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Storage on Ponderosa Pine Seedling Physiology and Quality

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Robert W. Rose

Two-year-old ponderosa pine (Pinus ponderosa Dougl.
ex Laws.) seedlings from two Oregon seed sources were
lifted three times in the fall, stored below freezing
(-1.5°C), and compared to seedlings that were handled
conventionally (spring lifting followed by short-term cold
storage, 2-4°C).

Based on patterns of budbreak in a greenhouse and
after outplanting, I concluded that freezer storage could
not totally substitute for natural chilling for September
and October-lifted seedlings. On the other hand, November
lift and stored seedlings had patterns of budbreak which
were similar to seedlings that had naturally overwintered
in the nursery beds.

During storage, cold-hardiness, dry matter, and
starch (both concentration, %, and content, mg) declined.
The majority of change occurred in the first 3 months of

storage.

Seedlings lifted in September and stored until planting had poor root initiation (<15% with new roots after 30 days), and had less than 25% field survival in the first and second years after outplanting. November and spring-lifted seedlings had greater than 62% of seedlings with new roots and greater than 75% field survival. October-lifted seedlings were generally intermediate in response between early and late fall-lifted treatments.

Initial root starch concentration or content at the time of planting was generally poorly related to subsequent root initiation, survival, and growth. Root starch concentration was greater in seedlings with new roots versus those without new roots, in both the greenhouse and field experiments. Seedling performance was apparently more closely associated with root initiation, and not starch. New roots improved the water status of seedlings and allowed greater conductance to water vapor.

Effects of Fall Lifting and Long-term Freezer Storage on
Ponderosa Pine Seedling Physiology and Quality

by

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CONTRIBUTION OF AUTHORS

Steven K. Omi is the senior author on all of the following manuscripts. The senior author designed the experiments, supervised the physical labor, completed data analyses, and wrote the first drafts of all manuscripts. Robin Rose is a co-author on all of the manuscripts. As major professor, he suggested modification of experiments and provided input on direction of the research. He also provided technical assistance and labor during several experiments, and he edited the manuscripts. Barbara Yoder is a co-author for Chapter IV. She helped with the porometer measurements, assisted with the experimental design, and had useful suggestions for the manuscript.

Effects of Fall Lifting and Long-term Freezer Storage on Ponderosa Pine Seedling Physiology and Quality

INTRODUCTION

Fall lifting and long-term freezer storage of coniferous nursery stock have become widespread in the Pacific Northwest (Hee 1986, Daniels and Simpson 1990). This is probably related to the high quality of seedlings (i.e., those that reach acceptable levels of survival and growth after planting, Duryea 1985) achieved through a knowledge of how seedling physiology can be altered culturally to meet reforestation objectives. Indeed, before 1970, fall lifting and long-term storage was discouraged (Hermann et al. 1972).

Since then, several studies showed that field performance of fall-lifted stock following long-term storage, can be as good or better than performance of spring-lifted seedlings (Mullin and Bunting 1972, Morby and Ryker 1979, Hinesley 1982, Ritchie et al. 1985, Tung et al. 1986); however, few studies have addressed the effects of fall lifting and long-term storage on ponderosa pine (Pinus ponderosa Dougl. ex Laws.) seedling quality (Morby and Ryker 1979) or physiology.

Despite its numerous operational and management advantages (Hocking and Nyland 1971), this practice can adversely affect plant growth and development. If seedlings are lifted before the buds will respond to

chilling, storage can be unsuccessful (Stone and Schubert 1959, Ritchie and Dunlap 1980). Because seedlings are no longer exposed to fluctuating temperature, daily photoperiod, and other environmental cues occurring overwinter, quality may decline with storage (Lavender and Wareing 1972, Ritchie 1986a).

Freezer storage, especially, may not provide the optimum temperature for fulfilling the chilling requirement (Ritchie 1984a, Ritchie et al. 1985, Deans et al. 1990). Thus, Chapter I describes a series of greenhouse and field experiments designed to investigate how lift date, seed source, and storage affect chilling requirements in ponderosa pine.

In addition, long-term storage has the potential to deplete carbohydrate reserves, which may ultimately decrease root and shoot growth and field survival (Duryea and McClain 1984, Marshall 1985). To our knowledge, there are no quantitative estimates of carbohydrate reserve dynamics for ponderosa pine. Therefore, Chapter II and Chapter III are investigations into quantifying starch using an enzymatic digestion method and using this method to evaluate changes in starch with storage, respectively.

Lifting date and storage have a strong influence on root growth potential (RGP), i.e., the seedling's ability to initiate or elongate new roots (Ritchie and Dunlap 1980). In Chapter IV, we test the null hypothesis that RGP would be similar for seedlings from a high and a low

elevation seed source. We also hypothesize that differences in root initiation should be reflected by changes in post-storage leaf water potential and stomatal conductance to water vapor.

In Chapter 5, growth potential at the time of spring planting was measured by monitoring changes in root initiation, fresh weight, and root starch concentration and content in a greenhouse. Field performance after fall lifting and freezer storage was compared to spring lifting for 2 years after planting. We examine the question of whether new root growth after planting is related to initial starch concentration. Chapter 5 is also a synthesis of the previous chapters, in an attempt to get an understanding of the mechanisms responsible for the treatment effects observed in the field.

CHAPTER I

EFFECTIVENESS OF FREEZER STORAGE IN FULFILLING THE
CHILLING REQUIREMENT OF PONDEROSA PINE SEEDLINGS

ABSTRACT

The degree to which freezer storage fulfills the chilling requirement of two sources of ponderosa pine (Pinus ponderosa Dougl. ex Laws.) seedlings was determined by monitoring development after potting or planting of seedlings which were lifted three times in the fall and subjected to storage. The fulfillment of chilling was assessed by measuring days to budbreak, cumulative percent of seedlings flushing, foliated shoot length, and bud abortion rates. The effect of freezer storage depended on stage of development, length of storage, and seed source. Storage could not totally replace winter conditions, especially for September and October lifting. The higher elevation seed source flushed sooner than the low in response to storage chilling. Delayed budbreak after planting for early lifting did not occur in the second year for the high seed source but continued into the second year for the low elevation seed source. November lifted and stored seedlings had patterns of budbreak which were similar to seedlings which had overwintered in the beds.

INTRODUCTION

Chilling requirement refers to the temperature and duration of exposure which dormant perennial plants from temperate regions must experience before spring growth (Lavender 1981). The temperature most effective in providing the chilling effect is about 3-5°C (Perry 1971) and the duration ranges from just 2 weeks to over 140 days for a variety of conifer species (Table I.1). A large number of factors (including age, budscale characteristics, chilling temperature, prechilling photoperiod, photoperiod during chilling, postchilling temperature regime, cultivar, sublethal stress; Hinesley 1982a, Fuchigami and Nee 1987) can affect the chilling requirement.

Fall lifting and long term storage present numerous operational and management advantages (Hocking and Nyland 1971). This practice, however, can adversely affect plant growth and development. If seedlings are lifted before the buds will respond to chilling, storage can be unsuccessful (Stone and Schubert 1959, Ritchie and Dunlap 1980). Several factors in storage affect the progression of seedling development. For example, seedlings are not exposed to fluctuating temperature or daily photoperiod, and the storage temperature may not be optimum for releasing seedlings from dormancy (Ritchie 1986a).

If seedlings are lifted in the fall at their proper

physiological condition, cold storage can fulfill, to a certain extent, the necessary chilling had they overwintered in the nursery beds. The results depend on genetic differences (Campbell 1978, Carlson 1985), duration of storage (Hinesley 1982a, Ritchie 1984a, Ritchie et al. 1985, Ritchie 1986b), stage of development (Nienstaedt 1966, Campbell 1978, Cannell et al. 1990) and temperature. In general, cold storage at above freezing temperatures (1-3°C) adequately satisfies chilling requirements (van den Driessche 1977, Carlson 1985); whereas, below freezing temperatures (-1°C) appear to be less efficient (Perry 1971, Ritchie 1984a, Ritchie et al. 1985, Ritchie 1986b, Fuchigami and Nee 1987).

Satisfaction of the chilling requirement is usually tested by bringing plants (which have already entered the rest phase of dormancy) into a favorable environment and measuring rate or percentage of seedling budbreak. Plants which flush quickly in response to winter chilling, are referred to as having been released from rest.

Abnormal bud flush and elongation can result from inadequate chilling (Hinesley 1982b). Ponderosa pine (<u>Pinus</u> <u>ponderosa</u> Dougl. ex Laws.) seedlings also display abnormal buds and stunted growth with inadequate chilling (Omi, unpublished data). Therefore, although chilling requirements for budbreak can be met with storage, the amount of elongation or foliar display following budbreak may be negatively affected. This aspect of chilling has

not been well addressed, and it has significant implications for forest regeneration.

The objective of this paper was to determine the effect of seed source, lift date, and storage on budbreak and flushing morphology. We hypothesized that freezer storage would satisfy the necessary requirements for budbreak, depending on development at lifting. We also hypothesized that freezer storage would reduce the frequency of abnormal buds and increase the foliated shoot length, depending on physiological condition at lifting. Finally, we wanted to determine if freezer storage increases the rate of budbreak after outplanting and how a natural winter affects budbreak in the second year in the field.

MATERIALS AND METHODS

Two-year-old bareroot ponderosa pine seedlings were selected from the Bend Pine Nursery, Oregon (44° 5' N, 121° 16' W; elevation, 100 m). Frozen soils prevent lifting in winter. Seedlings from two Oregon seed sources (Barlow Ranger District, Mt Hood National Forest (1100 m) and Lakeview Ranger District, Fremont National Forest (1800 m elevation)) were grown in different beds at the nursery under standard cultural practices until lifting.

Lifting and storage

Seedlings were shovel-lifted on September 22 (SEP), October 20 (OCT), November 17 (NOV), 1987, and March 1, 1988 (MAR), after 66, 315, 640, and 2,545 chilling hours (Figure I.1). Chilling hours were defined as the number of hours less than or equal to 5°C (Ritchie 1984a) since September 10. September 10 was an arbitrary starting date, coinciding with accumulation of chilling the previous year (Omi and Schuch 1987). Temperatures were recorded from a shielded thermistor located 20 cm above the soil surface. Chilling hours in 1987-1988 were similar to those in 1986-1987 (Figure I.1).

The design for lifting was a randomized complete block with 4 lifting dates assigned within each of 4 replications for the 2 seed sources (Fremont, Hood). After lifting, seedlings were graded according to nursery standards; root systems rinsed in water and pruned to

25 cm; and then seedlings were stored in polyethylene bags placed within wax-lined cardboard boxes. For fall-lifted seedlings, the inside bag temperature generally ranged from -0.1 to -3.4°C, with a mean of -1.5°C. A freezer malfunction on October 10-11 affected only the SEP seedlings. The malfunction caused the air temperature to drop at a rate of 1.1°C per hour, reaching a minimum of -7.2°C (minimum inside bag temperature -2.7°C), and then rising to -1.6°C at a rate of 1.4°C per hour. Fourteen hours later, room temperature dropped 0.7°C per hour to a minimum of -11°C (minimum inside bag temperature -6.9°C), and increased 1.2°C per hour to -1.6°C. MAR seedlings were stored at above freezing temperatures (2-4°C inside box) during the entire study.

Seedlings from each fall lift date were stored for either 1 day (0 months, cold storage), 90 days (3 months, freezer), 150 days (5 months, freezer), and until outplanting at Bend (freezer). Six days before end of the storage interval, the boxes were placed in a cooler (2 to 4°C) for thawing.

Greenhouse study

One day after lifting, and after storage, 40 seedlings per seed source x lift date x storage combination were potted (10 seedlings per 10.75-L pot, 4 replications) in a 1:1:1:2 soil:sand:peat:pumice (v:v) mixture and placed in a greenhouse with a 16 h extended

photoperiod supplemented with sodium vapor lamps (100 μmol m^{-2} s^{-1} at plant height). Seedlings were kept well-watered and a dilute maintenance application of fertilizer (1.32 g L^{-1} of 20-20-20) applied once every 4 weeks. Soil temperature generally ranged from 16-20°C, and air temperature from 16-23°C. Pots were moved weekly to remove any bias due to location on the greenhouse benches.

Days to budbreak was determined by scoring each seedling for terminal budbreak (separation of bud scales to reveal emerging needles, Burr et al. 1989) twice a week for 20 weeks. To evaluate the irregular growth and morphology of inadequately chilled seedlings, the percent of seedlings with foliated shoot length (Lanner and Connor 1988) less than 10 mm was determined at the end of 20 weeks (For some of the treatments, actual foliated shoot length (mm) was measured); and the percent of seedlings with abnormal buds (swelling of buds and terminal elongation without emergence of needles, referred to as aborted) was determined.

Cold hardiness

To get an estimate of relative hardiness at lifting and after 3 months of freezer storage for fall lifts, a random sample of 28-36 (mean=32) seedlings from all replications were selected for each assessment. Seedlings were tested for hardiness 2-3 days from lifting, and 5-6 days before the end of the 3 month storage period (fall

lifts only). Hood seedlings were measured for all 3 fall lift dates and MAR, whereas Fremont seedlings were assessed on OCT, NOV, and MAR. These tests were conducted with the assistance of the International Paper Company in Lebanon, Oregon.

The group of seedlings for each seed source was evenly divided among three 10.75-L pots so that 9-13 (mean=11) seedlings were in each pot. The medium used was 1:1 peat:vermiculite (v:v). These pots of seedlings were then subjected to controlled freezing tests. For each lifting date, three test temperatures were used at which 20%, 50%, and 80% injury was expected (based on previous experience). The temperatures used at each lifting date were used again for the 3 month storage test.

Each temperature run consisted of placing 1 pot of each seed source in the freezer, allowing the temperature to decrease at 5°C per hour until the desired low temperature (2 hours), and then thawing at 20°C per hour to 0°C. After controlled freezing tests, the pots were kept well-watered in a growth room (120-150 μmol m^{-2} s^{-1} light intensity at plant height from cool-white fluorescent lights, 13-h photoperiod, 18 °C night and 22 °C day temperature). Whole plant hardiness was assessed after 20 weeks (18 weeks for the MAR trees, due to a growth room malfunction). A seedling was considered dead if all needles had turned brown or if significant needle damage (visually estimated) coincided with dark

discoloration around the cambium at the stem base. The percent alive in each pot was determined.

Field study

Seedlings which had been stored since lifting were outplanted at the nursery on March 16 and 17, 1988 (Chapter V). Ammonium sulfate (21-0-0) and triple superphosphate (0-45-0) were applied on March 10-11, 1988, at a rate of 112 and 140 kg ha^{-1}, respectively.

A total of 640 seedlings (2 seed sources x 4 lifting dates x 4 replications x 20 seedlings) were shovel-planted at 0.76 x 0.76 m spacing in a randomized complete block design. Seedlings were watered immediately after planting and the site was handweeded continuously for the next two growing seasons. Thereafter, seedlings were watered as often as the adjacent transplant stock. Irrigation was determined by water potential measurements on the 1+1 transplants. Seedlings were measured for budbreak 9 and 7 times throughout the first and second growing seasons, respectively. Foliated shoot length was measured at the end of the growing seasons.

The Hood seedlings were also outplanted on April 19, 1988, on a clearcut site on the Mt Hood National Forest (Chapter V). Seedlings were shovel-planted at 1.2 x 1.2 m spacing in a randomized complete block design with 4 treatments (1 seed source x 4 lift dates) and 4 blocks. Fifteen seedlings were planted in a row for each treatment

x block combination, for a total of 240 trees. The site was handweeded thoroughly on May 31, 1989, before spring budbreak in the second year after outplanting. Budbreak and foliated shoot length were recorded once at the end of the two growing seasons.

Statistical analysis

Greenhouse study

The cumulative percent of buds flushing was analyzed by date of measurement in two ways. First, the lift date-store combinations were each defined as a treatment and the data analyzed as a split-plot design (4 replications) with seed source as the main plot and lift date-store treatment as the subplot (Appendix AI.1). To determine if interactions existed among seed source, lift date, and storage, a 2 (seed source) x 3 (lift dates) x 3 (0,3,5 months of storage) factorial analysis of variance was performed with seed source as the main plot, lift date as the subplot, and storage as the sub-subplot (Appendix AI.2). A least-squares means procedure was used to separate the means (Searle et al. 1980).

The analyses of variance for days to budbreak and percent of seedlings with foliated shoot length less than 10 mm were similar to percent budbreak, with two exceptions. A log transformation was applied to days to budbreak to help make the error normal and homogeneous (means presented are retransformed from the log). In

addition, the information for SEP-0 months storage was incomplete because only 5% of the seedlings flushed. Therefore, to make the analysis balanced, the data were analyzed excluding this treatment.

Foliated shoot length was analyzed for the 7 treatment combinations measured (Appendix AI.1). The percent of seedlings with aborted buds after 20 weeks was analyzed with all treatments included (Appendix AI.1).

Field study

The percent of seedlings flushing for any given measurement date, the percent of seedlings with foliated shoot length less than 10 mm (log transformed), and foliated shoot length at the end of the growing season were analyzed both years at Bend as a 2 (seed source) x 4 (lift date, stored until outplanting) factorial in a randomized complete block design with 4 replications (Appendix AI.3).

At the Mt Hood site, foliated shoot length and percent of seedlings with foliated shoot length less than 10 mm were analyzed as a randomized complete block design with 4 replications and 4 treatments (lift dates stored until outplanting, Appendix AI.4).

RESULTS

Greenhouse study

Days to budbreak

When the data were averaged over seed sources,
freezer storage reduced days to budbreak (Figure I.2).
Five months of storage tended to reduce days to budbreak
compared to 3 months for SEP (39 to 36 days, $p>0.05$) and
OCT (39 to 38 days, $p>0.05$); but significantly ($p<0.05$)
reduced the days to budbreak for NOV (39 to 31 days) so
that days to budbreak were less than seedlings that
overwintered in the nursery beds (MAR, 35 days).

Days to budbreak for Fremont seedlings was about 11%
earlier than Hood seedlings ($0.05<p<0.10$, Table I.2).

Cumulative percent flushing

Without storage, less than 40% of the seedlings
flushed after fall lifting (Figure I.3). Seedlings
responded to storage regardless of lift date, but SEP and
OCT seedlings did not respond as rapidly as NOV, causing a
lift date x storage interaction. Budbreak was not
complete after 135 days for SEP and OCT seedlings (Figure
I.3).

Five month storage generally did not increase the
percent of seedlings flushing relative to 3 month storage.
Virtually all of the seedlings flushed with storage for
NOV, and for MAR seedlings.

On the average, Fremont seedlings had a higher

proportion of seedlings flushing compared to Hood (Figure I.4). Additionally, during the period of budbreak (days 30-60), Fremont responded more rapidly with storage, compared to Hood, resulting in a seed source x storage interaction.

Foliated shoot length, bud abortion

The percent of seedlings with foliated shoot length less than 10 mm declined with storage (Figure I.5). Of the few seedlings that flushed without storage, more than 80% had short foliated lengths. Storage reduced the percent of seedlings with aborted buds for all lift dates, and eliminated bud abortion for NOV (Figure I.5).

There was no significant difference between seed sources for percent of seedlings with foliated shoot length less than 10 mm, foliated shoot length, or percent bud abortion (Table I.2).

<u>Cold hardiness</u>

The limitation of the testing procedure and the number of seedlings used did not allow replication and statistical analysis of the whole-plant freezing data; however, some strong trends were apparent. Hood seedlings lifted in SEP were hardy to -13°C without sustaining any visible damage (Figure I.6). Seedling hardiness was generally reduced when seedlings were freezer-stored for 3 months, and Fremont trees tended to have greater survival after freezing, compared to Hood.

<u>Field study</u>

Bend site

Seed source x lift date interactions were
nonsignificant for all measurement dates in the first year
after outplanting. When averaged over seed sources, OCT
had consistently fewer seedlings flushing than the other
lift dates, but was only significantly less in late August
(Julian date 244, Figure I.7). SEP seedlings had budbreak
patterns similar to NOV and MAR, but budbreak for SEP may
have been overestimated for SEP because only 24% of the
SEP seedlings survived in the first year (Chapter V).
Fremont seedlings had advanced budbreak relative to Hood
seedlings (Figure I.7).

In the second year after outplanting, the budbreak
response for seed source differed by lift date. There was
little difference among lift dates in budbreak percent for
Fremont seedlings for all dates; on the other hand, the
later fall lift dates (OCT and NOV) seemed to have delayed
budbreak relative to MAR for the Hood seedlings (Figure
I.8). The budbreak percent for SEP seedlings was again
skewed because of poor survival (17%).

The percent of seedlings with foliated shoot length
less than 10 mm did not differ by seed source, lift date,
or year (Table I.3). However, the short foliated lengths
were eliminated in the second year for all lift dates
except SEP, and Fremont seedlings generally had a higher

frequency of seedlings with short foliated shoot length.

