings before rewatering to saturation averaged -1.0 MPa. Seedlings in the no stress treatments received water and nutrients as described under materials and methods above. Following the dormancy induction treatments, the water-soluble fertilizer was changed to 1ON-52P-17K (Green Valley Fertilizer) at 500 mg-L^{-1} and the seedlings were grown under the above-described temperature regime and naturally-declining photoperiods.

Lifting Date/Cold Storage Treatments

The final phase of the nursery experiment studied the influence of lifting date and cold storage duration on seedling development. The objective was to create a range of physiological seedling qualities within each stock type by altering the lifting date and duration of cold storage. Seedlings were extracted from the styroblocks in mid-November 1986, mid-January and mid-March, 1987 and placed in 1°C rooms for 4, 2 and 0 months cold storage, respectively.

Nursery Experimental Design

A split-plot design was used in the layout of the experiment. Daylength was randomized between halves of the greenhouse and moisture regime between quarters within each half. Each quarter of the greenhouse was divided into eight blocks. Within each of the eight blocks, a group of styroblocks representing each container cavity size (S3 and S4) were randomly assigned to one of the three lifting dates. Analysis of variance of the morphological data were used to test for treatment effects and their interactions (Steele and Torrie 1980). The analyses are not presented in this paper although they are used in data interpretation. We present data means and their standard errors in the figures.

Measurements of Seedling Morphology

Shoot elongation of five seedlings within each of the eight blocks per treatment combination of container size, day length and moisture regime were measured at 1-2 week intervals from June 6 until October 17, 1986 (40 seedlings per treatment combination).

In another subsample, three seedlings from each of the eight blocks per treatment combination were randomly selected and extracted at each of the three lift dates for determination of shoot and root dry weights, and rootcollar diameter (24 seedlings per treatment combination per lift date = 192 seedlings per lift date).

Measurements of Seedling Physiology

At each lifting date, seedling subsamples were randomly extracted for testing in each of the lift/cold storage treatments (table 1). Root growth capacity (RGC) (Burdett 1979) and dormancy release index (DRI) (Ritchie 1984) were measured on equal numbers of seedlings at each of the three treatment periods A-C. For each test, eight replicates of three seedlings each per treatment combination (container size; photoperiod; moisture regime) were grown in pots containing a 3:1 mixture of peat and vermiculite with 2 kg-M^{-3} dolomite lime added. The pots were placed in a completely random design within growth rooms with day/night temperatures set at 22°/18°C, 55 % relative humidity, and a photoperiod of 16 h provided by mixed fluorescent and incandescent lighting with a photosynthetic photon flux density of 200 µmol-s^{-1}.m^{-2}. Growth room temperatures for the test were as prescribed by D. Simpson (Kalamalka Research

Table 1.—Summary of test periods for the lift date/cold storage treatment combinations.

Lift Date	Cold Storage Duration (Months)		
	0	2	4
Nov. 15	A	B	C
Jan. 15	B	C	
Mar. 15	C		

Period A: 8 treatments
Period B: 16 treatments
Period C: 24 treatments

Station, B.C. Forest Service, personal communication, Oct. 2, 1985).

Seedlings being tested for RGC were extracted from the pots 1 week later, the soil media carefully removed from around the root plugs and the new white growing tips (> 1 cm in length) scored using Burdett's (1979) semiquantitative scale of 0 to 5.

Seedlings being tested for DRI were placed in the growth rooms under similar conditions to the above. The pots were watered twice a week to maintain soil moisture level at, or near field capacity. Seedlings were assessed daily to determine the number of days to terminal budbreak (DBB); buds were considered to have broken when the bud scales parted and needles extended at least 1 to 2 mm. The dormancy release index values were calculated after the equation [1] given by Ritchie (1984). Fully chilled western hemlock seedlings take 9 days to break bud; hence the numerator = 9. Therefore, at the peak of winter dormancy, as defined by Doorenbos (1953), seedlings have a DRI value near 0; when nearing full release from winter dormancy, the DRI value approaches 1. Data from the RGC and DRI tests were subjected to analysis of variance according to a completely random design.

$$[1]\ DRI = \frac{9}{DBB}$$

RESULTS

Seedling Morphology

Container size had a significant (P<0.001) influence on seedling height growth with seedlings in the S3 container being shorter than those in the larger S4 container. As this trend was consistent across all morphological variables measured, only data from the S4 container (PSB 415B) are presented (fig. 1). There were no interactions among the main treatment effects of container size, daylength and moisture regime for seedlings sampled at the end of the growing season.