Foliated shoot length was greatest for NOV seedlings and least for SEP seedlings in the first year (Table I.3), and the same general pattern was observed in the second year after outplanting. The two seed sources did not differ in foliated shoot length in year 1; but Hood seedlings had significantly longer lengths in year 2.

Mt Hood site

The percent of seedlings with short foliated shoot lengths was numerically largest for SEP seedlings, but there were no statistically significant differences among lift dates in either the first or second year after outplanting at Mt Hood (Table I.3). Foliated shoot length was greatest for NOV seedlings and least for SEP seedlings after one year; in the second year, there were few differences (Table I.3).

DISCUSSION

The effectiveness of freezer storage in fulfilling the chilling requirement of ponderosa pine seedlings depended on lifting date and duration of storage. Three months of freezer storage reduced days to budbreak similar to (but statistically different than) seedlings that overwintered outdoors. An additional two months of storage was more effective in reducing days to budbreak for later fall lifting relative to earlier lifting.

Although chilling reduced days to budbreak for all lift dates, freezer storage could not totally substitute for natural winter conditions. Earlier fall lifting (SEP and OCT) never reached 100% flushing after storage, whereas all seedlings flushed for NOV and MAR. Additionally, although days to budbreak was reduced with storage for earlier fall lifting, bud abortion frequency or short foliated shoot length could not be totally overcome with storage chilling. In contrast, seedlings that had been stored after late fall lifting and those lifted in spring responded similarly for the percent of seedlings with foliated shoot length less than 10 mm (less than 30%) and bud abortion frequency (0%).

The difference in response between early (SEP and OCT) and later (NOV) lifting was probably due to disruption of the normal dormancy sequence. Douglas-fir

seedlings require short-mild days to prepare for chilling (Lavender and Stafford 1985); thus, early fall lifting could prevent exposure of seedlings to the necessary environmental signals needed for development. Another reason could be that the freezer storage temperature was not optimum for development with earlier lifting. The rate of development for woody species changes with temperature and plant condition during the rest-developing and rest-breaking phase (Kobayashi et al. 1982, Kobayashi and Fuchigami 1983, Hanninen and Pelkonen 1988). SEP seedlings may have been damaged by the freezer breakdown, but this was considered unlikely due to the minor fluctuation in temperature. This did not rule out the possibility of significant deacclimation in storage (Figure I.6), however, so that seedlings were more susceptible to damage after storage (Faulconer 1988).

After experiencing a natural winter, budbreak occurred over a much shorter time period in the second year after outplanting (Figures I.7, I.8); however, the delayed budbreak for OCT (also observed in the greenhouse study) continued into the second year for Hood seedlings (NOV also lagged behind MAR), and disappeared for Fremont seedlings. If Fraser fir, noble fir (Abies procera), and shasta red fir (Abies magnifica) seedlings are inadequately chilled in one year, they are able to resume normal growth after winter chilling in the subsequent year (Hinesley 1982a, Tung 1987).

Fremont seedlings consistently flushed sooner than Hood seedlings in the greenhouse study and the field study. The difference was maintained even into the second year after outplanting. This effect could be related to preconditioning (resulting from bed effects) or seed source elevation (see Campbell and Sugano 1979). Plants from high elevation which are unlikely to receive mid-winter warming often flush sooner than low elevation when grown in a common environment, although the results are not universal (Lavender 1981, Ritchie 1984a). An explanation is that at low elevation, a mechanism is needed to guard against early flushing and spring frost damage (see Silen 1978, Fuchigami and Nee 1987). Campbell and Sugano (1979) concluded that selection for drought (in addition to cold) resistance is another important factor determining seed source response to chilling for Douglas-fir.

Seed sources also responded differently to storage—Fremont trees were more responsive. This agreed with Ritchie (1984a) who showed that high elevation Douglas-fir broke rest more rapidly with freezer storage compared to low elevation. However, since there were only two seed sources (with possible confounding by bed differences) used in our study, responses due to seed source alone must be considered with caution.

The concept that days to budbreak was not the only indicator of fulfillment of the chilling requirement is

depicted in Figure I.9. We incorporated the degree growth stage model (°GS) of Fuchigami and coworkers (Fuchigami et al. 1982, Fuchigami and Nee 1987) to indicate the progress of plant development. The model is useful because it describes development in terms of physiological condition of the plant, independent of calendar date.

The °GS model quantifies development for temperate zone woody species. The stages from 0°GS to 180°GS describe the events from spring budbreak to the onset of rest. Maximum rest is achieved at 270°GS, when days to budbreak is maximized for plants placed in a favorable growth environment. During the rest phase, buds are dormant because of internal biochemical factors (Lavender and Stafford 1985). End of rest occurs at 315°GS, when the chilling requirement is fulfilled and buds remain dormant because of cool temperature. During this quiescent phase, seedlings will flush if the environment is favorable (Lavender and Stafford 1985). End of quiescence occurs at 360°GS or 0°GS (spring budbreak) and the cycle occurs again.

The °GS shown in Figure I.9 were not calculated, but were our best estimates of plant development based on days to budbreak, cumulative percent flushing, bud abortion rates, and foliated shoot length. Chilling hours included natural chilling plus those accumulated in storage. Chilling hours were used as the x-axis to indicate that plant development progressed with low temperatures through

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the 180 to 360°GS (Kobayashi et al. 1982).

When Fremont seedlings were lifted in the fall, maximum rest (270°GS) had already occurred (Figure I.9). In contrast, development was not as advanced for Hood seedlings earliest in the fall (260°GS) because maximum rest had not been achieved (data not shown). Nonetheless, °GS advanced from 260 to beyond 270°GS with additional chilling hours (Kobayashi et al. 1982).

Plant development was enhanced with storage chilling, as °GS progressed beyond 300°GS. After 3 months of storage, °GS advanced either because days to budbreak was reduced, cumulative percent budbreak was more complete, bud abortion rates declined, or the percent of seedlings with foliated shoot length less than 10 mm dropped.

As other researchers have found (see INTRODUCTION), the number of chilling hours (equivalent to hours with natural chilling) achieved through freezer storage did not generally increase development to the same °GS as natural chilling (MAR). One exception, however, was 3 months of storage for Fremont-NOV seedlings, where we estimated °GS to be equivalent to natural chilling (Figure I.9).

Ritchie (1984b) and Cannell and Smith (1983) suggest that chilling sums can be adequate for a given location, species, and seed source; however, modeling chilling requirements based on simple accumulation of chilling hours may be questionable--fluctuating temperatures affect budbreak (Campbell 1978, Lavender 1981) and starting dates

for accumulation of hours are determined without consideration of plant physiological condition. Sarvas (1974) and Hanninen et al. (1985) (using Sarvas' model) attempted to model forest tree development based on differential temperatures during the chilling requirement phase.

Similar research with horticultural crops has been more extensive (Richardson et al. 1974, Norvell and Moore 1982, Ebert et al. 1986, del Real Laborde 1987, Fuchigami and Nee 1987). Ultimately, chilling models that depend on °GS or developmental rate (for example, Campbell and Sugano 1975) and not calendar date should provide the most accurate guidelines for predicting the effectiveness of fall lifting and long-term storage.

Table I.1. Estimated chilling requirements for the completion of rest in conifers, from various studies.

Species	Chilling estimate	Chilling initiated	Chilling requirement[1]	Reference
Picea glauca	weeks, 2-4˚C	July, September	4-8 weeks	Nienstaedt 1966
7 _Picea_ species	weeks, 2-4˚C	imposed[2]	4-8 weeks	Nienstaedt 1967
Pseudotsuga menziesii	hours, <4.4˚C	October1	2000 hours	van den Driessche 1975
Pseudotsuga menziesii	days at 4.4, 7.2, 10˚C	imposed	--	Campbell and Sugano 1975
Abies fraseri	days, 4˚C	imposed	2-6 weeks	Hinesley 1982
Picea sitchensis	days, <5˚C	November 1	>140 days	Cannell and Smith 1983
Pinus taeda	hours, 0-8˚C	?	_	Carlson 1985
Pseudotsuga menziesii	hours, <5˚C	--	1400 hours	Ritchie and Dunlap 1980
Pseudotsuga menziesii	hours, <5˚C	late September		Ritchie 1984
Pinus contorta, _Picea glauca_	hours, <5˚C	September 1	--	Ritchie et al. 1985
Pseudotsuga menziesii	hours, <6˚C	?	--	Ritchie 1986b
Pseudotsuga menziesii	hours, <7˚C	imposed	1960 hours	Burr et al. 1989
Picea engelmannii	hours, <7˚C	imposed	2800 hours	Burr et al. 1989
Pinus ponderosa	hours, <7˚C	imposed	0 hours	Burr et al. 1989
Pinus ponderosa	hours, <5˚C	September 10	--	Omi and Schuch 1987
Pinus strobus	hours, 0-7˚C	October	2800 hours	Johnsen et al. 1989

[1]chilling requirement=length of chilling necessary to provide "adequate" budbreak under favorable environments, determined by authors.

[2]imposed=chilling began at the start of the chilling treatment (controlled conditions).

Table I.2. Budbreak and subsequent growth morphology of
Fremont and Hood seedlings in the greenhouse study. Seed
source means (standard errors in parentheses, number of
seedlings in brackets) with different letters are
significantly different (p<0.05).

Seed source	Budbreak (%) after 45 days	Days to budbreak[1]	Seedlings (%) with foliated shoot length<10mm	Foliated shoot length (mm)	Seedlings (%) with aborted buds after 20 wk
Fremont	54.5 (2.7) a [381]	42.1 a [261]	55.6 (3.3) a [261]	14.5 (.8) a [209]	3.3 (1.1) a [369]
Hood	42.7 (2.7) b [382]	47.2 a [247]	54.2 (3.8) a [247]	15.1 (.8) a [205]	6.2 (1.1) a [365]

[1]Data retransformed from log, 0.05<p<0.10.

Table I.3. Foliated shoot length and percent of seedlings with foliated length less than 10mm for two years after outplanting at Bend (Fremont and Hood seed sources) and at Mt Hood (Hood seedlings). Seed source or lift date means (n=number of seedlings, standard errors in parentheses) with different letters are significantly different (p<0.05).

		End of year1			End of year2	
Seed source	n	Seedlings (%)[1] with foliated shoot length<10mm	Foliated shoot length (mm)	n	Seedlings (%)[1] with foliated shoot length<10mm	Foliated shoot length (mm)
Site: Bend						
Fremont	181	2.4 a	31.6 (1.9) a	155	0.2 a	79.1 (3.9) b
Hood	105	0.4 a	32.6 (2.9) a	83	0.02 a	122.8 (5.9) a
Lift date						
SEP	27	2.4 a	19.7 (5.3) c	18	0.6 a	74.6 (11.3)b
OCT	71	0.4 a	34.1 (2.9) b	60	0.0 a	106.8 (5.8) a
NOV	99	0.6 a	43.5 (2.4) a	82	0.0 a	115.6 (4.7) a
MAR	89	1.6 a	31.2 (2.6) bc	78	0.0 a	106.8 (4.9) a
Site: Mt Hood (Hood seedlings)						
Lift date						
SEP	17	52.3 (11.1) a	10.1 (3.3) c	13	53.6 (10.9) a	17.1 (3.0) ab
OCT	45	26.7 (6.8) a	19.1 (2.0) ab	43	33.8 (6.2) a	16.9 (1.7) b
NOV	50	17.8 (6.4) a	22.4 (1.9) a	40	28.6 (6.0) a	19.0 (1.6) ab
MAR	49	28.4 (6.5) a	15.9 (1.9) bc	40	19.6 (6.2) a	23.7 (1.7) a

[1]Data retransformed from log for Bend site; untransformed for Mt Hood site.
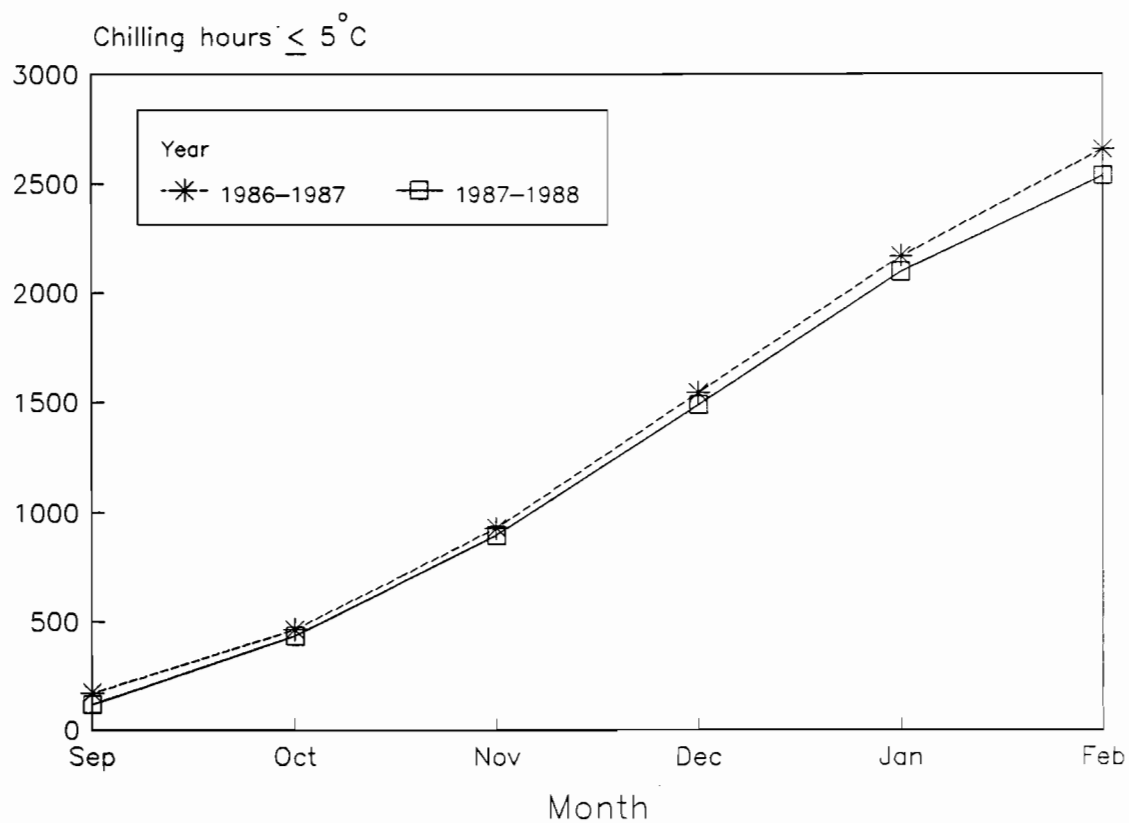
Figure I.1. Cumulative chilling hours (less than or equal to 5°C) at the Bend Pine Nursery in 1986-1987 and 1987-1988.
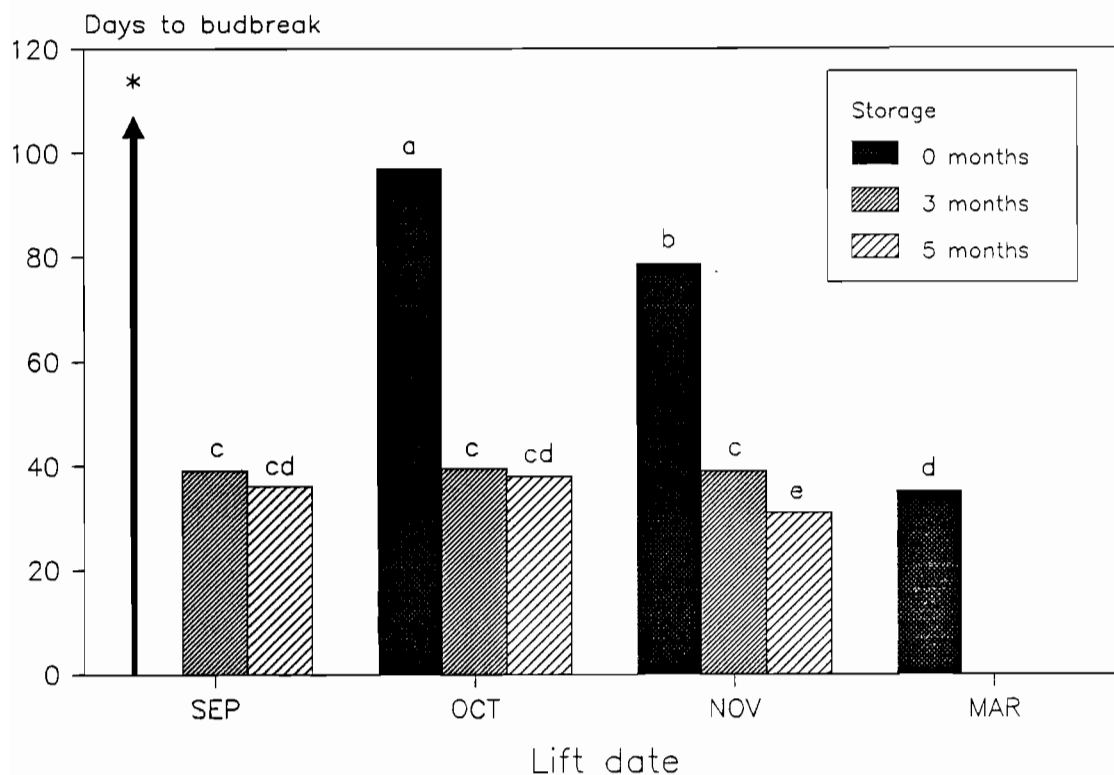
Figure I.2. Days to budbreak for Fremont and Hood
seedlings combined. Data retransformed from the log.
Means with different letters are significantly different
at p<0.05 (* Only 5 of 74 seedlings flushed during the
140-day measurement period; data excluded from analysis).