Seedlings exposed to short days ceased shoot elongation by the end of the dormancy induction treatment period (week 26). Shoots of seedlings under long days continued to extend until mid-October when they formed a terminal bud (week 35) (fig. 1). Moisture stress significantly (P<0.001) reduced shoot length but not to the same degree as was achieved with short days. Moisture availability primarily influenced the rate of growth whereas short days affected the phenology of growth.

By mid-January (Lift 2), short days, moisture stress, or both of them produced seedlings with significantly smaller shoot dry weights that those grown under no moisture stress and long days (fig. 2a). In relative terms, moisture stress usually resulted in a greater reduction of shoot dry weight and stem diameter than exposure to short day lengths (fig. 2a and 2c). Exposure to short days did not result in a significant reallocation of dry matter to

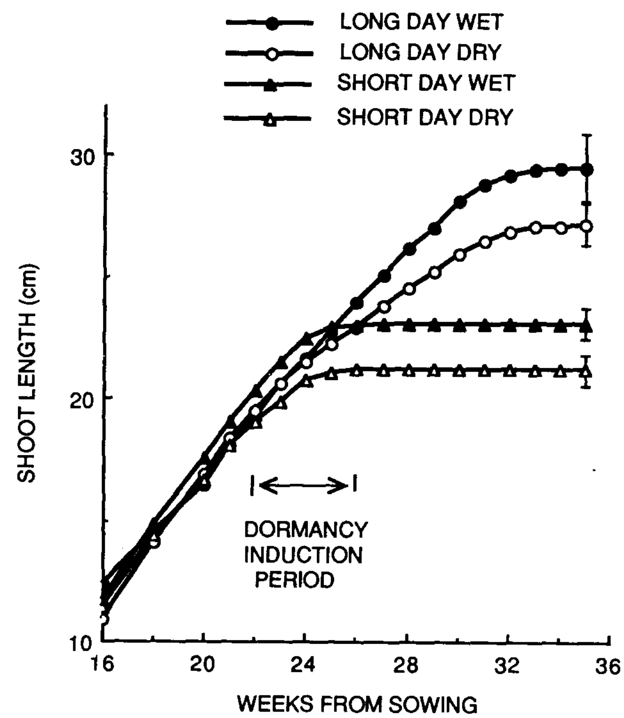

Figure 1.-Shoot length of western hemlock seedlings grown in the larger S4 container (PSB 415B) subjected to dormancy induction treatments, applied for 4 weeks beginning in mid-July (shown by the horizontal arrow). Vertical lines indicate t 1 SE.

the roots and moisture stress under long or short days simply reduced root dry weight (fig. 2b)

Seedling Physiology

Lifting date and length of cold storage had a highly significant (P<0.001) effect on RGC (table 2 and fig. 3). Container size and day length had a small, but significant effect, respectively; the larger S4 container and the short day treatments gave higher RGC (2.6 each) than the smaller cavity size and the longer days (RGC 2.4 each). For seedlings that were not cold-stored, RGC values increased significantly (P<0.01) between mid-November and mid-March. Seedling RGC values also significantly (P<0.01) increased with time in cold storage with the exception of the November-lifted stock that was stored for two months.

Later lifting dates and longer lengths of cold storage both significantly (P<0.001) increased DRI (fig.4). Some seedlings lifted in November (Lift 1) took more than 65 days to break bud resulting in a very low mean DRI value of 0.30. Those lifted in January (Lift 2) had lost a considerable amount of dormancy with a mean DRI value of 0.56 while those lifted in March (Lift 3) were fully released from dormancy with a DRI value of 1.00. While there were several significant treatment interactions