a) 30 days after potting
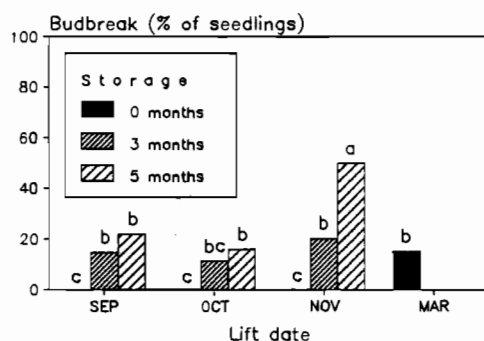
b) 45 days after potting
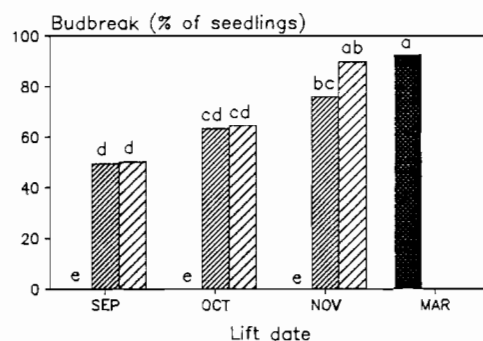
c) 90 days after potting
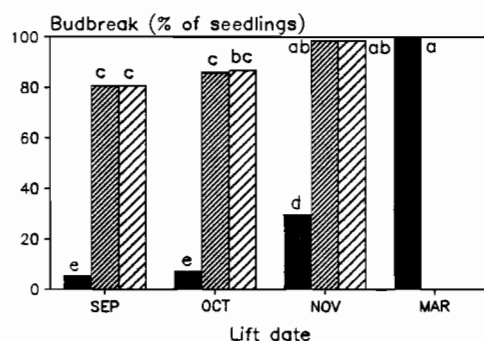
d) 135 days after potting
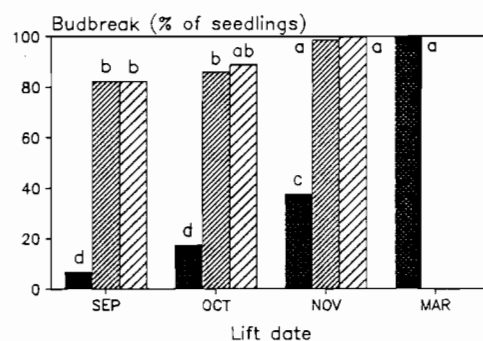
Figure I.3. Percent budbreak in the greenhouse a) 30, b) 45, c) 90, and d) 135 days after potting for seedlings (Fremont and Hood combined) after lifting and freezer storage (0, 3, 5 months). Means on a given day with different letters are significantly different at p<0.05.
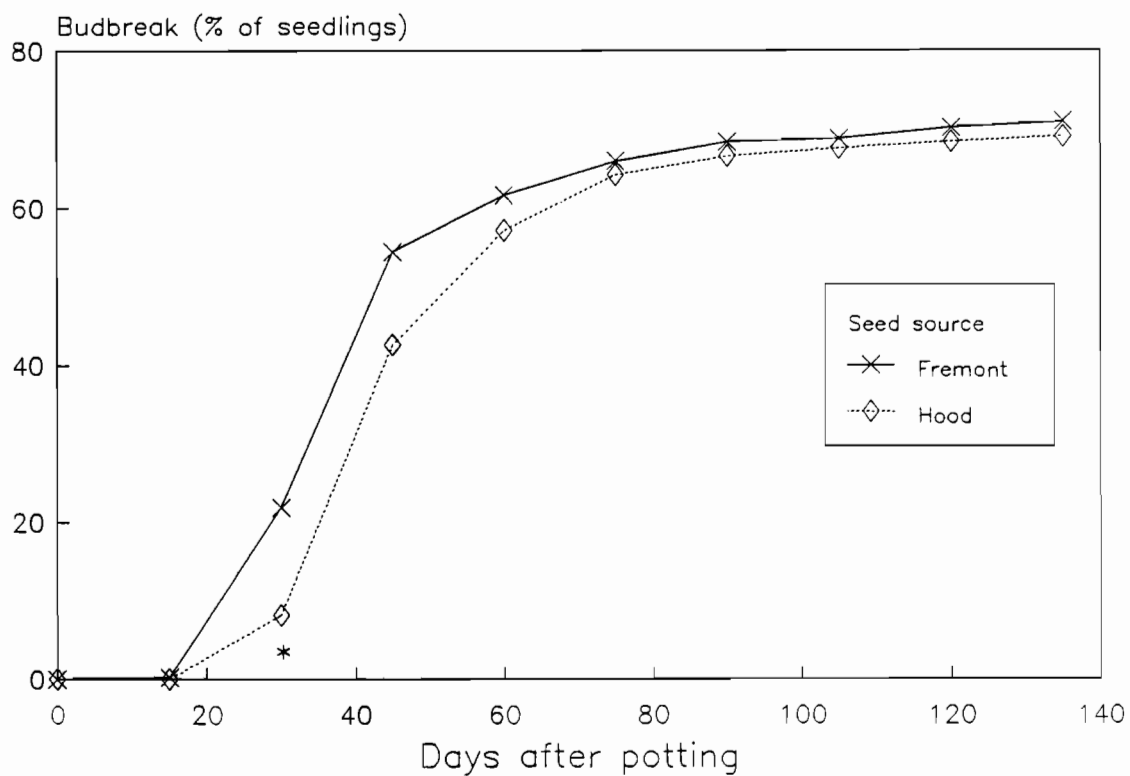
Figure I.4. Cumulative budbreak in the greenhouse for Fremont and Hood seedlings (all lift dates and storage intervals combined). Asterisk (*) represents significant difference between means (p<0.05).
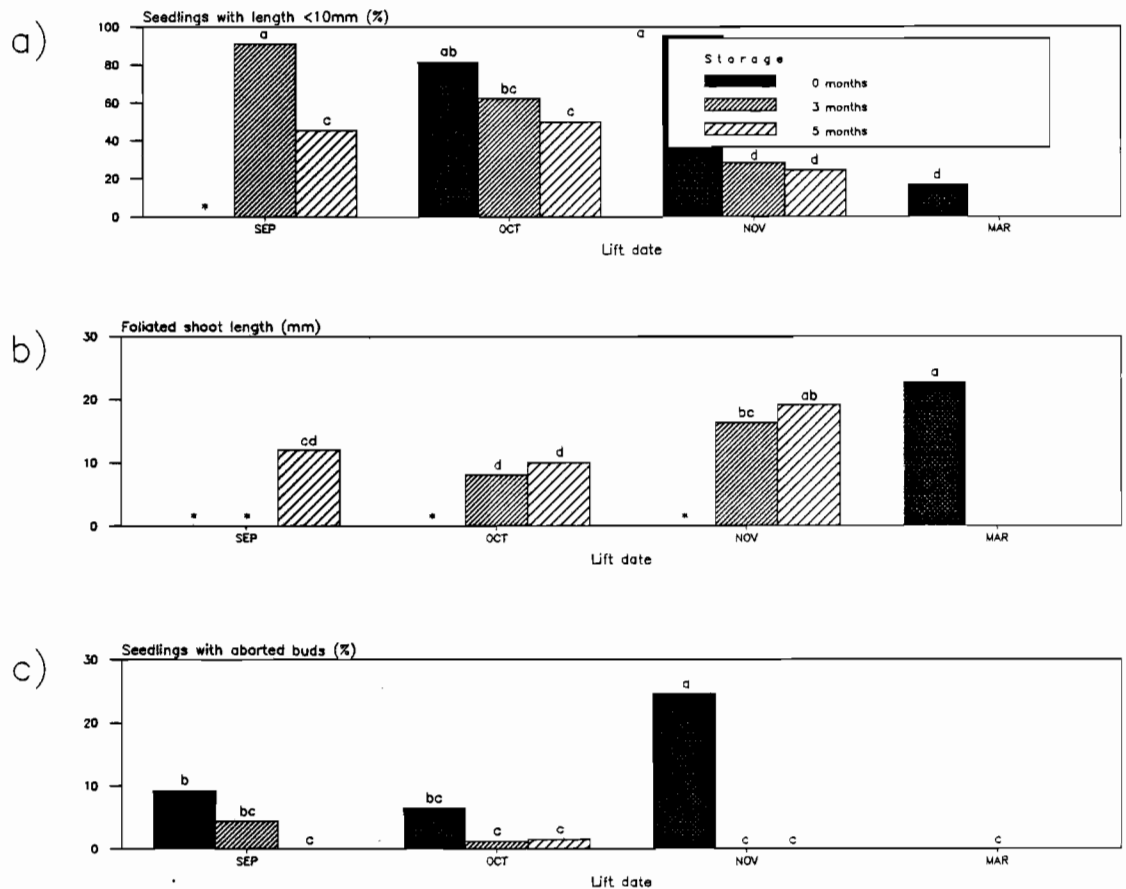
Figure I.5. Shoot morphology after 20 weeks in the greenhouse for lift date and storage treatments (Fremont and Hood seedlings combined): a) percent of seedlings with foliated shoot length<10mm, b) foliated shoot length, and c) percent of seedlings with aborted buds. Means with different letters are significantly different at p<0.05 (Asterisks (*) represent treatments not included in analysis).
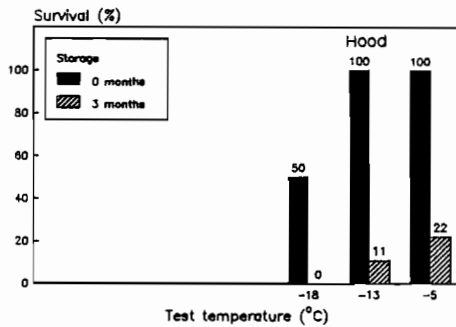
Figure I.6. Twenty week growth room survival after controlled freezing to specified test temperatures of Fremont or Hood seedlings at the time of lifting or after 3 months freezer storage for a) SEP lifting, b) OCT lifting, c) NOV lifting, and d) MAR lifting (no storage test for MAR).

a)

b)
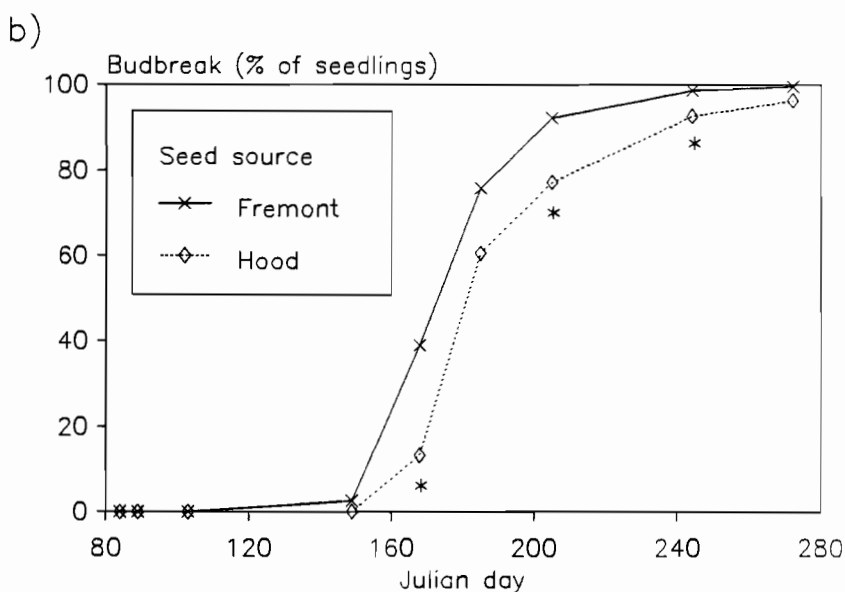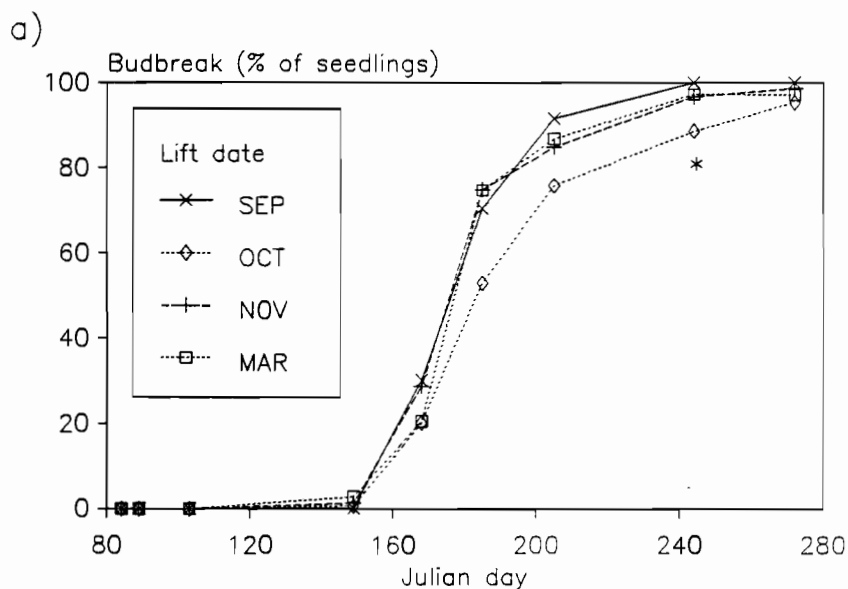
Figure I.7. Cumulative budbreak at Bend after outplanting, 1988, for: a) lift dates (seed sources combined), and b) seed sources (lift dates combined). Asterisk (*) represents significant difference among means (p<0.05).
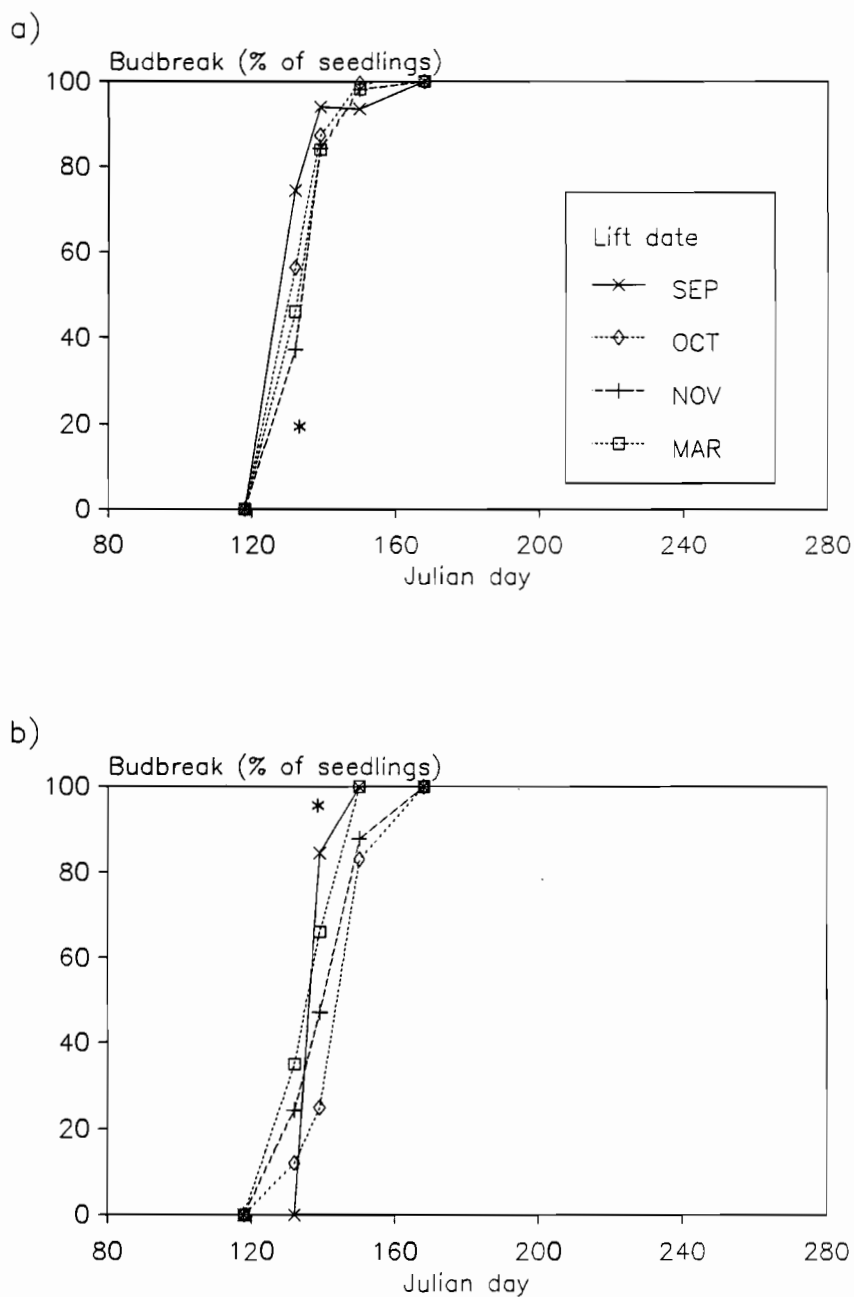
Figure I.8. Cumulative budbreak among lift dates at Bend in the second year after outplanting, 1989, for a) Fremont seedlings and b) Hood seedlings. Asterisk (*) represents significant difference among means (p<0.05).

a)   Days to budbreak

b)   DEGREE GROWTH STAGE MODEL
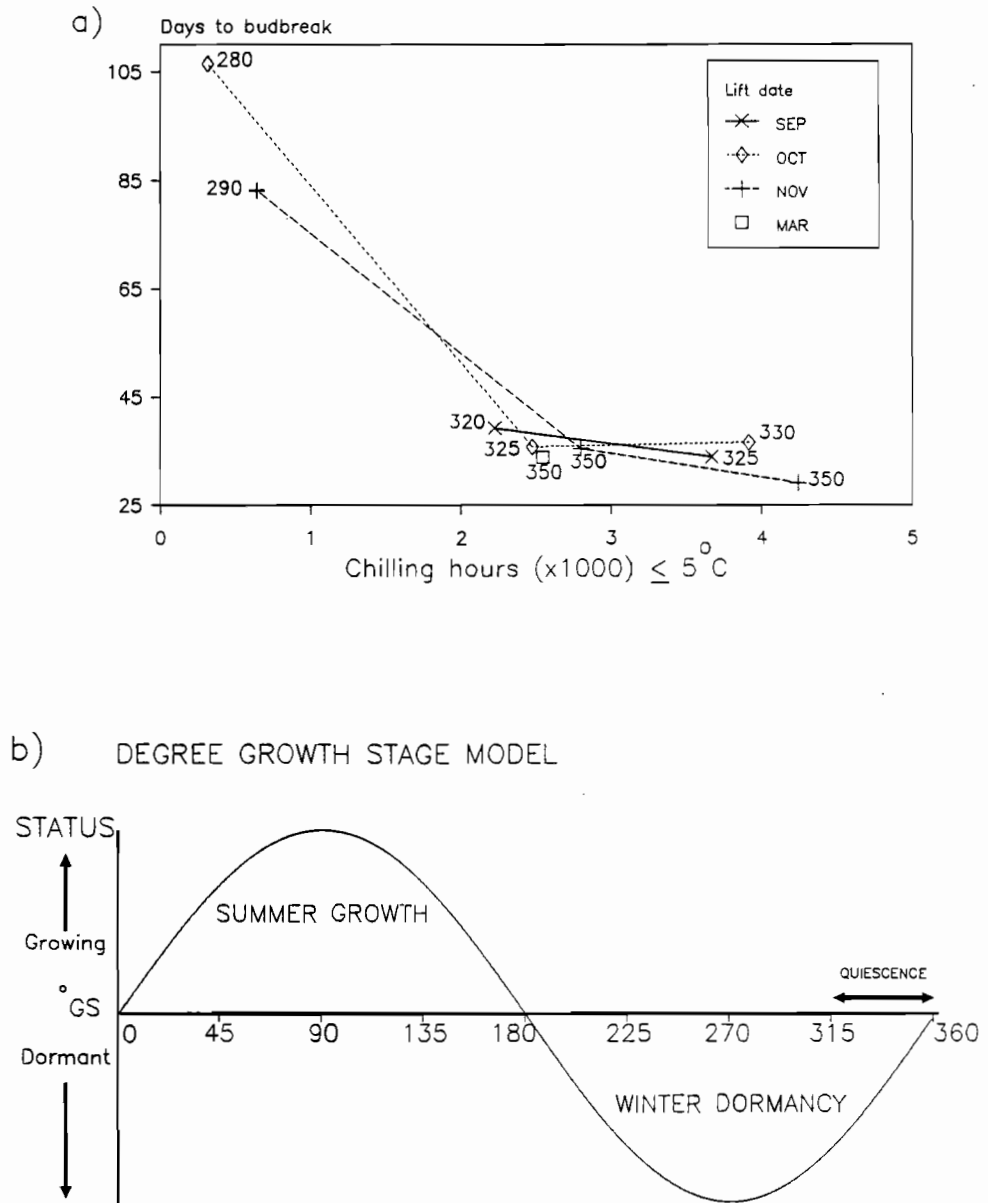
Figure I.9. a) Cumulative chilling hours, days to budbreak, and °GS (numbers besides points) for Fremont seedlings after lifting and 3 or 5 months of freezer storage, and b) °GS model for temperate woody perennials (adapted from Fuchigami and Nee 1987).

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CHAPTER II

AMYLOGLUCOSIDASE AND α-AMYLASE ENZYMES FOR DETERMINING STARCH CONCENTRATION IN PONDEROSA PINE TISSUE

ABSTRACT

Purified combinations of α-amylase and amyloglucosidase were compared with combinations obtained directly from the supplier to determine their relative precision in estimating starch concentration in ponderosa pine (<u>Pinus</u> <u>ponderosa</u> Dougl. ex Laws.) root tissue. Estimates of starch by an enzymatic digest method were nearly identical for all preparations, suggesting that an insignificant amount of contaminants was present in the unpurified enzyme combination. We concluded that the purity of purchased enzymes was sufficient for use in the enzyme digest method.

INTRODUCTION

Quantifying the starch stored in tissue is a useful basis for examining the mechanisms of growth, carbon allocation, and forest ecosystem productivity. The amount of stored starch provides an estimate of tree growth after insect defoliation (Webb 1981) and of seedling vigor (Duryea and McClain 1984, Marshall 1985). Of the many laboratory techniques available to quantify starch in plant tissues, enzymatic digestion has been a favored method due to its specificity for starch (see Haissig and Dickson 1979). A combination of the enzymes α-amylase and amyloglucosidase provides an efficient catalyst in the hydrolysis of starch because it attacks both the α-1,4 and α-1,6 glycosidic linkages of amylose and amylopectin (Greub and Wedin 1969).

Commercial preparations of α-amylase and amyloglucosidase are usually subjected by researchers to further purification procedures to eliminate contaminants, such as cellulases and hemicellulases, that degrade structural carbohydrates. The presence of these enzymes and of sugar contaminants could lead to an overestimate of the amount of starch present. The objective of this paper was to compare the precision of estimating starch by commercially prepared enzymes and enzymes that had undergone extensive purification.

Preliminary work with three different combinations of

amylase and amyloglucosidase suggested that these enzymes were appropriate for analyzing starch concentration in root tissue from ponderosa pine (<u>Pinus</u> <u>ponderosa</u> Dougl. ex Laws.). We tested the hypothesis that sample to sample differences in starch concentration would remain unchanged, regardless of the enzyme combination. Secondly, we hypothesized that the precision in estimates of starch concentration would be unaffected by enzyme combination. The same analytical technique (Rose et al. 1990, modified from Haissig and Dickson 1979) was used throughout the tests.

MATERIALS AND METHODS

Root tissue from ponderosa pine was oven-dried at 70°C, ground in a Wiley Mill (420μ screen), and then stored at -18°C until analysis. To remove soluble sugars, phenolics, and other compounds that might hinder the determination of starch, the tissue was extracted three times with methanol:chloroform:water (12:5:3, v:v). Starch concentration was estimated after enzymatic hydrolysis, followed by colorimetric reaction with glucose oxidase-peroxidase-o-dianisidine dihydrochloride (details in Rose et al. 1990).

The three enzyme combinations were as follows 1) COM, commercial products consisting of α-amylase (Sigma no. A-2643) and amyloglucosidase (Sigma no. A-3514), both without further purification after purchase from the supplier. A protease inhibitor, difructo-phosphokinase (DFP), had been added by the supplier because of contaminants in the α-amylase preparation; 2) PUR, a combination of α-amylase (Sigma no. A-0273), and amyloglucosidase (Sigma no. A-3423). Both were bought as crude preparations and were purified following dialysis and acetone precipitation (K. R. Forry, unpublished); and 3) ACE, a combination of α-amylase (Sigma A-0273) and amyloglucosidase (Sigma no. A-3514) with no further purification. Each of the three combinations was suspended in 0.05M sodium acetate buffer (pH 5.1).

Assayed enzyme activities and final digestion solutions are given in Table II.1.

To purify the α-amylase for ACE, 2.4 g of α-amylase powder was dissolved in 12 ml water and 0.05 g of calcium acetate monohydrate was added to make the final solution 0.025M calcium acetate. Equal volumes (1 ml each) of α-amylase solution and ice cold acetone were mixed and centrifuged. The supernatant was removed by aspiration and 3 ml of acidified water (pH 4.2) was added. After mixing, the solution was spun on a centrifuge and the supernatant was removed by aspiration. The acetone/water wash was repeated 2 more times, and the final pellets were combined together and held in suspension with 0.05M sodium acetate buffer (ph 5.1) and a pinch of sodium chloride. The purified α-amylase was then frozen until used.

Statistical analysis

Experiment 1 consisted of four sample of root tissue and three draws per sample (replication) in a completely randomized design for each of the three different enzyme combinations. Each replication was subsampled (two aliquots per replication). Experiment 2 was similar to Experiment 1 except that root tissue was from three samples known to have a wider range of starch concentration. These values were determined in preliminary work with the enzymatic digest method.

To test the hypothesis that the differences among

samples were similar regardless of enzyme source, data were analyzed as a split-plot design with three replications (Appendix AII.1)--samples were the main plots (four for Experiment 1, three for Experiment 2) and enzyme combinations were sub-plots (three for both experiments).

Data were left untransformed for Experiment 1. A log transformation of starch percent was necessary to help normalize the error in Experiment 2. Means and 95% confidence intervals reported for Experiment 2 were retransformed from the log, and the mean separation was based on the log transformed data. Fisher's protected LSD test was used to test the separation of means.

To test the hypothesis that the precision of the enzyme combinations was similar, aliquot errors and replication errors were compared using the F-test of variances described in Dixon and Massey (1951). For each experiment and enzyme combination, aliquot error was estimated as the mean square error, after accounting for variation due to samples and replication.

Replication error was determined by averaging aliquots over each replication and was then estimated by the mean square error, after accounting for variation due to samples. Errors were compared in the untransformed scale for Experiment 1 and in the log-transformed scale for Experiment 2.

RESULTS AND DISCUSSION

Enzyme combination x treatment interaction was nonsignificant (p>0.05) in either experiment, indicating that the differences among samples were similar for all enzyme combinations. In both experiments, the relative ranking of enzymes was the same (Table II.2) with the highest starch percentage consistently associated with COM and the lowest with ACE. There was no evidence to reject our first hypothesis that sample difference would remain unchanged regardless of enzyme combination. This held true for both a small range of starch (Experiment 1) and a wider range (Experiment 2). We concluded that the ability to detect sample differences for all enzyme combinations was similar.

Both experiments resulted in a similar aliquot-to-aliquot and replication-to-replication variability among the enzymes (Table II.3). The only significant difference among the error was the replication error for Experiment 1 where the F-ratio was sufficiently large to warrant further investigation (F=2.68, 0.05<p<0.10). A pairwise comparison of replication errors (Dixon and Massey 1951) suggested no significant difference between COM and PUR (F=2.27, p>0.10), greater variability in ACE versus PUR (F=6.26, p<0.01), and perhaps a slightly greater error for ACE versus COM (F=2.75, 0.05<p<0.10). We could find little evidence to reject our second hypothesis that

precision would be unaffected by enzyme combination. The only exception may have been a slightly greater variability for ACE relative to the other two combinations.

The selection of which enzymes to use can, therefore, be based on other factors. Although the assayed activities of PUR were quite reasonable (Table II.1), this combination required a lengthy purification procedure for both enzymes. ACE utilized a simpler purification procedure, but the yield for α-amylase was quite low, suggesting that more time would be necessary to purify a large quantity. COM was clearly the best method because it was precise and required no further purification beyond that carried out by the manufacturer. Although the presence of contaminants in the commercial preparations was not checked directly, our data indicated no significant contamination. Overestimation of starch, which could result from either the presence of sugar contaminants or enzymes capable of degrading structural carbohydrates, was not observed. In another study, starch determination in ponderosa pine root tissue using a commercial enzyme source (without purification) results in slightly higher starch values, relative to purified enzymes (Rose et al. 1990).

Based on the results of these experiments, COM was used in this thesis for the analysis of starch. Subsequent to the completion of starch analysis, an

additional experiment was conducted to determine if amyloglucosidase alone would yield results similar to the combination of enzymes used for COM (Appendix B). Preliminary results were encouraging and suggested further investigation.

The availability of commercial enzymes with sufficient purity makes the enzymatic method of starch determination convenient and desirable. Knowledge of these preparations should aid researchers in their continuing investigations into carbohydrate metabolism and tree growth.

Table II.1. Sources for enzymes, assayed activities, and activities in final enzyme solution, for 3 combinations of α-amylase and amyloglucosidase (U ml^{-1}=uM glucose released per ml enzyme solution).

Combi-nation	Enzyme	Assayed activity (U ml^{-1})	Activity in final solution (U ml^{-1})
COM	α-amylase	19,302,975	1900
	amyloglucosidase	71.7	5
ACE	α-amylase	5,518.4	400
	amyloglucosidase	71.7	2
PUR	α-amylase	960,000	400
	amyloglucosidase	60	2

Table II.2. Starch (percent dry weight) in ponderosa pine
root tissue, by enzyme combination (combined over samples)
and sample (combined over enzyme combination), for
Experiment 1 (standard errors in parentheses) and
Experiment 2 (data retransformed from log, 95% confidence
intervals in parentheses). Means for an enzyme or sample
down a column denoted by different letters are
significantly different ($p < 0.05$).

Experiment 1:

Enzyme	n	Starch %	Enzyme	n	Starch %
COM	12	9.0 (0.2) a	COM	9	4.9 (2.9-8.3) a
PUR	12	8.9 (0.2) a	PUR	9	4.6 (2.6-8.1) b
ACE	12	7.6 (0.2) b	ACE	9	4.4 (2.5-7.8) b

Experiment 2:

Sample #	n	Starch %	Sample #	n	Starch %
1	9	9.3 (0.2) a	5	9	9.9(9.5-10.2)a
2	9	8.6 (0.2) ab	6	9	5.4(5.3-5.6) b
3	9	8.4 (0.2) bc	7	9	1.9(1.7-2.0) c
4	9	7.8 (0.2) c			

Table II.3. F-test of aliquot and replication errors, for Experiment 1 and Experiment 2. All F values are nonsignificant (p>0.05).

Experiment 1:

Enzyme	df	Aliquot error	F
COM	12	0.0049	0.84
PUR	12	0.0089	
ACE	12	0.0043	

Enzyme	df	Rep. error	F
COM	8	0.43000	2.69[1]
PUR	8	0.18914	
ACE	8	1.18396	

Experiment 2:

Enzyme	df	Aliquot error	F
COM	9	.000075	0.43
PUR	9	.000039	
ACE	9	.000105	

Enzyme	df	Rep. error	F
COM	6	.00127	1.48
PUR	6	.00530	
ACE	6	.00657	

[1]$0.05 < p < 0.10$.

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CHAPTER III

STARCH AND DRY MATTER PARTITIONING AFTER FALL LIFTING AND
FREEZER STORAGE OF PONDEROSA PINE SEEDLINGS

ABSTRACT

Starch, and dry matter partitioning and allocation in
bareroot ponderosa pine (Pinus ponderosa Dougl. ex Laws.)
seedlings were investigated during their second growing
season in the nursery to determine how these responses
were affected over time, by seed source, and with fall
lifting and freezer storage. Height growth was completed
by the earliest fall lift (September), and seedlings from
both sources generally continued to increase in root,
fascicle, and shoot dry weight during the winter. Stem
and needle starch concentration (%) and content (mg)
declined in the fall and accumulated before the spring
lift, while root starch changes depended on seed source.
Dry weight and root starch decreased 5-13% and 82-99% in
storage, respectively, with the majority of change
occurring in the first 3 months of storage. The high
elevation source generally had greater allocation to roots
as evidenced by the smaller allocation coefficient
(2.8-3.9 vs 4.1-6.1 g shoot weight g^{-1} root weight^{-1}) and
greater relative growth rates of roots (0.02-0.05 vs
0.02-0.03 g g^{-1} wk^{-1}). These seedlings also tended to have
greater starch in all tissue components compared to the
low elevation source.

INTRODUCTION

Fall lifting and long-term storage of conifer seedlings have proven to be as effective as conventional practices (e.g., midwinter or spring lifting and short-term storage) in terms of field performance for a variety of species (Mullin and Bunting 1972, Mullin and Parker 1974, Morby and Ryker 1979, Hinesley 1982, Ritchie et al. 1985, Tung et al. 1986). Fall lifting and long-term storage are especially convenient for nurseries where soil freezes in the winter, and for nursery customers who have soils ready for planting before the nursery soil thaws.

Freezer storage has become a common practice following fall lifting in the Pacific Northwest (Hee 1986, Daniels and Simpson 1990). Many of the underlying physiological processes affected by fall lifting and long-term storage have been examined (van den Driessche 1979, Ritchie 1982, Ritchie et al. 1985, Ritchie 1986, Faulconer 1988, Cannell et al. 1990); however, little is known about how ponderosa pine (Pinus ponderosa Dougl. ex Laws.) seedling physiology is altered. Of particular interest is the potential of long-term storage to deplete carbohydrate reserves, which may ultimately decrease root and shoot growth and field survival (Duryea and McClain 1984, Marshall 1985). Reserves are utilized for maintenance and repair of tissue, as well as providing substrates for growth; and reserves have been suggested to be predictors

of seedling quality for over 20 years (Winjum 1963, Puttonen 1980, Marshall 1985).

Carbohydrate reserves generally decline in cold storage because maintenance respiration continues. The rate of depletion depends on species (McCracken 1979), time of year (Hocking and Ward 1972), tissue analyzed (Hocking and Ward 1972), physiological condition at the time of lifting (Navratil 1973), temperature (Ritchie 1982), and duration (McCracken 1979, Ritchie 1982). Although Hellmers (1962) observed carbohydrate reserves in ponderosa pine with storage, there are few quantitative estimates for this species.

Carbohydrate reserves occur primarily in the form of starch and sugars, with starch generally being the most abundant form of carbohydrate reserve in tree species (Little 1970, Glerum 1980); however, other compounds can function as reserves, including fats and hemicellulose (Glerum and Belatinecz 1980). The time of year or phenology can influence the ratio of the various carbohydrate reserves (Kreuger and Trappe 1967, Fege and Brown 1984).

The partitioning of photosynthate into starch may be related to carbon allocation between shoots and roots (Huber 1983). Roots generally contain the largest concentration of nonstructural carbohydrate and are often considered the primary storage organ (Loescher et al. 1990). Accumulated starch reserves in roots may

supplement spring root growth (Wargo 1979), while twig and stem starch may be an indicator of recovery from damage (Waring and Schlesinger 1985). Starch concentration in fine roots, when first formed, determines how long they survive (Marshall and Waring 1985).

The objective of this paper was to determine how fall lifting and long-term freezer storage affect the distribution of starch and dry matter in ponderosa pine, and to compare the response of a high and a low elevation seed source.

MATERIALS AND METHODS

Two-year-old seedlings from two Oregon seed sources were selected at the Bend Pine Nursery in central Oregon (44° 5' N, 121° 16' W; elevation, 1100 m). This nursery was chosen because frozen soils prevent lifting in winter, and fall lifting with long-term storage had not been attempted. One seed source represented seeds from the Barlow Ranger District, Mount Hood National Forest (Hood, 1100 m elevation). The other seed source represented seeds collected from the Lakeview Ranger District, Fremont National Forest (Fremont, 1800 m elevation). Seeds were sown in the spring of 1986.

Summer lifting and morphology before fall lifting

Seedlings were shovel-lifted on May 22, June 18, and August 4, 1987, from four blocks (replications) within beds for each seed source, and stored overnight at 2°C. The next day, seedlings (10 per replication) were measured for height and stem diameter, frozen on dry ice, and stored at -18°C. Root, stem, fascicle, and total needle weight were subsequently determined on 5-10 seedlings per replication after drying at 70°C. About 50-60 fascicles from the current year's growth were removed per seedling to get an estimate of individual fascicle weight.

Fall lifting and storage

Seedlings were shovel-lifted on September 22 (SEP),

October 20 (OCT), November 17 (NOV), 1987, and March 1, 1988 (MAR) after 66, 315, 640, and 2,545 chilling hours (Chapter I), and graded according to nursery standards. The design for lifting was described in Chapter I.

Second-year height growth had been completed by SEP (Table III.1). All fall-lifted seedlings were in varying degrees of dormancy, with SEP seedlings being close to the maximum rest phase of dormancy (characterized by having maximum days to budbreak in a favorable environment, Chapter I). OCT and NOV seedlings were progressing further through the dormancy cycle (reduced days to budbreak as lift date became later in the fall). Plant development progressed with additional storage chilling for all fall lift dates. MAR seedlings were quiescent, and they flushed rapidly in response to warming temperature (Chapter I).

Conditions of storage were those described in Chapter I. Seedlings from each fall lift date were stored for 1 day (0 months, cold storage), 90 days (3 months, freezer), 150 days (5 months, freezer), and until outplanting at Bend (freezer) on March 16-17. Freezer storage temperature averaged -1.5°C inside the storage bags. Six days before the end of the storage interval, the boxes were placed in a cooler (2-4°C, cold storage) for thawing. MAR seedlings remained in cold storage until planting.

After lifting and after storage, seedling morphology was measured similarly as that for the summer harvests,

with a few exceptions. Seedling morphology of MAR
seedlings was not assessed until the time of planting;
plus, all seedlings stored until spring planting (destined
for morphological assessment) were frozen in dry ice when
trees were outplanted (Chapter V) and subsequently stored
at -37°C, before processing for dry weight determination.

Winter sampling

Shoots of seedlings (5-9 per block) were removed for
each seed source on January 14, 1988, while the soil was
frozen at the nursery. Handling of seedlings and
morphological measurements were similar to that described
for fall lifts. Lifting for all dates was accomplished
before 0900 h, with exception of May-lifted seedlings
(1500-1600 h).

Allocation coefficient and relative growth rate

To determine seed source differences in relative
growth partitioning between roots and shoots, an
allocation coefficient (Campagna and Margolis 1989) was
calculated as :

$$k = (SDW_2 - SDW_1)/(RDW_2 - RDW_1),$$

where SDW is shoot dry weight and RDW is root dry weight
at measurement times 2 and 1. Large values of k indicate
greater allocation to shoot growth relative to root
growth, over a specific time. If k is less than 1, dry
matter allocated to roots is greater than shoots.

Dry weights for block means were used to determine $\underline{k}$ for 4 periods during the second-year growth:

$\underline{k}_1$ = May 22 to SEP,

$\underline{k}_2$ = May 22 to OCT,

$\underline{k}_3$ = May 22 to NOV, and

$\underline{k}_4$ = May 22 to MAR.

Relative growth rates (RGR) were calculated for the same 4 periods for needles, stems, and roots. The formula was:

RGR = (ln W_2 - ln W_1)/(weeks),

where W_2 and W_1 are weights at measurement times 2 and 1, and weeks are the number of weeks (17, 21, 25, and 40) between times of measurement.

Both the allocation coefficient and RGR of seedlings were approximations because nursery cultural practices and grading selectively choose individuals.

Starch analysis

After dry weights were measured, root, stem, and fascicle tissues were stored with a desiccant at -18°C, ground in a Wiley Mill (420μ screen) and stored with a desiccant at -18°C until chemical analysis. During grinding, samples were pooled (5-6 seedlings per treatment and replication). Starch was not estimated for the summer harvests. Starch was determined in roots for all other combinations of lift date and storage (except January), but for only 2-3 of the storage periods for stems and

fascicles.

Ground material was extracted with methanol:chloroform:water (v:v) (3 times for root tissues, 4 times for fascicles and stems), and then analyzed for glucose concentration after starch solubilization and incubation with a combination of amyloglucosidase and α-amylase enzymes (Chapter II, Rose et al. 1990). Although similar in their ability to detect treatment differences, the enzyme digest method is more precise than perchloric acid methods (Rose et al. 1990). Each sample was analyzed twice.

Glucose concentration was corrected to starch concentration (% dry weight) using 0.9 as a hydrolysis factor (glucose x 0.9, Volenec 1986). Starch content of tissue was calculated by multiplying dry weight x starch concentration. Starch content in leaf tissue was expressed as μg starch per fascicle and mg starch in all needles per tree.

Statistical analysis

To determine the differences in morphology throughout the second growing season, the data were analyzed as a split-plot design with seed source as the main plot, lift date and storage treatment as sub-plot, and 4 replications. The analysis of variance had the form shown in Appendix CIII.1. A log transformation was necessary for fascicle weight to help make the error normal and

homogeneous. Means presented for fascicle weight were retransformed from the log. A least squares mean procedure was used to separate the means (Searle et al. 1980).

To determine the effect of storage after fall lifting, the morphology data were analyzed as a 2 (seed source) x 3 (lift date) x 4 (storage) factorial with 4 replications (Appendix CIII.2). Mean separation was accomplished with a least squares mean procedure (Searle et al. 1980).

Seed source differences in $\underline{k}$ or RGR were analyzed as a completely randomized design with 4 replications. The analysis of variance had the form shown in Appendix CIII.3. Fisher's protected LSD was used to separate means.

The analyses for the effect of lift date (Appendix CIII.4) or seed source and storage (Appendix CIII.5) on starch concentration or content were similar to those for morphology. A log transformation was necessary for all starch data in order to make the error normal and homogeneous. All means presented were retransformed from the log.

Differences were tested at the $\alpha=0.05$ level unless otherwise noted.

RESULTS

Morphology at lifting and after storage

Date of lifting affected morphology in the same way for both seed sources. Height growth was completed by SEP, while stem, root, and fascicle weight continued to increase over the winter (Table III.1). Root:shoot dry weight ratio declined through the previous summer for both seed sources. For Fremont seedlings, the ratio increased in the fall and declined in spring, while for Hood seedlings, root:shoot ratio tended to drop over time, resulting in a significant seed source x lift date interaction (Table III.1). At the time of lifting, root:shoot ratio for Fremont seedlings was 19 (May) to 50 (SEP, OCT) percent greater than Hood seedlings ($p < 0.01$).

In general, Fremont seedlings were shorter and lighter in stem weight than Hood seedlings, but greater in root weight and fascicle weight (Table III.2).

The tendency for dry weight to decline in storage (about 5-13%) was similar for both seed sources. Not all of the tissue components dropped significantly in weight, but the trends were consistent for all response variables (Table III.2). The majority of weight loss occurred in the first 3 months of storage.

Allocation and relative growth rate

Allocation coefficients were greater than 1 for both seed sources over all time periods, indicating that

allocation to shoot was greater than to roots; however, Hood seedlings had a greater allocation to shoots than Fremont for 3 of the 4 periods, and for the final period, the tendency was Hood > Fremont (Table III.3).

Relative growth rates for needles and stems was not significantly different between seed sources for any time period. On the other hand, root RGR was 41 (May to SEP), 48 (May to OCT) and 29 (May to NOV, $p < 0.10$) percent greater for Fremont compared to Hood (Table III.4).

Starch at lifting and after storage

Root starch concentration and content declined through the fall for Fremont seedlings, while remaining constant for Hood seedlings; root starch then tended to increase to the MAR date (Table III.5). Stem and needle starch changed similarly for both seed sources--declining in the fall and increasing after January. Root starch concentration and content were generally greater than that found in stems and needles.

Although root starch concentration (Figure III.1) and content (Table III.6) decreased consistently for both seed sources after storage (Figure III.1, Table III.6), the magnitude of the decline differed by seed source (95-99% for Fremont, 73-95% for Hood) causing a seed source x lift date interaction. As with dry weight, the greatest decrease in starch occurred in the first three months of storage, and there were few differences thereafter.