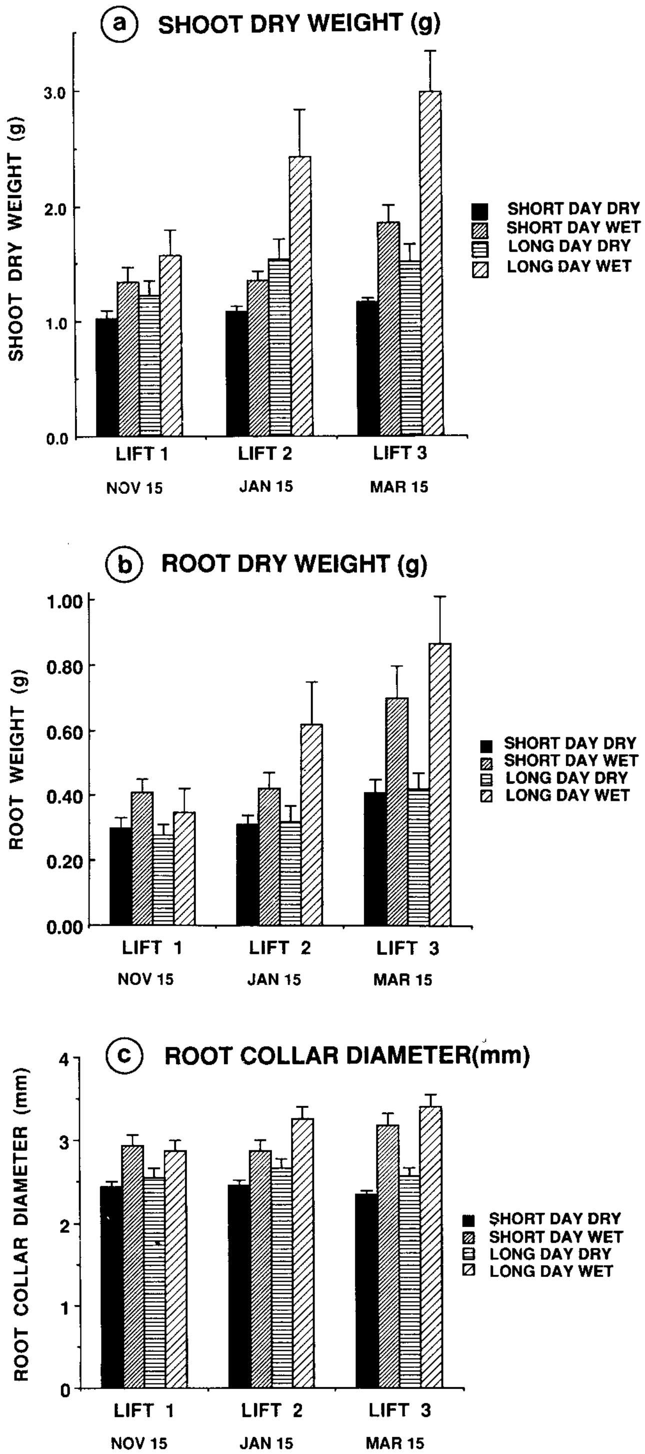

Figure 2.-Shoot dry weight (a), root dry weight (b) and root collar diameter (c) of western hemlock seedlings grown in the S4 container (PSB 415B) that had been subjected to four dormancy induction treatments (short day dry, short day wet, long day dry and long day wet) applied for 4 weeks beginning in mid-July, and three lifting dates (Nov. 15, Jan. 15, Mar. 15). Vertical lines indicate 1 SE within lifting dates.

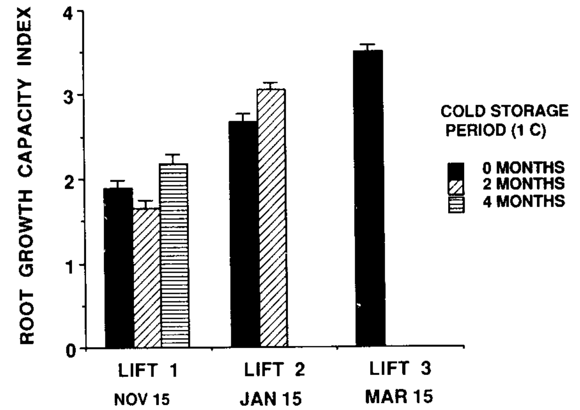

Figure 3.-Root growth capacity index of western hemlock seedlings for all treatment combinations lifted at three dates and cold stored for various lengths of time. Vertical lines represent 1 SE.