For the storage periods measured, stem and needle starch concentrations or content generally changed similarly with storage as the roots (Table III.7).

DISCUSSION

Starch partitioning among tissue

Starch partitioning among tissue was consistent with results in Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco, Ritchie 1982), loblolly pine (Pinus taeda L., Reid 1986), mugo pine (Pinus mugo Turra) and radiata pine (Pinus radiata D. Don) (McCracken 1979). Root starch was generally greater than stem or needle starch, even after accounting for the larger biomass of stems and needles. This was not surprising, considering that leaf tissue has higher maintenance costs associated with enzyme turnover (Waring and Schlesinger 1985); in addition, all sampling took place early in the morning before any appreciable diurnal accumulation of leaf starch (Huber 1983, Fondy and Geiger 1983). Therefore, needle starch estimates in this study were minimal; however, all tissues can be important as storage reserves, depending on tree age and time of year. A greater amount of experimentally applied ^{14}C is stored in jack pine (Pinus banksiana (Lamb.)) seedling roots in the fall, but later in the winter, needles are storing large amounts (Glerum and Belatinecz 1980).

The mechanisms controlling the allocation of carbon among the various tissues are not well understood. Traditionally, the "strength" of source (synthesis or mobilization of photosynthate) and sink (utilization) has been used to explain allocation (Wardlaw 1968, Fondy and

Geiger 1983, Larcher 1983). Another possibility is the photosynthate partitioning into leaf starch. Leaf starch accumulation appears to be associated with sucrose phosphate synthetase, i.e., the formation of sucrose (Huber and Israel 1982). Increased leaf sucrose could be related to increased root respiration and nutrient uptake (Wardlaw 1980, Huber and Israel 1982). Allocation may be controlled by nitrogen uptake (Vessey and Layzell 1987), or the ratio of biochemically available carbohydrate to available nitrogen (Campagna and Margolis 1989).

Seasonal patterns

The decline in stem and needle starch through the fall may be related to the conversion of starch to sugar and the acquisition of frost hardiness (Chapter I). Although the mechanism responsible for the hydrolysis of starch is unknown (Yamashita 1990), it is hypothesized that it is due to the synthesis of hydrolytic enzymes or the activation of pre-existing enzymes (Dear 1973). Peak stem, shoot, or foliar sugar concentrations, however, coincide with mid-winter in a variety of species (Ericsson 1979, Fege and Brown 1984, Kreuger and Trappe 1967, Ritchie 1982, McNabb 1985, Margolis and Waring 1986, Yamashita 1990).

The decline in needle starch could also reflect the translocation of carbohydrate to roots during the fall (Shiroya et al. 1966, Little 1970, Glerum and Belatinecz

1980). This could explain why root starch concentrations were relatively high in SEP and OCT for Fremont. Fremont seedlings allocated more dry matter ($\underline{k}_1$, $\underline{k}_2$, $\underline{k}_3$) and had a higher relative growth rate for roots (RGR) compared to Hood seedlings in the fall.

Root carbohydrate reserves also display the starch to sugar phenomenon during overwintering (Halmer and Bewley 1982) and this could explain the tendency for root starch to decline for Fremont seedlings in the fall. In contrast, root starch in Hood seedlings stayed constant, a result that was in agreement with Kreuger and Trappe (1967) and van den Driessche (1978) for Douglas-fir. We have no explanation for this difference, other than genetic, bed or environmental conditions that alter photosynthetic efficiency; or differences between seed sources in relation to timing of acclimation.

Both starch and dry weight for all tissues increased in MAR, probably coinciding with low growth rate and high photosynthetic rates before shoot elongation. Starch or total nonstructural carbohydrate increases in the spring before budbreak are common (Kreuger and Trappe 1967, van den Driessche 1978, Ritchie 1982, McNabb 1985, Margolis and Waring 1986, Cannell et al. 1990).

Effects of storage

The decline in starch during cold dark storage was consistent with Douglas-fir (Ritchie 1982, Cannell et al.

1990), Sitka spruce (<u>Picea</u> <u>sitchensis</u>, Cannell et al. 1990), mugo and radiata pine (McCracken 1979), and Engelmann spruce (<u>Picea</u> <u>engelmannii</u>, Ronco 1973). Winjum (1963), however, found that starch and reducing sugar concentration did not change in Douglas-fir and noble fir seedlings during storage. Dry weight losses in storage have also been reported by DeWald and Feret (1988) and Ronco (1973). The decrease in root starch could be associated with increases in root sugar (Ritchie 1982), in response to storage temperatures near freezing (Halmer and Bewley 1982); respirational losses are also a factor.

Most of the dry weight and starch decline occurred in the first three months of storage, similar to McCracken (1979), Ritchie (1982), and Cannell et al. (1990). It is hypothesized that this pattern of depletion is due to a steady state of respiration to base levels of carbohydrates (Cannell et al. 1990), or as a result of differential respiration rates during storage. Ritchie (1982) suggested that respiration rate is high initially because substrates or oxygen are non-limiting, and then the rate slows because of a reduction in reserves, oxygen, or both. In contrast, whole seedling carbohydrate (starch and mostly sugars) and dry weight of Engelmann spruce stayed constant after two months in storage (1-2 $^{\circ}$C), and then declined (Ronco 1973).

Seed source differences

There were strong seed source differences in starch and dry matter partitioning. Fremont seedlings were generally greater in starch concentration and content, shorter, lighter in stem weight and shoot weight, and greater in fascicle and root weight, than Hood seedlings. The same trends in starch between Fremont and Hood were found in seedlings after they were outplanted in March (Chapter V). Some differences may be influenced by cultural practices or soil differences between beds. On the other hand, dry matter partitioning may reflect genetic adaptations associated with high and low elevation environments.

The root:shoot ratio for the higher elevation Fremont seedlings was much greater (19-54%) than Hood at the time of lifting, and Fremont seedlings in general allocated more resources toward roots (k, RGR). Live fine root mass or root growth in coniferous forest species has been related to northern latitude (Vogt et al. 1986), high altitude (Vogt et al. 1986), or cold temperatures (Turner et al. 1982), suggesting a greater allocation of carbohydrate to root growth for these environments. The response may be the result of nutrient or moisture limitation at high altitudes (Waring and Schlesinger 1985).

Other reserves may be competing for the available sucrose pool. Fructan storage, for example, may prevent

starch accumulation in the chloroplast and allow photosynthetic activity at low temperatures (Nelson and Spollen 1987). Sugars can make up a large component of the total nonstructural carbohydrate pool (McCracken 1979, Ritchie 1982). Another factor could be nursery management practices. High seedbed densities or witholding water to induce dormancy, for example, could reduce photosynthesis and the accumulation of reserves (McNabb 1985, Marshall 1985).

Storage until planting clearly depleted starch. Whether this depletion affects subsequent root initiation or future survival and growth, is addressed in Chapter V.

Table III.1. Morphology of seedlings throughout the second growing season for two seed sources. Date or seed source means (n=number of seedlings for each mean, s.e.=standard error of a date mean) within a column with different letters are significantly different (p<0.05).

	Height (cm)	Stem diameter (mm)	Stem weight (g)	Root weight (g)	Fascicle weight[1] (mg)	Total needle weight/tree (g)	Shoot weight (g)	Root:shoot weight ratio[2]	
Date								Fremont	Hood
May	18.0 c	4.3 d	0.77 d	0.86 d	6.2 e	1.23 d	2.00 d	0.49 a	0.41 a
June	20.0 b	4.8 c	1.05 d	0.96 d	18.6 d	1.99 c	3.04 c	0.35 c	0.30 b
August	20.4 b	5.3 b	1.49 c	1.26 c	43.1 c	2.90 b	4.56 b	0.34 c	0.28 b
SEP	23.4 a	5.7 a	2.15 ab	1.75 ab	50.6 b	3.36 ab	5.56 a	0.40 b	0.26 b
OCT	22.7 a	5.5 ab	2.01 b	1.68 b	50.0 b	3.28 ab	5.44 ab	0.40 b	0.26 b
NOV	23.3 a	5.6 ab	2.24 ab	1.72 b	55.8 ab	3.66 a	6.09 a	0.35 c	0.26 b
MAR	24.2 a	5.6 ab	2.44 a	1.92 a	59.9 a	3.65 a	6.15 a	0.35 c	0.28 b
n	78-80	34-40	40-80	78-80	39-42	37-79	37-79	19-39	18-40
s.e.	0.60	0.12	0.11-.16	0.07	--	0.04-.19	0.22-.23	0.01	0.01-.02

[1]Means for fascicle weight were retransformed from the log.

[2]Significant seed source x date interaction. Ratios are shown for each seed source and date.

Table III.2. Morphology of seedlings from two seed sources after three fall lift dates and four storage periods (n=number of seedlings for each mean, s.e.=standard error of a seed source or storage mean). Seed source or storage means within a column with different letters are significantly different (p<0.05)[1].

Seed source	Height (cm)	Stem diameter (mm)	Stem weight (g)	Root weight (g)	Fascicle weight (mg)	Total needle weight/tree (g)	Shoot weight (g)
Fremont	17.5 b	5.5 a	1.6 b	1.74 a	56.2 a	3.14 a	4.85 b
Hood	28.4 a	5.6 a	2.5 a	1.49 b	45.3 b	3.09 a	5.62 a
n	473-479	474-479	474-479	474-477	240-242	236-240	236-240
s.e.	0.23	0.05	0.05	0.03	0.80	0.08	0.14
Storage[2]							
0 months	23.1 a	5.6 a	2.13 a	1.72 a	52.6 a	3.43 a	5.69 a
3 months	23.0 a	5.7 a	2.10 a	1.61 ab	50.7 a	3.07 b	5.18 ab
5 months	22.8 a	5.5 a	1.95 a	1.56 b	50.1 a	2.96 b	5.02 b
Planting	22.8 a	5.3 b	2.00 a	1.58 b	49.7 a	3.01 b	5.04 b
n	237-239	237-239	237-239	236-239	120-122	116-120	116-120
s.e.	0.32	0.07	0.07	0.04	1.2	0.11	0.19

[1]Mean differences for SEP, OCT, and NOV lift dates represented in Table III.1.

[2]Seedlings were stored for 0, 3, and 5 months, and until planting (approximately 6, 5, and 4 months for SEP, OCT, and NOV, respectively) in March.

Table III.3. Allocation coefficients ($\underline{k}$=g g^{-1}) for two seed sources for the period from May 22, 1987 to : 1) September 22, 1987 (SEP), 2) October 20, 1987 (OCT), 3) November 17, 1987 (NOV), and March 1, 1988 (MAR). Each coefficient is a mean of 4 replications (s.e.=standard error of a seed source mean).

Time period	Seed source[1]		s.e.
	Fremont	Hood	
May to SEP	2.77 *	6.13	0.94
May to OCT	3.27 **	5.49	0.33
May to NOV	4.00 *	6.07	0.57
May to MAR	3.88 ns	4.10	0.46

[1]Seed source means separated by * are significantly different ($p<0.05$); **=$p<0.01$; ns=not significant.

Table III.4 Relative growth rates (RGR=g g^{-1} wk^{-1}) for needles, stems, and roots for two seed sources for the period from May 22, 1987 to : 1) September 22, 1987 (SEP), 2) October 20, 1987 (OCT), 3) November 17, 1987 (NOV), and March 1, 1988 (MAR). Each RGR is a mean of 4 replications (s.e.=standard error of a seed source mean).

Tissue	Time period	Seed source[1]			
		Fremont		Hood	s.e.
Needles	May to SEP	0.055	ns	0.061	0.008
	May to OCT	0.050	ns	0.042	0.003
	May to NOV	0.046	ns	0.040	0.002
	May to MAR	0.029	ns	0.025	0.002
Stems	May to SEP	0.058	ns	0.061	0.004
	May to OCT	0.044	ns	0.046	0.002
	May to NOV	0.042	ns	0.042	0.003
	May to MAR	0.027	ns	0.030	0.001
Roots	May to SEP	0.048	*	0.034	0.003
	May to OCT	0.037	**	0.025	0.002
	May to NOV	0.031	ns[2]	0.024	0.002
	May to MAR	0.020	ns	0.019	0.001

[1]Seed source means separated by * are significantly different (p<0.05); **=p<0.01; ns=not significant.

[2]0.05<p<0.01.

Table III.5. Starch concentration (% dry weight) and content (% starch x dry weight) for roots, stems, and needles of two seed sources (Fremont, Hood, or both combined) at the time of lifting. Date or seed source means (n=number of seedlings for each mean) with different letters down a column are significantly different (p<0.05). Means were retransformed from the log.

| | Roots | | | | Stems[1] | | Needles[1] | | |
| | Concentra-tion (%) | | Content (mg) | | | | | Content[2] | |
Date	Fremont	Hood	Fremont	Hood	Concentra-tion (%)	Content (mg)	Concentra-tion (%)	Fasci-cle (μg)	Needles (mg)
SEP	4.29 a	0.21 b	78.05 a	3.27 b	0.32 a	6.77 b	0.10 a	51 a	3.32 a
OCT	3.37 ab	0.19 b	67.41 a	2.71 b	0.15 b	3.06 c	0.04 bcd	19 c	1.21 c
NOV	1.43 c	0.20 b	28.16 b	3.15 b	0.10 c	2.47 c	0.03 d	17 c	1.13 c
Jan[3]	--	--	--	--	0.05 d	--	0.05 c	29 b	1.86 b
MAR	2.14 bc	1.56 a	43.37 ab	28.31 a	0.42 a	10.12 a	0.07 b	43 a	2.58 ab
n	20	20	20	20	40-41	40	40-41	40-41	40-41
			Seed source[4]						
			Fremont		0.11 a	2.21 a	0.05 a	28 a	1.60 a
			Hood		0.07 b	1.82 a	0.04 a	18 b	1.20 a
			n		180-181	160	180-181	180-181	180-181

[1]Data combined for both seed sources.

[2]Starch content in needles estimated as μg per fascicle and mg in all needles per tree.

[3]No root sample for January because of frozen soil.

[4]Data combined for dates. Stem content means betweeen Fremont and Hood are significantly different at 0.05<p<0.10.

Table III.6. Root starch content (mg per root=% starch x dry weight) for two seed sources after fall lifting and four storage periods (0, 3, and 5 months, and until planting in March). Means were retransformed from the log. Within a seed source and lift date, storage means (n=20) with different letters are significantly different (p<0.05).

Lift date	Storage	Seed source	
		Fremont	Hood
SEP	0 months	78.05 a	3.27 a
	3 months	0.49 b	0.18 b
	5 months	0.55 b	0.17 b
	Planting	0.66 b	0.20 b
OCT	0 months	67.41 a	2.71 a
	3 months	2.40 b	0.55 bc
	5 months	1.14 b	0.25 c
	Planting	1.36 b	0.09 d
NOV	0 months	28.16 a	3.15 a
	3 months	1.31 b	0.55 bc
	5 months	0.71 b	0.86 b
	Planting	1.01 b	0.32 c

Table III.7. Stem and needle starch concentration (%) and content (% starch x dry weight) for two seed sources (Fremont, Hood, or both combined) after fall lifting and after storage. Means were retransformed from the log. Storage means (n=number of seedlings for each mean) within a lift date with different letters are significantly different (p<0.05).

| | | Stem | | | | Needles[1] | | |
| | | Concentra-tion (%) | | Content (mg) | | | Content[2] | |
Lift date	Storage[3]	Fremont	Hood	Fremont	Hood	Concentra-tion (%)	Fascicle (μg)	Needles (mg)
SEP	0 months	0.45 a	0.23 a	7.41 a	6.18 a	0.10 a	51 a	3.32 a
	Planting	0.04 b	0.03 b	0.70 b	0.67 b	0.04 b	16 b	0.99 b
OCT	0 months	0.27 a	0.08 a	4.74 a	1.97 a	0.04 a	19 a	1.21 a
	Planting	0.06 b	0.04 b	0.93 b	0.99 b	0.03 a	15 a	0.95 b
NOV	0 months	0.14 a	0.08 a	2.71 a	2.25 a	0.03 a	17 a	1.13 a
	3 months	0.05 b	0.03 b	0.84 b	0.83 b	0.03 a	15 a	0.95 a
	Planting	0.06 b	0.04 b	1.07 b	0.78 b	0.03 a	16 a	0.98 a
n		20	20	20	20	40	40	40

[1]Data combined for both seed sources.

[2]Starch content in needles estimated as μg per fascicle and mg in all needles per tree.

[3]Storage period until planting was approximately 6, 5, and 4 months for SEP, OCT, and NOV, respectively.

a) Fremont
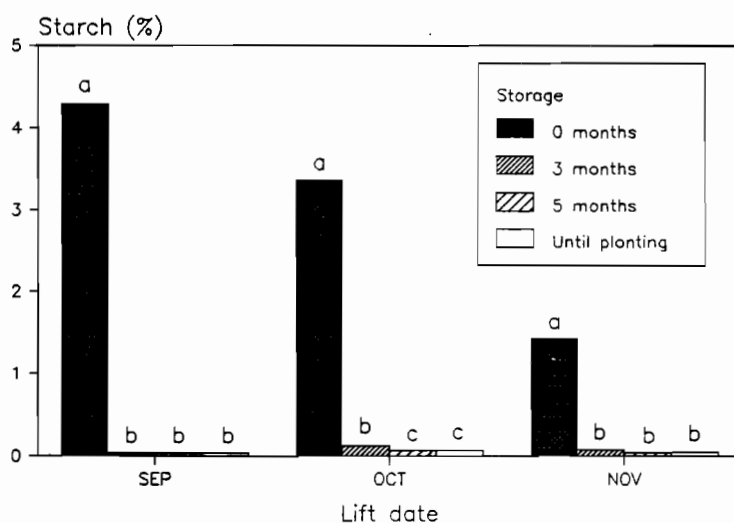
b) Hood
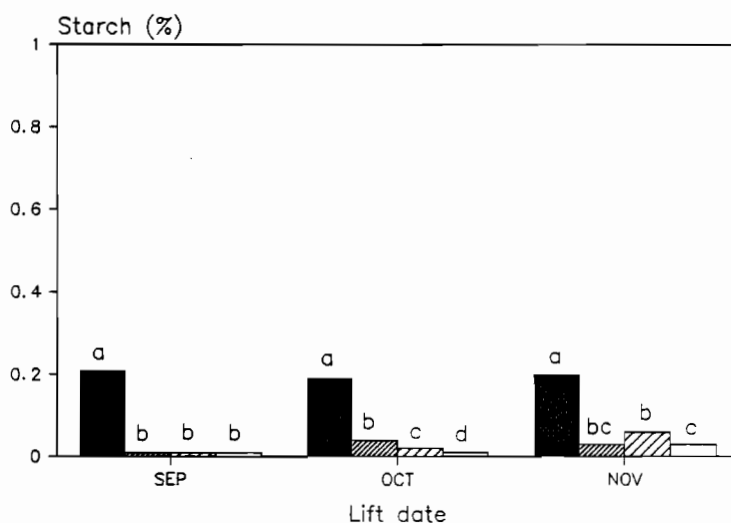
Figure III.1. Root starch concentration (% dry weight) for a) Fremont and b) Hood seed sources after fall lifting and four storage periods. Means are retransformed from the log. Within a seed source and lift date, means with different letters are significantly different (p<0.05).

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CHAPTER IV

FALL LIFTING AND LONG-TERM STORAGE OF PONDEROSA PINE
SEEDLINGS: EFFECTS ON POST-STORAGE LEAF WATER POTENTIAL,
STOMATAL CONDUCTANCE, AND ROOT GROWTH POTENTIAL

ABSTRACT

Post-storage water relations, stomatal conductance to
water vapor, and root growth potential were investigated
for ponderosa pine (Pinus ponderosa Dougl. ex Laws.)
seedlings from a high- and a low-elevation seed source.
Seedlings were either lifted in October or November and
freezer stored, or were lifted in March. Seedlings were
grown hydroponically in a greenhouse for 31 days after
storage. Seedlings lifted in October had poor root
initiation (<17 new roots per seedling), low predawn water
potential (<-1.5 MPa), and low stomatal conductance
(7.10 mmol m^{-2} s^{-1}). There was little difference in post-
storage water relations and conductance between seedlings
lifted in November and those lifted in March. Throughout
the 31 days, seedlings from the higher elevation source
had consistently greater root production (3-9 times more
new roots), higher water potential (about 2 times more
positive predawn MPa), and greater conductance (1.3-5
times greater) than those from the lower elevation source.
Number of new roots on day 31 was significantly related to
water potential (r^2=0.65 for predawn) and conductance
(r^2=0.82), Similarly, the dry weight of new roots on day

31 accounted for a significant amount of the variation in predawn water potential (r^2=0.81) and conductance (r^2=0.49).

INTRODUCTION

Fall lifting of coniferous nursery stock followed by freezer storage is becoming a widespread practice in the Pacific Northwest (Hee 1986, Ritchie 1986). The effects of freezer storage on the subsequent physiology of seedlings has been the subject of several studies (Ritchie 1984, Grossnickle and Blake 1985, Ritchie et al. 1985, Grossnickle and Blake 1987), but few have addressed ponderosa pine (Pinus ponderosa Dougl. ex Laws.). An increase in the length of time seedlings are stored in the dark is associated with a decrease in photosynthesis of Mugo pine (Pinus mugo Turra) and Monterey pine (Pinus radiata D.Don) after storage (McCracken 1978). White spruce (Picea glauca (Moench) Voss) stored overwinter may be preconditioned to drought because stomata close sooner in response to drought than do those of spring-lifted stock (Blake 1983). Although species difference in physiology after freezer storage have been addressed (Grossnickle and Blake 1985, Ritchie et al. 1985, Grossnickle 1988), few studies (Ritchie 1984) have examined provenance response to freezer storage.

Lifting date and storage have a strong influence on the seedling's ability to initiate or elongate new roots (root growth potential, RGP, Ritchie and Dunlap 1980). Root growth potential is an often-used indicator of seedling quality, but its positive correlation to field

performance is not universal (Ritchie and Dunlap 1980, Burdett 1987). Root growth potential after storage is influenced by physiological condition of the seedling at the time of lifting (Stone 1970). If seedlings are lifted in the phase of dormancy where they are not ready to respond to chilling, then storage may reduce RGP. On the other hand, storage chilling can contribute to increased RGP if the seedlings are lifted later in the dormancy cycle and are responsive to chilling (Ritchie and Dunlap 1980); however, RGP can decline with storage chilling even if dormancy is released (Carlson 1985).

Our objectives were to examine the effects of fall lifting and long-term freezer storage on seedling root growth potential and physiology. We hypothesized that ponderosa pine seedlings lifted in October and subjected to long-term freezing would initiate fewer new roots than seedlings lifted in late fall or spring. Because field studies suggest that seed source may determine the success of fall lifting and long-term storage, we tested the null hypothesis that RGP would be similar for seedlings from a high and a low elevation seed source. We hypothesized that differences in root initiation would result in changes in post-storage water potential and stomatal conductance to water vapor. In transplanted seedlings, root growth is critical to the return of a favorable water balance (Rietveld 1989). Therefore, if lifting date or seed source alters RGP, the difference in root growth

should be reflected by changes in post-storage water relations.

MATERIALS AND METHODS

Seedling materials

Two-year-old bareroot ponderosa pine seedlings were selected at Bend Pine Nursery, Oregon (44° 5' N, 121° 16' W; elevation, 1100 m). Frozen nursery soils typically prevent lifting of seedlings in the winter. Seedlings from two Oregon seed sources (Barlow Ranger District, Mt Hood National Forest (1100 m) and Lakeview Ranger District, Fremont National Forest (1800 m)) were grown in different beds at the nursery under standard cultural practices until lifting.

Hood seedlings were shovel-lifted on October 20 (OCT) and November 17 (NOV), 1987, or on the operational lifting date, March 1, 1988 (MAR). Fremont seedlings were lifted on NOV and MAR dates only. At the nursery, seedlings were put in 1.5-ml polyethylene bags and placed in 30 x 75 x 40 cm wax-lined cardboard boxes filled with ice for transport to Corvallis, Oregon. Lifted seedlings were stored (2-4°C, cold storage) overnight.

Fall-lifted seedlings were transferred to the freezer (freezer storage) the day after lifting. Ambient and inside-bag temperatures during freezer storage were monitored with a hygrothermograph and thermocouples attached to a recorder. Inside-bag temperature generally ranged from -0.1 to -3.4°C, with a mean of -1.5°C. MAR seedlings remained in cold storage until the greenhouse

test began.

Greenhouse test

Fall-lifted seedlings were removed from the freezer
on March 16, 1988, and placed in cold storage. On March
21, these seedlings, plus MAR seedlings, were transferred
to the greenhouse.

To determine seedling moisture status at the end of
storage, 25 seedlings (five from each seed source x lift
date) were removed individually from the storage box.
Needle water potential (MPa) was immediately measured on a
needle fascicle with a pressure chamber. White root tips
were removed to facilitate identification of subsequent
new root growth. Seedlings were then randomly assigned to
three 37.8-l tanks filled with water in which a commercial
fertilizer (0.26 g L^{-1} of 15-30-15) was dissolved.
Seedlings (8-9 per tank) were placed in plywood templates
(1.87 cm thick) with the root collars secured by foam
plugs so that the root systems were totally immersed in
the nutrient solution. An air pump attached to a bubble
wand that extended along the base of the tank, provided
aeration.

Growing conditions in the greenhouse monitored during
the 31-day test included: air temperature (day, generally
21-24°C; night, 17-19°C); pH (6.4-7.2); and water
temperature (14-24°C). Oxygen concentration of water was
measured with a Yellow Springs Instrument (Yellow Springs,

OH) dissolved oxygen meter. Oxygen concentration in the root zone directly above the bubble wand was 8.5 ppm (oxygen saturation = 9.0 ppm) and ranged from 6.5-7.4 ppm in the adjacent spaces. Natural light was supplemented by 300-watt incandescent light bulbs suspended about 0.5 m above the seedling tops (30-70 μmol m^{-2} s^{-1}), extending the daylength to 16 h. Water and fertilizer were changed weekly, and seedling tops were misted up to four times daily.

The number of new root tips (up to 50) was counted every 5-6 days. Predawn (0230-0400 h) needle water potential was measured every 9-11 days. Mid-morning (1030-1200 h) needle conductance to water vapor, concurrent needle water potential, temperature, relative humidity, and light intensity were measured every 4-6 days. After 31 days, the roots were separated into new root tips and remaining root, frozen in dry ice, placed in a -18°C freezer, dried at 70°C, and then the two root fractions were weighed.

Needle resistance and light intensity were measured with a Licor Li-1600 steady state porometer (Lincoln, NE). Needle resistance measurements were taken on two adjacent fascicles. These same two fascicles were measured for the duration of the test. Resistance values (s cm^{-1}) from the porometer were later converted to conductance (mmol m^{-2} s^{-1}). The needle surface area was calculated by measuring a cross-sectional dimension of the needle and

assuming the three needles per fascicle comprised a cylinder.

Statistical analysis

To determine differences among the five lift date x seed source treatment combinations, the data were analyzed by sampling day as a completely randomized design with 5 replications (seedlings) for each sampling day (Appendix CIV.1). Initial, predawn, and midmorning water potential values (-10 MPa) were log transformed to achieve a normal and homogeneous distribution of the variance. Dry weight of old roots on day 31, conductance, and number of new roots were left untransformed, with the exception of a log transformation applied to conductance on day 20. Fisher's protected LSD was used to separate the means at the 0.05 level. Means presented for the treatment effects on predawn water potential were retransformed from the log. Seed source x lift date interactions were determined by analyzing NOV and MAR data as a 2 (seed source) x 2 (lift date) factorial with 5 replications in a completely randomized design (Appendix CIV.2).

Transformation of the data was also necessary to determine the relationship among root number or weight and water relations (Day 31). Weight of new roots, water potential (-10 MPa), conductance, and number of new roots (n + 0.5) were log transformed. Slopes and intercepts for the treatments were tested under the hypothesis that there

was no difference among the regression parameters.
Because the slopes and intercepts were not significantly
different (p>0.05) among treatments, there was no evidence
that the relation between water relations and root number
or weight was different by treatment. Therefore, the data
were combined over all treatments, and regression analyses
were based on all observations (n=20-25).

RESULTS

Analysis of treatment effects

Seedlings had relatively high water potential
(>-0.78 MPa) when they came out of the storage (Figure
IV.1). Initial water potential in Hood NOV seedlings
(-0.78 MPa) was about two times lower than for the other
treatments (-0.44 to -0.32 MPa). Hood OCT seedlings began
with the highest water potential (-0.32 MPa), but after
day 5 consistently ranked lowest for both predawn (-1.90
to -1.52 MPa) (Figure IV.1) and midmorning readings
(-2.94 to -2.24 MPa). Hood OCT also had the lowest values
in conductance ($0.81-7.14$ mmol m^{-2} s^{-1}, Figure IV.1).
Conductance values were low for all treatments on days 10
and 20, coinciding with clear days when relative humidity
was low (30%) and light intensity (>400 μmol m^{-2} s^{-1}) and
temperature ($>25°C$) were high.

Hood OCT seedlings showed the fewest (15) new roots
(Figure IV.1), but on day 31 no significant differences
were apparent among treatments for number of new roots or
for dry weight (data not shown) of new or old roots.

Seed source x lift date interactions were generally
nonsignificant, indicating that lift date affected the
variables in the same manner for both seed sources. The
most pronounced difference was between seed sources
(Figure IV.1). NOV was not significantly different from
MAR for most responses and days; however, Fremont

seedlings had, on the average, more positive predawn (e.g., day 31:-0.59 vs -1.13 MPa) and midmorning (-1.31 vs -1.76 MPa) water potential (p<0.05) and greater stomatal conductance (24.05 vs 18.15 mmol m^{-2} s^{-1}) and more new roots (36 vs 27) than did Hood seedlings.

Regression analysis of physiological responses

Seedlings with new roots tended to have higher water potential and conductance compared to seedlings that did not initiate new roots (Figure IV.2). Seedlings without new roots had negligible conductance (<0.16 mmol m^{-2} s^{-1}) after day 20, whereas conductance increased eightfold from day 20-31 for seedlings that produced new roots (2.53-20.77 mmol m^{-2} s^{-1}).

Water potential and conductance were closely related to the number of new roots on day 31 (Figure IV.3), accounting for 65, 64, and 82 % of the variability in predawn water potential, midmorning water potential, and conductance, respectively. The weight of new roots was a slightly better predictor of predawn water potential than the number (r^2=0.81 vs 0.65, Figure IV.3). Weight and number were about the same for predicting midmorning water potential (r^2=0.57 vs 0.64, Figure IV.3). Weight was not as good as number for accounting for the variation in conductance (r^2=0.49 vs 0.82, Figure IV.3). The weight of the root excluding new tips was not related to any water relations variable on Day 31, nor was it a predictor of

the weight of new roots or the number of new roots
(r^2 less than .02).

Stomatal closure on day 31 had begun at predawn water
potentials as high as -0.5 MPa. Complete closure occurred
when predawn water potential reached -1.5 to -2.0 MPa, or
when mid-morning water potential ranged from -2.0 to
-2.5 MPa (Figure IV.4).

DISCUSSION

October lifting for Hood seedlings followed by long-term freezer storage resulted in low water potential and root production when tested in March. Water potential stayed consistently low even after conductance declined, demonstrating that either low conductance did not sufficiently limit water loss, or high root resistance to uptake. In contrast, post-storage physiology and root initiation of seedlings lifted in late fall were generally more favorable for potential growth.

Post-storage root production, changes in water potential, and patterns of conductance to water vapor, were similar between seedlings lifted in late fall (NOV) and spring (MAR). Root initiation and post-storage physiological responses may contribute to the rapid establishment of these seedlings. Scots pine (_Pinus sylvestris_ L.) seedlings that are fall-lifted and stored at -4°C or 2°C, have a higher photosynthetic capacity at planting initially, compared to seedlings that overwinter outdoors; but after 20 days in a growth chamber, there is no effect due to length of storage or date of lifting (Mattson and Troeng 1986).

Fremont seedlings had consistently higher root production and more favorable water relations than did Hood seedlings. Fremont seedlings tended to allocate more dry weight to the roots, relative to Hood (Chapter III).

This could be due to the preconditioning effects of differences in nursery bed culturing or to the environment before lifting.

Alternatively, differences in root production could represent ecophysiological adaptations related to seed source. Live fine root mass or root growth in coniferous forest species has been related to latitude (Vogt et al. 1986), altitude (Vogt et al. 1986), or cold temperatures (Turner et al. 1982), suggesting a higher allocation of carbohydrate to root growth for these environments. A drought-resistant provenance of Pinus pinaster Ait. seedlings has a higher rate of root elongation in a hydroponic system than a drought-sensitive one, in response to moisture stress (Nguyen and Lamant 1989), indicative of an adaptation to contrasting soil moisture environments. Ponderosa pine seedlings differ in RGP patterns due to both latitude and elevation of the seed source (Jenkinson 1980).

Root initiation was a significant predictor of water relations variables, and seedlings which produced new roots had a markedly different pattern of water potential and conductance over time (Figure IV.2). The positive effect of new root production on water relations in ponderosa pine was in agreement with other studies. New roots correspond to reduction in plant water flow resistance (Grossnickle and Blake 1985, Grossnickle 1988), increases in root hydraulic conductivity (Carlson 1986,

Colombo and Asselstine 1989), and increases in needle water potential (Nambiar et al. 1979, Rietveld 1986) and stomatal conductance (Grossnickle 1988). When grown hydroponically for 30 days, loblolly pine (Pinus taeda L.) families which produce the greatest number of new roots do not lose as much fresh weight as families that have fewer new roots (Rose and Whiles 1984).

It was difficult to determine whether new root production enabled favorable water relations, or whether favorable water relations and greater conductance allowed more roots to grow. Many factors influence stomatal opening, including light intensity, vapor pressure deficit, temperature, abscisic acid, and carbon dioxide concentrations (Lassoie et al. 1985); and the effect of roots on stomatal conductance is probably regulated by the interaction of these factors. Also, root water status and subsequent root activity may stimulate cytokinin metabolism in the root, which tends to increase stomatal opening (Schulze 1986). The fact that root initiation was related to conductance supported this idea.

Our data indicated, however, that root initiation allowed a return to more favorable water potential, which in turn allowed greater conductance. Conductance was high on day 1, and declined when needle water potential began to drop. Water potential became more positive and conductance was higher in seedlings that produced new roots. Furthermore, Fremont seedlings tended to have

higher water potential than Hood seedlings, and therefore higher conductance (Figure IV.1, IV.4).

Because new roots were initiated at low conductance (presumably low photosynthetic rates), root growth in ponderosa pine may occur at the expense of stored carbohydrates. Alternatively, new root growth may only require a low initial assimilation rate; however, in our study, all seedlings had relatively high conductance initially. Some of these seedlings later produced new roots and others did not. This would support the hypothesis that others factors (e.g., foliage translocated materials) besides carbohydrate status influence new root growth in conifers (Zaerr and Lavender 1970).

Root growth in Sitka spruce (Picea sitchensis (Bong.) Carr.) relies, at least initially, on stored carbohydrates (Philipson 1988). In contrast, new root growth in Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) depends on currently assimilated carbon for carbohydrate substrates (Marshall and Waring 1985, van den Driessche 1987, Philipson 1988) and would therefore be expected to require relatively high conductance. In both cases, new root growth could increase water uptake, leading to favorable conditions for conductance and carbon assimilation for increased root growth.

Our values for complete stomatal closure were in general agreement with those found by Lopushinsky (1969) and Running (1976). Our study showed that stomatal

closure was initiated at a much higher water potential (-0.5 MPa) than reported by Running (-1.8 MPa), possibly because other factors (e.g., low photosynthetic rates) were controlling stomatal closure. In addition, conductance declined in a linear fashion with decreasing water potential, as opposed to a threshold value for water potential initiating closure (Lopushinsky 1969, Running 1976). The different methods and test conditions used to evaluate stomatal closure make a comparison between our results and these studies difficult.