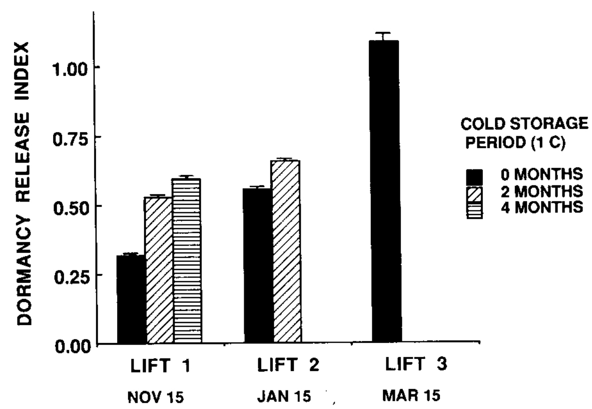

Figure 4. -Dormancy release index of western hemlock seedlings for all treatment combinations lifted at three dates and cold stored for various lengths of time. Vertical lines represent 1 SE.

(table 2) seedlings in the S3 containers had generally higher DRI values than those in the larger S4 containers and those seedlings subjected to short days had higher DRI values than those in the long day treatments. Moisture stress effects were also significant ($P<0.05$), an average of one day more being needed to break bud than in plants grown without moisture stress.

DISCUSSION

All treatment combinations in the nursery - container size, day length and degree of moisture stress - had a significant effect on seedling morphology. The larger container provided the seedlings with 80 % greater rooting volume and growing space. As a result, seedlings grown in the S4 container were taller (26 vs 24 cm), had greater

Table 2.—Anaylsis of variance summary of treatment effects on root growth capacity (RGC) and dormancy release index (DRI).

Treatment		RGC	DRI
Lift/Storage	(L)	***[1]	***
Container size	(S)	**	***
Daylength	(D)	*	***
Moisture	(M)	NS	*
C x L		NS	**
D x L		***	***
M x L		NS	NS
C x D		NS	NS
C x M		NS	NS
D x M		NS	***
C x D x M x L		NS	NS

[1]P<0.001 (***); <0.01 (**); <0.05 (*)

shoot dry weight (1.3 vs 0.8 g at Lift 1) and a larger root collar diameter (2.7 vs 2.2 mm at Lift 1) than those seedlings grown in the S3 container. As the effect of container size was so consistent throughout the study, it will not be discussed further.

Short days, applied in mid-July, rapidly arrested shoot elongation in western hemlock seedlings but moisture stress did not and, when used in combination with short days significantly reduced the number of needle primordia formed in the bud (O'Reilly et al. 1989a). Moisture stress also reduced shoot dry weight and stem diameter, most likely the result of reduced rates of photosynthesis caused by stomatal closure (Osonubi and Davies 1980). Moisture stress has been shown to produce terminal buds (Cheung 1973) and significantly reduce shoot growth of western hemlock seedlings (Cheung 1973; Nelson and Lavender 1976); unfortunately, the degree of moisture stress was not documented in these studies. In the present study, moisture stress (predawn average of -1.0 MPa) did not trigger bud development. In previous studies of other conifers (Lavender et al. 1968; Macey and Arnott 1986; Young and Hannover 1978), more severe stress levels have caused the formation of a terminal bud and arrested shoot elongation. Bud induction may require higher levels of moisture stress than those used in our study; however, this could result in mortality of western hemlock seedlings as they are sensitive to water stress. Some seedlings in our experiment died when predawn shoot water potentials decreased to -1.5 MPa.

Short days arrested shoot growth but did not result in a significant reallocation of dry matter to the roots as observed in pine seedlings (Ledig et al. 1970). Results similar to ours with western hemlock have been reported by Burdett and Yamamoto (1986) for Pinus contorts Dougl. and by Heide (1974) for Pices abies (L.) Karst.

Seedling quality assessment should be based on measurement of several physiological parameters (Ritchie 1984). In our experiment, we used RGC (Burdett 1979) and DRI (Ritchie 1984) to measure the impact of lifting date and cold storage duration on the seedling quality of western hemlock. The intensity of seedling dormancy weakened over the winter with DRI values rising consistently from mid-November (DRI= 0.3) to mid-March (DRI= 1.0). Western hemlock seedlings were released from dormancy at a slower rate in cold storage than those that were held in the nursery throughout the winter. Similar results were found for Douglas-fir and Ritchie et al. (1985) speculated that this was because (a) the temperature in cold storage (1°C) is below the optimum for dormancy release (4°C), (b) intermittent warm periods during the winter accelerates dormancy release and (c) absence of daily photoperiod may retard dormancy release in storage.

Subjecting seedlings to short day lengths in July to arrest height growth in the nursery will tend to result in an earlier release from dormancy in the next growing season; short day seedlings lifted in March flushed 2-3 days sooner than those grown under long days. This effect was enhanced by duration of cold storage. November-lifted seedlings under short day treatments flushed from 10 to 15 days sooner than those grown under long days. Using moisture stress as a means of controlling shoot growth in the nursery had a weakly significant (P<0.05) effect on the number of days it took the seedlings to break bud. Trees lifted in March that that been subjected to moisture stress flushed on average one day sooner than those grown under no moisture stress but considering the risk of mortality in western hemlock, the treatment is not recommended. In addition, seedlings planted in the spring that are predisposed to flush sooner stand a greater chance of being damaged by late-spring frosts.

RGC values were low for seedlings lifted in mid November and gradually increased throughout the winter with later lifting dates. Similar observations have been made by D. Simpson (personal communication) for Picea glauca (Moench) Voss and by Mattson (1986) for Pinus gilvestris L. In general, RGC values also rose while in cold storage, something also noted by Burdett and Simpson (1984) for Pinus contorts L. Therefore, a positive relationship exists between dormancy intensity and RGC in western hemlock seedlings. This is supported by the observation of consistently lower RGC and DRI values for seedlings that were cold stored versus those that remained in the greenhouse and were not stored. It is possible that the mechanisms controlling a seedling's RGC, DRI and chilling requirement are linked as suggested by Ritchie et al. (1985). Moisture stress during the growing season, had no significant effect on seedling RGC; short days resulted in a small, but significant increase.

CONCLUSION

The results of this study indicate that short days are the most effective method of controlling shoot growth of western hemlock seedlings in the nursery. Moisture stress did not do this effectively and significantly reduced the final values for all morphological variables measured. In terms of seedling physiological quality as indicated by RGC and DRI values, the results indicate a preference for spring lifting of this species either immediately before planting in March or after two months of cold storage from a mid-January lifting date.

ACKNOWLEDGMENTS

We wish to thank Mr. G. Goodmanson, and Mr. M. Hooper for technical assistance, Mr. J. Wiens for the graphics and Mr. D. Beddows for statistical services.

LITERATURE CITED

Bunting, W. R. 1980. Seedling quality: growth and development - soil relationships, seedling growth and development, density control relationships. p. 21-42 In_ Proceedings North American forest tree nursery soils workshop (L.P. Abrahamson and D. H. Bickelhaupt, eds.). State Univ. New York Coll. Environ. Sci. and Forestry, Syracuse, N.Y.

Burdett, A. N. 1979. New methods for measuring root growth capacity: their value in assessing lodgepole pine stock quality. Can. J. For. Res. 9:63-67.

Burdett, A. N. and D. G. Simpson. 1984. Lifting, grading, packaging, and storing. p.227-234 In Forest Nursery Manual: production of bareroot seedlings (M. L. Duryea and T. D. Landis, eds.) Martinus Nijhoff/Dr. W. Junk Publishers, The Hague/ Boston/Lancaster.

Burdett, A. N. and S. Yamamoto. 1986. Growth rate and shoot:root allometry in Psuedotsuea menziesii (Mirb.) Franco and P nua contorta Dougl. seedlings raised under two photoperiodic regimes. Scand. J. For. Res. 1:397-402.

Cheung, Kin-Wah 1973. Induction of dormancy in container-grown western hemlock (Tsuea heterophvlla (Raf. Sarg.). British Columbia Forest Service Res. Notes. No. 59. 5 p.

Doorenbos, J. 1953. Review of literature on dormancy in buds of woody plants. Landbouwhoogesch. Meded. 53:1-23.

Downs, R. J. and J. M. Bevington. 1981. Effect of temperature and photoperiod on growth and dormancy of Betula papyrifera. Am. J. Bot. 68:795800.