In summary, October lifting was detrimental for Hood seedlings, whereas measured physiological differences were minimal between seedlings lifted in November and freezer-stored and conventionally handled seedlings. The post-storage physiology of Fremont seedlings was generally different from that of Hood seedlings. The effect may have been due to differences in new root initiation, which in turn affected water relations and stomatal conductance.
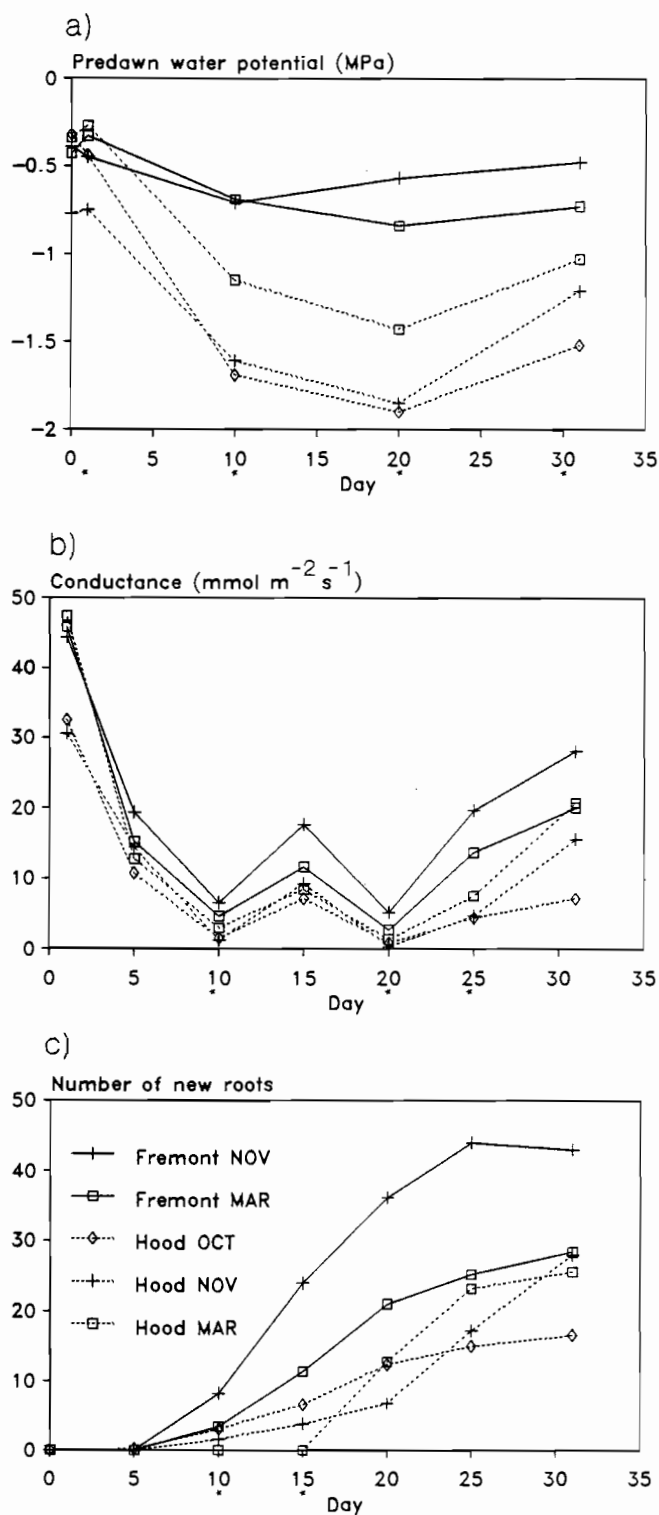
Figure IV.1. Mean a) predawn water potential, b) midmorning conductance to water vapor, and c) number of new roots, 0-31 days after planting. Asterisks below Day denote significant differences among treatments (p<0.05).
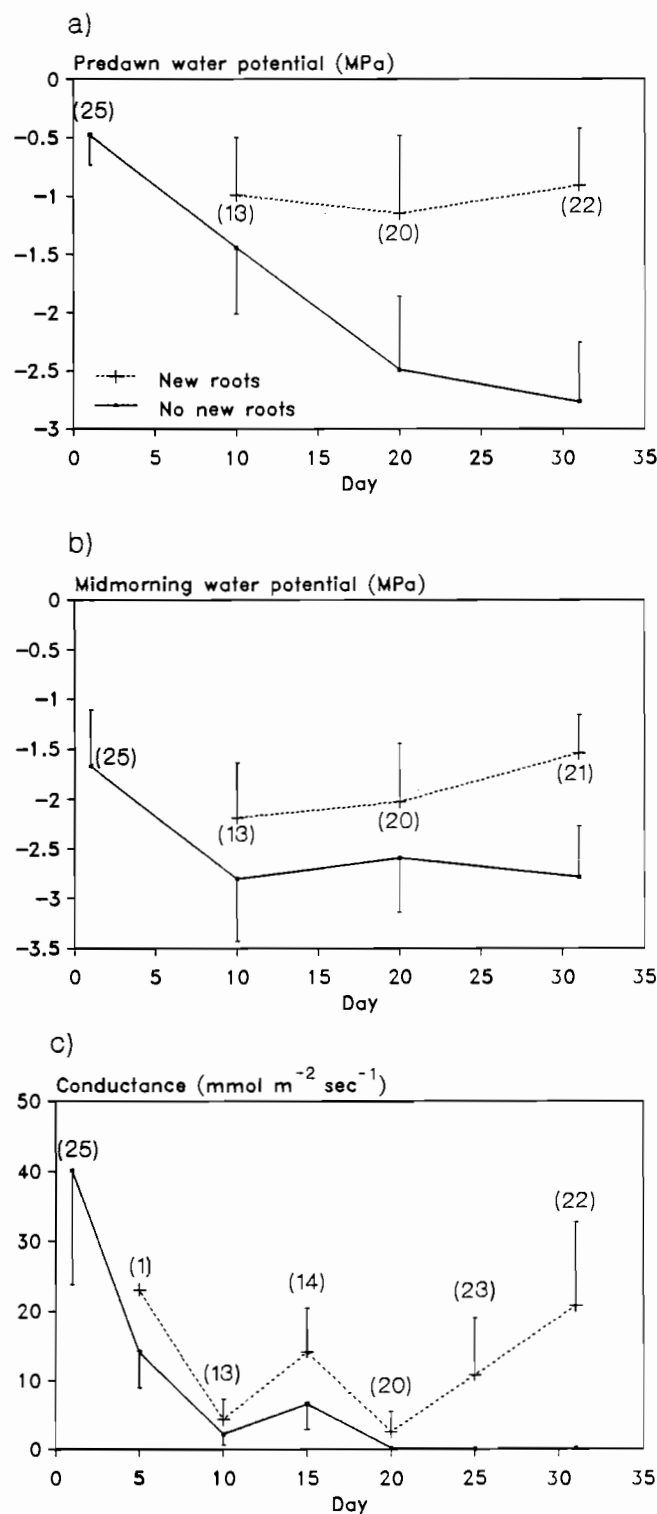
Figure IV.2. Mean a) predawn water potential, b) midmorning water potential, and c) midmorning conductance for seedlings with or without new roots. Numbers in parentheses are numbers of seedlings with new roots out of a total of 25 (after day 5). Bars=1 s.d. of the mean.
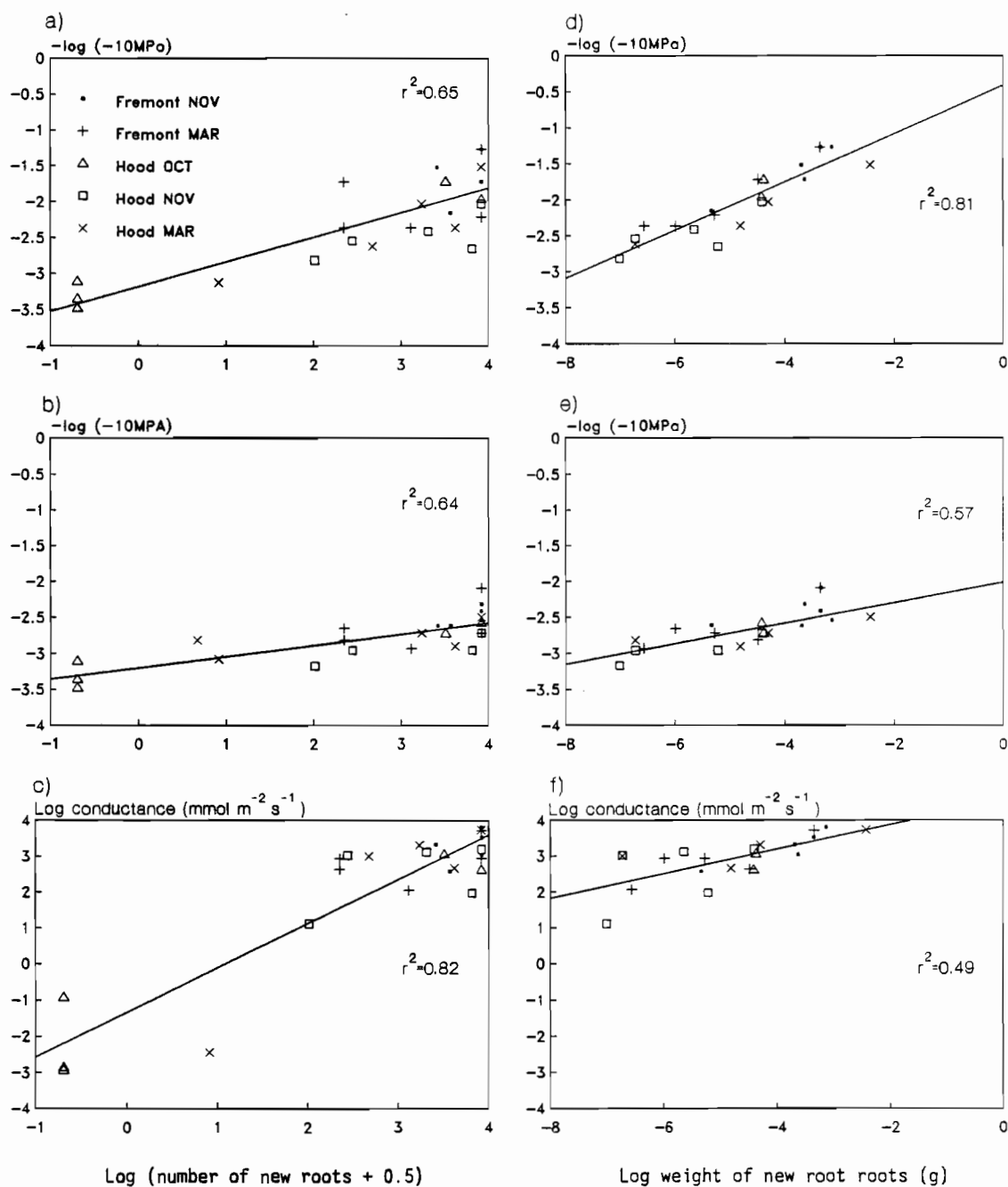
Figure IV.3. Number of new roots related to a) predawn water potential, b) midmorning water potential, and c) conductance, and weight of new roots related to d) predawn water potential, e) midmorning water potential, and f) conductance.
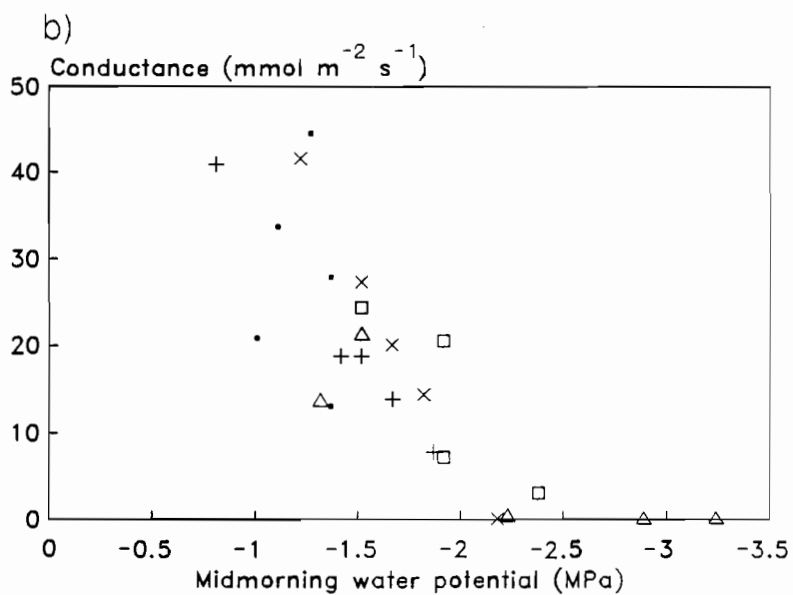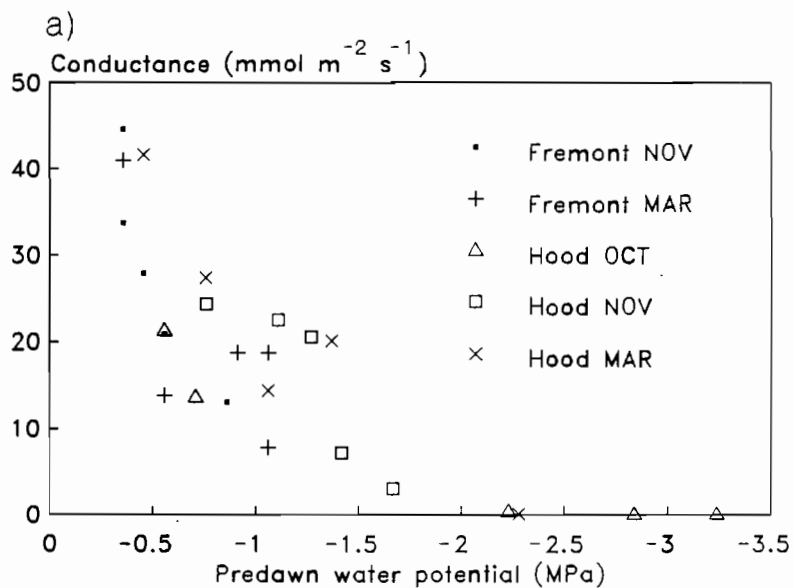
Figure IV.4. Conductance to water vapor versus a) predawn water potential and b) midmorning water potential on day 31.

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CHAPTER V

FALL LIFTING AND LONG-TERM FREEZER STORAGE AFFECT FRESH
WEIGHT, ROOT INITIATION, STARCH, AND FIELD PERFORMANCE

ABSTRACT

To determine the response of seedlings lifted in the
fall and stored below freezing until outplanting,
ponderosa pine (Pinus ponderosa Dougl. ex Laws.) seedlings
from two Oregon seed sources were harvested in September,
October, and November (stored below freezing until late
winter/spring planting), and compared with seedlings
lifted in March (stored above freezing until planting).
Growth potential at the time of planting was measured by
monitoring changes in root initiation, fresh weight, and
root starch concentration (% dry weight) and content (mg)
in a greenhouse. Field performance was determined by
measuring survival and growth at two field sites. Less
than 15% of the seedlings from the September lift and
store treatment initiated new roots, and fresh weight
decreased more than 30% during the greenhouse test.
Seedlings from this treatment had less than 25% field
survival in the first and second years after outplanting.
The late fall and spring-lifted treatments had greater
than 62% of seedlings with new roots, less than 14% loss
in fresh weight, and greater than 75% field survival.

In the greenhouse test, initial root starch
concentration was not significantly related to the weight

of new roots on day 30 (r^2=0.07, n=27), or days to root initiation (r^2=0.01, n=29). In the field, initial root starch concentration or content at the time of planting were not significantly related (r^2<0.07) to first-year height, growth, or fascicle length, but concentration and content were significantly related to first-year survival (r^2=0.21-0.22).

INTRODUCTION

Fall lifting and long-term storage increase harvesting options for nurseries where soil freezes in winter. Fall lifting and long-term storage are also favorable for seedling customers who have forest soils ready for planting before the nursery soil thaws or who have forest sites still covered with snow when seedlings are conventionally lifted. Low-temperature storage can help satisfy the chilling requirement necessary for release of seedlings from dormancy (Chapter I, van den Driessche 1977, Ritchie 1984, Ritchie et al. 1985). Below freezing temperature decreases plant respiration rate, and reduces storage mold associated with higher storage temperatures (Hocking and Nyland 1971, van den Driessche 1979).

The success of fall lifting and long-term storage depends on seedling physiology at the time of lifting. Storage can be unsuccessful if seedlings are lifted before the period of deep dormancy, when buds are not responsive to chilling (Ritchie and Dunlap 1980), or if seedlings have not achieved adequate resistance to stresses of handling and cold storage (Daniels and Simpson 1990). Seedlings in storage are in the dark at a constant temperature, and the temperature may be too cold to adequately satisfy the chilling requirement; and seedlings may experience desiccation, and lose frost and drought

resistance (Lavender and Wareing 1972, Ritchie 1982, Ritchie 1986a, Faulconer 1988). Furthermore, carbohydrate reserves decline during long-term storage (Chapter III, Ritchie 1982, Cannell et al. 1990), possibly leading to reduced root initiation, lower seedling survival, and poor growth (Duryea and McClain 1984).

Although previous attempts at fall lifting and long-term storage of western conifers were not successful (Hermann et al. 1972), the practice has now become common in the Pacific Northwest for a variety of species (Hee 1986, Daniels and Simpson 1990). Few studies have examined the response of ponderosa pine (Pinus ponderosa Dougl. ex Laws.) to fall lifting and long-term freezer storage (Morby and Ryker 1979). The objectives of this paper were to determine the effect of fall lifting and overwinter freezer storage on ponderosa pine: 1) field performance potential, by monitoring changes in fresh weight and root initiation in a greenhouse, and 2) field performance, by evaluating survival and growth of outplanted seedlings. Additionally, we hypothesized that low root starch concentration after storage would decrease root initiation under controlled conditions, and decrease survival and growth in the field.

METHODS

Seedling materials

The Bend Pine Nursery, Oregon (44° 5' N, 121° 16' W; elevation, 1100 m) was selected because desiccating east winds, snow, and frozen soils prevent harvest in winter. Fall lifting and long-term storage had not been attempted at the nursery.

Two-year-old seedlings from two Oregon seed sources (Barlow Ranger District, Mount Hood National Forest (1100 m) and Lakeview Ranger District, Fremont National Forest (1800 m)) were grown in different beds at the nursery under standard cultural practices until the time of lifting.

Lifting and storage

Seedlings were shovel-lifted on September 22 (SEP), October 20 (OCT), and November 17 (NOV), 1987, and March 1, 1988 (MAR) after 66, 315, 640, and 2,545 chilling hours ($<5°C$ since early September, Chapter I).

Second-year height growth had been completed by SEP (Chapter III). All fall-lifted seedlings were in varying degrees of dormancy, with SEP seedlings being close to maximum rest, and OCT and NOV seedlings advanced slightly further through the dormancy cycle (Chapter I). Plant development progressed with additional storage chilling (Chapter I). Seedlings lifted later in the fall were more frost-hardy than earlier lifted seedlings; however, even

SEP seedlings were able to withstand temperatures down to -13°C with no visible damage (Chapter I). MAR seedlings were quiescent at the time of lifting, and flushed rapidly in a favorable environment (Chapter I).

The fall-lifted seedlings were stored at -1.5°C and the March lifted seedlings were stored at 2-4°C in Corvallis, Oregon, until the root initiation and outplanting experiments described below. A freezer malfunction on October 10-11 affected only the SEP seedlings. Room air temperature dropped at a rate of 1.1°C per hour, reaching a minimum of -7.2°C (minimum inside bag temperature -2.7°C), and rose to -1.6°C at a rate of 1.4°C per hour. Fourteen hours later, room temperature dropped 0.7°C per hour to a minimum of -11°C (minimum inside bag temperature -6.9°C), and increased 1.2°C per hour to -1.6°C.

Greenhouse root initiation experiment

The greenhouse test began 5 days after planting at Bend (see below) and lasted for 30 days. Seedlings for the greenhouse test and representing the fall lift dates were removed from the storage freezer on March 16, 1988, and transferred to the cooler. On March 21, these seedlings, plus the MAR trees stored in the cooler since March 2, were removed from the storage boxes and placed in water-filled buckets.

Subsequently, any white root tips (and a lateral root

segment about 3 to 4 cm [roughly 62 mg dry weight] for starch analysis) were removed; seedlings were blotted dry and measured for fresh weight (g), height (cm), and stem diameter (mm). Root volume (cm^3) was measured by displacement of water (Burdett 1979). Seedlings were then placed in 37.8-L tanks (painted on the outside to prevent light transmission) filled with water and a commercial fertilizer (0.26 g L^{-1} of 15-30-15). An air pump attached to a bubble wand, extending along the base of the tank, provided aeration. Tanks were fitted with a template to contain 20 seedlings per tank, and seedlings were held in place by foam plugs.

A total of 320 seedlings in 16 tanks were used (2 seed sources x 10 seedlings x 4 blocks x 4 lift dates). The 10 seedlings representing each seed source x nursery block x lift date combination were assigned to each half of a tank in a completely randomized design.

Air temperature (generally, day, 21-24°C; night, 17-19°C); pH, 6.5-6.9; and water temperature, 14-24°C) were monitored over the 30-day test period. Oxygen concentration of water was measured with a Yellow Springs Instrument (Yellow Springs, OH) dissolved oxygen meter. Oxygen concentration in the root zone directly above the bubble wand was 8.5 ppm (oxygen saturation=9.0 ppm) and ranged from 6.5-7.4 ppm in the adjacent spaces. Natural light was supplemented by 300-watt incandescent light bulbs suspended about 0.5 m above the seedling tops

(30-70 μmol m^{-2} s^{-1}), extending the daylength to 16 h. Water and fertilizer were changed weekly, and seedling tops were misted up to four times daily.

Presence of new root tips (new roots present vs absent) on each individual seedling was measured every 5 days, fresh weight was measured every 10 days, and height and stem diameter were measured after 30 days. Seedlings were divided into root and shoot, and roots were separated into new root tips and old roots. Root fractions were immediately frozen in dry ice, placed in a -18°C freezer, and subsequently dried at 70°C in preparation for starch analysis.

Outplanting at Bend Pine Nursery

The planting site had never been used for tree production, but it had been planted in rye (Secale sp.) in September of 1987 and was destined for 1988 1+1 transplants. The site was plowed and harrowed in February, 1988. Ammonium sulfate (21-0-0, 112 kg ha^{-1}) and triple superphosphate (0-45-0, 140 kg ha^{-1}) were incorporated into the soil on March 10 and 11, 1988.

On March 11, 1988, fall-lifted seedlings were removed from the freezer and placed in a cooler in Corvallis. On March 16, seedlings were transported to the nursery. Seedlings were shovel-planted at 0.76 x 0.76 m spacing in a randomized complete block design with 8 treatments (2 seed sources x 4 lifting dates) and 4 blocks, on March 16

(blocks 1 and 2) and 17 (blocks 3 and 4). Air temperature during planting was 10-13°C; soil temperature at 25 cm depth was 5°C.

Twenty seedlings were planted as two 10-tree rows for each treatment x block combination, for a total of 640 trees. A row of buffer trees surrounded each block. Seedlings were watered immediately after planting, and the site was handweeded continuously throughout the next two growing seasons. Seedlings were watered as often as the adjacent transplant stock.

Starch, dry matter partitioning, and root initiation

To determine morphology and starch at the time of planting, a subsample of trees (10 per seed source, nursery lift date, and replication) representing the fall lift dates were immediately frozen on dry ice simultaneous with planting. These were stored at -37°C, and subsequently rinsed with water, roots pruned to 25 cm, measured for height and stem diameter, weighed after drying at 70°C, and then stored with a desiccant at -18°C. Root, stem, fascicle, and total needle weight were determined on 5-10 seedlings per replication. Approximately 40-50 fascicles were removed per seedling to get an estimate of individual fascicle weight.

During the first growing season, outplanted trees were also harvested three times: at 4 weeks (3 trees per treatment per block, 12 trees total per seed source and

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nursery lift date), and at 10 and 28 weeks (2 trees per treatment per block, 8 trees total per seed source and nursery lift date) from planting. These were handled similar to those sampled at planting. Data for the first harvest (4 weeks) were not shown because of similarity in response to the harvest at planting. The second harvest (10 weeks) occurred before budbreak (Chapter I), and the third harvest was at the end of the growing season. To get an estimate of root initiation in the field, seedling root systems were observed at harvest and categorized into two root classes: those with new root tips, and those without. All sampling took place before 0900-1000 h.

Field performance

Seedlings were measured for initial height (cm) one week after planting. Height, growth, and survival (% alive) were subsequently measured for two years after planting, at the end of each growing season. End of season measurements also included current season fascicle length (mm) and stem diameter (mm). Hood seedlings were also planted at a site within their own seed zone (see below); Fremont seedlings were only planted at Bend.

Outplanting at Mt Hood

This site was tractor-logged in 1986 and burned in the fall of 1987. The unit had a south exposure with 0 to 25% slope (0% at the planting site), and an elevation of 1,036 m. The Douglas-fir site index was 80 (site

class V). Annual precipitation is 125 cm per year.

Hood seedlings in freezer storage were transferred from the freezer to the cooler on April 14, 1988. Four days later, these seedlings, plus the March-lifted ones stored in the cooler, were transported to a cooler (2-4°C) at the Bend Nursery for temporary overnight storage. On April 19, the seedlings were taken to the Hood site and planted. Conditions during planting included rain, 9-11°C air temperature, and 7-8°C soil temperature. Seedlings were shovel-planted at 1.2 x 1.2 m spacing in a randomized complete block design with 4 treatments (1 seed source x 4 lifting dates) and 4 blocks. Fifteen seedlings were planted in a row for each treatment x block combination, for a total of 240 trees. A row of buffer trees surrounded each block. Height and survival were measured for two years. At the end of the growing season, fascicle length of current year's growth was measured, and at the end of the second year, stem diameter was also measured.

Starch analysis

After dry weights were determined, tissues were stored with a desiccant at -18°C, ground in a Wiley Mill (420μ screen), and stored with a desiccant at -18°C until chemical analysis. Root samples on day 0 of the greenhouse root initiation experiment were bulked (10 per replication and treatment) as were the seedlings sampled

at the time of outplanting (5 per replication and
treatment). All other samples destined for starch
analysis were kept separate by seedling. For the
greenhouse test, root tissue was analyzed. There was
insufficient mass to analyze starch in the new roots after
30 days.