Duryea, M. L. 1985. Evaluating seedling quality: principles, procedures, and predictive abilities of major tests. For. Res. Lab., Oregon State Univ., Corvallis, Oregon. 143 p.

Heide, O. M. 1974. Growth and dormancy in Norway spruce ecotypes (picea abies) I. Interaction of photoperiod and temperature. Physiol. Plant. 30:1-12.

Lavender, D. P., K. K. Ching and R. K. Herman. 1968. Effect of environment on the development of dormancy and gro wth of Douglas-fir seedlings. Bot. **Gaz.** 129:70-83.

Ledig, F. T., F. H. Bormann, and K. F. Wenger. 1970. The distribution of dry matter growth between shoots and roots in loblolly pine. Bot. Gaz. 131:349-359.

Macey, D. M. and J. T. Arnott. 1986. The effect of moderate moisture and nutrient stress on bud formation and growth of container-grown white spruce seedlings. Can. J. For. Res. 16:949-954

Mattson, A. 1986. Seasonal variation in root growth capacity during cultivation of container-grown Pinus Bvlvestris seedlings. Scand. J. For. Res. 1:473-482.

Nelson, E. A. and D. P. Lavender. 1976. Dormancy of western hemlock seedlings. p. 103-107 In Proceedings of the Western Hemlock Management Conference (W. A. Atkinson and R. J. Zasoski, eds.). Coll. Forest Resources, Univ. Wash., Inst. For. Prod. Contrib. No. 34.

O'Reilly, C., J. N. Owens and J. T. Arnott. 1989a. Bud development in difference seedling types of western hemlock during nursery growth. (Submitted to Forestry).

O'Reilly, C., J. T. Arnott and J. N. Owens. 1989b. Effect of photoperiod and moisture availability on shoot growth, seedling morphology, and cuticle and epicuticular wax features of container-grown western hemlock seedlings. (Submitted to Can. J. For. Res.)

O'Reilly, C., J. N. Owens, J. T. Arnott and B. G. Dunsworth. 1989c. Effect of nursery treatments on shoot length components of western hemlock seedlings during the first year of field establishment. Paper presented at the combined WFNA/ FNABC meeting, Vernon, B.C. August 8-11, 1988.

Osonubi, O. and W. J. Davies. 1980. The influence of water stress on the photosynthetic performance and stomatal behaviour of tree seedlings subjected to variation in temperature and irradiance. Oecologia 45:3-10.

Ritchie, G. A. 1984. Assessing seedling quality. p. 243259. In Forest Nursery Manual: production of bareroot seedlings (M.. L. Duryea and T. D. Landis, eds.) Martinus Nijhoff/ Dr. W. Junk Publishers, The Hague/Boston/Lancaster.

Ritchie, G. A., J. R. Roden and N. Kleyn. 1985. Physiological quality of lodgepole pine and interior spruce seedlings: effects of lift date and duration of freezer storage. Can. J. For. Res. 15:636-645.

Scarratt, J. B., C. Glerum and C. A. Plexman (eds.) 1981. Proceedings of the Canadian Containerized Tree Seedling Symposium. [Toronto, Ontario, September 14-16,19811. COJFRC Symp. Proc. O-P-10, Can. For. Serv., Great Lakes For. Res. Cent., Sault Ste. Marie, Ont. 460 p.

Schmidt-Vogt, H. 1981. Morphological and physiological characteristics of planting stock: present state of research and research tasks for the future. p. 433446 In Proceedings IUFRO XVII World Congress, Kyoto, Japan.

Steele, R. G. D. and J. H. Torrie. 1980. Principles and procedures of statistics. 2nd Ed. McGraw-Hill, New York.

Sutton, R. F. 1979. Planting stock quality and grading. Forest Ecology and Management 2:123-132.

Tinus,R. W. and S. E. McDonald. 1979. How to grow tree seedlings in containers in greenhouses. USDA Forest Service, General Tech. Report RM-60, Rocky Mountain Forest and Range Experiment Station, Ft. Collins, Colorado. p. 256

Young, E. and J. W. Hanover. 1978. Effects of temperature, nutrient and moisture stress on dormancy of blue spruce seedlings under continuous light. For. Sci. 24:458-467.