For the outplanting experiment, all tissue components
(root, stem, needle) were analyzed at the time of
planting; roots were analyzed for all subsequent harvests
and treatment combinations; stems were analyzed for NOV
and MAR seedlings (4 weeks from planting--Fremont and
Hood, 10 and 28 weeks from planting--Fremont only); and
needles were analyzed for Fremont NOV and MAR seedlings.

Ground material was extracted with
methanol:chloroform:water (3 times for root tissues, 4
times for stems and fascicles), and then analyzed for
glucose concentration after incubation with a combination
of amyloglucosidase and α-amylase enzymes (Chapter II,
Rose et al. 1990). Each sample was analyzed twice.
Glucose concentration was corrected to starch
concentration (% dry weight) using 0.9 as a hydrolysis
factor (glucose x 0.9, Volenec 1986). Starch content was
calculated by multiplying dry weight x starch
concentration. Starch content in leaf tissue was
expressed as μg starch per fascicle and mg starch in all
needles per tree.

Statistical analyses

Greenhouse root initiation experiment

To determine if morphology, root initiation, or root starch varied significantly by seed source or lift date, greenhouse data (fresh weight, root volume, height, stem diameter, percent with new roots, root dry weight, days to root initiation, root starch concentration on day 0) were analyzed by day as a 2 (seed source) x 4 (lift and store) factorial experiment with 4 replications in a completely randomized design. The starch data required a log transformation, and the means presented were re-transformed from the log. The analysis of variance had the form shown in Appendix CV.1. Fisher's protected LSD was used to separate the means for those responses with equal number of observations per mean. A least squares means procedure (Searle et al. 1980) was used to separate means (new root dry weight, days to root initiation) having unequal number of observations.

To determine how fresh weight changed over time, the data were averaged by seed source or lift date (nonsignificant interaction for any day) and plotted to observe trends. The change in fresh weight from day 0 to day 10 was analyzed as a split plot design with seed source and lift date as the main plot, and day as the sub-plot (Appendix CV.2).

The change in fresh weight from day 10 to day 30 was analyzed with a repeated measures design. The test for

the Huynh-Feldt (1970) condition was significant (chi-square=12.37, 2 df, p=0.0021), suggesting the criterion of independence may be violated; however, the Greenhouse-Geisser (1959) adjustment for the violation showed that it was not altering the conclusions differently from an analysis assuming independence. Nonetheless, the Greenhouse-Geisser adjustment was used to adjust the degrees of freedom in the following analysis.

Contrasts associated with the linear and quadratic terms of a polynomial equation were computed for seed sources or lift dates. These contrasts were used to test the hypothesis that the linear or quadratic relationship between fresh weight and day were not significantly different from 0 for the seed source and lift date treatments.

Because each seedling had been measured for the presence of new roots on day 30, the data were analyzed as a split-plot design with seed source and lift date as the main plot, and presence of new roots as the sub-plot (Appendix CV.3). Both the concentration and content data required log transformations to normalize the error. Mean separation of the transformed data was determined by a least squares means procedure (Searle et al. 1980).

To determine the relationship between initial root starch and subsequent growth of new roots, replication means (n=32 maximum) were plotted and regressed between starch and percent of seedling with new roots, dry weight

of new roots, and days to root initiation. Initial root starch concentration required a log transformation to satisfy assumptions of regression.

Outplanting at Bend Pine Nursery

Starch, dry matter partitioning, and root initiation:

The starch (Appendix CV.4) and dry matter (Appendix CV.5) data were analyzed by harvest date as a randomized complete block design with 4 replications. The starch data required a log transformation, and the means presented were re-transformed from the log. A least squares means procedure (Searle et al. 1980) was used to separate the means.

There were too many missing cells in the analysis of variance to determine if there were seed source or lift date differences in starch between seedlings with or without new roots after planting; thus, the data were collapsed and treated as completely randomized design with root category (with or without new roots) as the only treatment.

Because of the small sample size associated with the number of seedlings with new roots after planting, an analysis of contingency tables was performed on the counts, by harvest date (Everitt 1977). The null hypothesis was that there was no association among presence of new roots, seed source, and lift date. If the null hypothesis of mutual independence was rejected, then

hypotheses of partial independence were performed to determine the nature of the association.

Field performance:

Field data at Bend were analyzed as a 2 (seed source) x 4 (lift date) factorial in a randomized complete block design with 4 replications (Appendix CV.5). For the Mt Hood site, data were analyzed as a randomized complete block design with 4 (lift date) treatments and 4 replications (Appendix CV.6). A least squares means procedure (Searle et al. 1980) was used to separate the means.

Root initiation and starch as predictors

To determine the relationship between greenhouse root initiation and field performance, or between initial root starch and field performance, seed source x lift date x block means of the field response variables were regressed against decline (%) in fresh weight from day 0 to day 30, seedlings (%) with new roots on day 30, weight of new roots and root weight (total) on day 30, and initial root starch (log transformation) at planting.

RESULTS

Greenhouse root initiation experiment

Morphology and root growth

At day 0 and day 30, there were no significant interactions between seed source and lift date for all variables measured, indicating that the beginning and final morphology of the two seed sources were affected in the same way by the lift and store treatments. At day 0, Fremont trees were 16% lighter, 36% shorter, and 11% greater in root volume, compared to Hood seedlings (Table V.1, Figure V.1).

During the first ten days, fresh weight declined 17 and 21% for Fremont and Hood seedlings ($p<0.01$, Figure V.1), respectively. From day 10 to day 30, linear effects for seed sources were highly significant ($p<0.01$; quadratic effects nonsignificant), indicating that the slope for the linear relationship between seed source and time was significantly different from 0. Fremont seedlings increased linearly (6%) in fresh weight from day 10-day 30, while Hood seedlings decreased (4%). As a result, fresh weight differences between Fremont and Hood seedlings became nonsignificant on day 30 (Figure V.1).

Fremont consistently had a greater percent of seedlings with new roots (15-70%) compared to Hood (2-43%) for all days (Figure V.1). After 30 days, height and stem diameter rankings between seed sources remained similar to

those at day 0 (Table V.1). Fremont trees had more than
1 1/2 times the percentage of seedlings with new roots,
and double the average dry weight of new roots per
seedling, but the latter difference was not significant.

On day 0, MAR trees had the greatest fresh weight,
root volume, and stem diameter, but the lift dates were
all equal in height (Table V.1, Figure V.1). The decline
in fresh weight during the first 10 days was similar (17-
23%, p<0.01) to seed source losses (Figure V.1).

Quadratic effects for lift dates from day 10 to day
30 were nonsignificant, with linear effects depending on
lift date. SEP seedlings declined 11% in fresh weight;
OCT did not change; and NOV and MAR increased 7 and 5%,
respectively (Figure V.1).

From day 10 to day 30, the percent of seedlings with
new roots was greatest for NOV and MAR (62.5-82.5%),
followed by OCT (35-47.5%), then SEP (11.2-15%, Figure
V.1). By day 30, the most obvious change in rankings
among lift dates was the small stem diameter, and old-root
dry weight for SEP seedlings (Table V.1). New-root dry
weight of OCT, NOV, and MAR seedlings were four to five
times greater than SEP, but the difference was not
significant. Days to root initiation were not affected by
either seed source or lift date (Table V.1).

Starch

On day 0, there were large differences in root starch

concentration (Figure V.2). MAR seedlings had nearly 32
times the starch concentration, relative to the fall-
lifted seedlings (1.60 vs 0.04-0.05%). By day 30, the
ranking among lift dates was the same, but the relative
difference between lift dates was greater for Fremont
seedlings, resulting in a seed source x lift date
interaction (Figure V.2). At the end of the experiment,
Fremont seedlings lifted in OCT or MAR had nearly double
the root starch concentration relative to Hood (0.11% vs
0.04%, 0.97% vs 0.52%, p<0.01), but the differences
between seed sources for the other lift dates were
nonsignificant (p>0.05). The results for root starch
content were very similar to that found for concentration
(data not shown).

Seedlings with new roots had nearly 3 times the
starch concentration than seedlings without new roots for
MAR (1.03 vs 0.38), but the difference was not significant
between the root classes for the other lift dates (Figure
V.4); however, the trend was in the same direction (root
starch concentration greater in seedlings with new roots).
On the average, root starch content in seedlings with new
roots (3.6 mg) was significantly greater than in seedlings
without new roots (1.0 mg).

Initial root starch concentration was not
significantly related to the weight of new roots on day 30
(r^2=0.07, n=27) or days to root initiation (r^2=0.01,
n=29). Initial root starch concentration accounted for

about 36% of the variation in the percent of seedlings with new roots (p<0.01, Figure V.4).

Outplanting at Bend Pine Nursery

Starch

Root starch concentrations were generally low (<2.1%) after storage (Chapter III), increased to a peak (4-8%) 10 weeks from planting, and then declined at the end of the first growing season (1.5-2.2%, Figure V.5). Exceptions to this included a gradual increase of SEP (Fremont and Hood) and OCT (Hood only) seedlings to a maximum at the end of the season. Initial starch concentration differences among the nursery lift date treatments were minimized at the end of the season, and Fremont seedlings tended to have greater starch concentration than Hood seedlings (Figure V.5).

Root starch content roughly followed the same pattern as concentration for Fremont seedlings, except that NOV seedlings tended to have the greatest, but not statistically different, content (264 mg vs 130-144 mg) 28 weeks from planting (Figure V.5). In contrast to concentration patterns, root starch content in Hood seedlings reached a maximum (119-213 mg) at the end of the growing season. The trends in stem and needle starch concentration and content were similar to the differences among treatments for root starch (Table V.2, V.3).

Four and 10 weeks from planting, seedlings with new

roots had 4-67, and 4-124 times the root starch concentration and content, respectively, than seedlings without new roots (Table V.4). At the end of the season, there was no difference. In contrast, with a limited sample there was little difference in stem or needle starch between seedlings with or without new roots (Table V.4).

Dry matter partitioning

Through the first 10 weeks after planting, Fremont seedlings were lighter in stem weight (27-33%), greater in root weight (16-52%), fascicle weight (24-31%) and root:shoot dry weight ratio (25-68%), and not significantly different in total needle weight or shoot weight, compared to Hood seedlings (Tables V.6, V.7). These differences were generally maintained through the end of the growing season, with the exception that individual fascicle weight was not affected by seed source 28 weeks from planting (Table V.6).

Initially, MAR seedlings tended to have the greatest weight in tissue components and SEP seedlings the lightest (Table V.6); however, by the end of the season, NOV seedlings tended to have the greatest (but not always statistically different) weight among the lift dates, followed by MAR, OCT, and SEP.

Root initiation

Four and 10 weeks from planting, the null hypothesis

of mutual independence was rejected (p<0.01, Table V.7) for the number of seedlings with new roots, indicating that root activity was affected by seed source, lift date, or the interaction. Further tests of partial independence and examination of means suggested that NOV and MAR tended to have more seedlings with new roots than would be expected under independence, while the number of seedlings with new roots was less than expected for SEP and OCT. An exception was for Fremont OCT, where the number of seedlings with new roots tended to exceed the expected.

At the end of the first growing season (28 weeks), the test of mutual independence for the number of seedlings with new roots was not rejected (Table V.5), indicating that there was no significant difference between seed sources or among lift dates for new root activity.

Field performance

At the end of the first growing season at Bend, Fremont seedlings had 1.6 times greater the survival and similar fascicle length, compared to Hood (Table V.8). First-year growth was not affected by seed source for any given lift date, with the exception of SEP, where Hood growth was nearly 4 times greater than Fremont (p<0.01, Table V.9). Second-year results were similar to the first, with exception that there was no significant interaction between seed source and lift date for growth

(36% less for Fremont) and stem diameter (no difference between Fremont and Hood).

There were strong lift date differences in field performance (Table V.9). Most survival and growth responses were in the order NOV > MAR = OCT > SEP. OCT seedlings tended to have fewer surviving seedlings and less growth than MAR, but the differences were generally not significant.

For Hood seedlings planted at Mt Hood, SEP and OCT responses were similar to that found at Bend (Table V.10)--these lift date treatments had the poorest survival and growth for two years. The NOV and MAR seedlings had the greatest survival and growth and were not statistically different for any of the field performance responses; however, in contrast to the Bend results, MAR seedlings tended to have slightly better survival, growth and fascicle length relative to NOV.

Root initiation and starch as predictors

The best predictors (greenhouse data) of first-year survival at Bend were decline (%) in fresh weight (day 0 to day 30), and seedlings (%) with new roots on day 30 ($p<0.01$, Figure V.6), accounting for 74% and 69% of the variation in survival. The weight of new roots and total root dry weight on day 30 accounted for 16% and 32% ($p<0.01$) of the variation in first-year survival, while days to root initiation was unrelated ($p=0.55$).

Greenhouse variables were generally poor predictors of first-year growth at Bend, explaining less than 29% of the variation in first-year growth. Decline (%) in fresh weight and seedlings (%) with new roots on day 30 had the strongest correlations with growth (Figure V.6). The weight of new roots on day 30 (r^2=0.15, p=0.05), days to root initiation (r^2=0.02, p=0.38), or total dry weight (r^2=0.08, p=0.12) were not strongly related to growth.

Initial root starch concentration or content at the time of planting were not significantly related (r^2<0.07) to first-year height, growth, or fascicle length. Concentration and content were significantly (p<0.05) related to first-year survival, accounting for 21% and 22% of the variation, respectively.

DISCUSSION

Field performance and root initiation

Early fall lifting was detrimental to field
performance. The detrimental effect of early fall lifting
and storage on field performance agreed with results in
ponderosa pine (Stone and Schubert 1959) and other conifer
species (Hermann et al. 1972).

The poor survival and growth of the SEP seedlings
could be due to a variety of reasons. The initiation of
stem units and needle primordia laid down in the winter
bud for spring growth (Lanner 1976) may have been
disrupted by early fall lifting. At the time of lifting,
SEP seedlings were in a phase of rest (Chapter I), when
coniferous seedlings have not acquired sufficient
hardiness and stress resistance (Hermann et al. 1972,
Lavender 1985, Ritchie 1986b). The stress could be
lifting, handling, freezing damage associated with
mechanical breakdown (Chapter I), desiccation during
storage, and the interaction of these factors.

The freezer malfunction that affected only SEP
seedlings was probably a minimal influence on poor SEP
performance. The Hood seedlings had already begun to
acclimate by mid-September, and were not visibly damaged
by controlled freezing down to -13°C (Fremont seedlings
were probably more cold-hardy than Hood seedlings for SEP,
Chapter I). This temperature was lower than the minimum

recorded during the malfunction. Furthermore, the rate of decline and rise in temperature, known to affect photosynthetic recovery in ponderosa pine (Pharis et al. 1970) and degree of cold injury (Glerum 1985), was very gradual. This did not rule out the possibility that significant deacclimation occurred during storage (Chapter I, Ritchie et al. 1985, Faulconer 1988) so that seedlings were damaged when the malfunction occurred.

The poor field performance of SEP seedlings appeared to be related to the poor root initiation measured in both the greenhouse and the field. Less than 20% of the seedlings from SEP initiated new roots in the greenhouse; similarly, SEP generally had the lowest root initiation in the field site at Bend. When root growth is poor, water uptake is limited (Chapter IV, Rietveld 1989). This was morphologically expressed as a continuous decline in fresh weight in the greenhouse, and physiologically resulted in high plant moisture stress and low stomatal conductance (Chapter IV).

In contrast to the poor performance of early fall-lifted seedlings, the survival and growth of NOV seedlings were as good or better than conventionally handled stock (MAR). NOV seedlings, especially, consistently ranked among the highest in dry matter accumulation, survival, and growth, irrespective of seed source or planting site. Seedlings lifted later in the fall most likely had achieved a greater degree of cold hardiness and stress

resistance, enabling them to endure long-term freezer storage. OCT seedlings, on the other hand, had slightly depressed field responses relative to NOV and MAR, which may be related not only to lower stress resistance, but also to inadequate fulfillment of the chilling requirement (Chapter I). The field performance of fall-lifted and stored stock can be comparable to spring-lifted stock for several conifers (Mullin and Bunting 1972, Morby and Ryker 19979, Hinesley 1982, Ritchie et al. 1985, Tung et al. 1986).

The underlying reasons for the more favorable field responses were associated with greenhouse root initiation and fresh weight changes, as well as field root initiation. In the greenhouse, OCT seedlings had intermediate root initiation, conciding with a non-significant change in fresh weight from day 10 to day 30. Up to 10 weeks in the field after planting, OCT seedlings were also intermediate in the percent of seedlings with new roots, depending on seed source. NOV and MAR, however, had the greatest greenhouse root initiation, and were the only 2 treatments that increased significantly in fresh weight. This suggested that greater root initiation led to greater water uptake and hence increase in fresh weight.

In fact, root initiation (percent of seedlings with new roots on day 30 of the greenhouse test) was a significant predictor of the percent change in fresh

weight (p=.0001, r^2=0.76, n=32)--treatments with poor root initiation lost the most fresh weight. This was similar to loblolly pine (Pinus taeda L.), where families that produce a large number of new roots in a 30-day hydroponic test do not lose as much fresh weight as those with fewer new roots (Rose and Whiles 1984).

The high correlation between first-year survival and percent change in fresh weight (Figure V.6) was probably related to the high correlation between new root initiation and change in fresh weight. New roots correspond to reduction in plant water-flow resistance (Grossnickle and Blake 1985, Grossnickle 1988), and increases in root hydraulic conductivity (Carlson 1986, Colombo and Asselstine 1989), needle water potential (Chapter IV, Nambiar et al. 1979, Rietveld 1986), and stomatal conductance (Chapter IV, Grossnickle 1988). Water stress in newly planted seedlings is alleviated by the production of new roots (Blake and Sutton 1987).

The effect of seed source could also be related to relative stress resistance, cold hardiness, and root initiation. Compared to Hood, Fremont survival was 60-78% greater at Bend in the first two years after outplanting. Fremont tended to have a higher degree of cold hardiness at the time of lifting (Chapter I); Fremont was also significantly greater in root initiation throughout the entire greenhouse test, corresponding to a significant increase in fresh weight while Hood decreased. The

difference between seed sources in root initiation could be related to differences in preconditioning (e.g., nursery bed effects), or adaptations related to seed source. The higher altitude Fremont seedlings may allocate more resources to root growth (Turner et al. 1982, Vogt et al. 1986) compared to Hood (Chapter III). Root volume differences before planting (11% greater for Fremont) was more evidence for this hypothesis.

Field performance, root growth, and starch

In contrast to root initiation, root starch was not a strong predictor of field survival or growth. Ronco (1973) and Ritchie (1982) also found that carbohydrate reserves were not strongly related to field performance, although carbohydrate reserves have been qualitatively linked to field performance (Hellmers 1962, Winjum 1963, Puttonen 1980). No research has definitively supported the use of carbohydrate reserve as a predictor of seedling quality (Duryea and McClain 1984, Loescher et al. 1990).

The poor correlation may be related to the poor correlation between initial root starch and subsequent root growth (weight of new roots, days to root initiation). Although initial root starch concentration was significantly related to the percent of seedlings with new roots (Figure V.5), the relationship was likely to be weaker because the spread in the data for each treatment was limited. The positive relationship appeared to be

strongly influenced by the grouping of MAR points and the grouping of SEP-OCT-NOV points, together which may cause a spuriously large correlation.

Carbohydrate reserves and new root growth are often poorly related (van den Driessche 1978, Ritchie and Dunlap 1980, Ritchie 1982, Rose and Whiles 1984, McNabb 1985, Reid 1986, Cannell et al. 1990, Deans et al 1990). Current photosynthate (Marshall and Waring 1985, van den Driessche 1987) or other shoot factors (Zaerr and Lavender 1970) may control new root growth in conifers, although there are likely to be differences in species response (Philipson 1988).

Other factors which could influence root growth include auxin or other plant growth regulators. Auxin stimulates root primordia in tree roots (Coutts 1987). In ponderosa pine, exogenous applications of auxin to seedlings positively affected new root growth, but not the elongation of old roots (Zaerr 1967); however, Lavender and Hermann (1970) could find no positive effect on root growth from external application of growth regulatory compounds to Douglas-fir. They concluded that a translocatable substance from foliage was necessary for root growth. Zaerr and Lavender (1970) concluded that the substance controlling root growth was not carbohydrate alone.

In our study, root growth occurred even though initial starch concentrations were close to zero,

suggesting that current photosynthate may be more important for new root growth; however, we also found that root growth occurred when stomatal conductance was low (Chapter IV), which might suggest that root growth occurred at the expense of stored carbohydrates. The discrepancy in conclusions may be related to the mobilization of sugars as stored reserves. Sugars can comprise a large fraction of the total nonstructural carbohydrate pool (McCracken 1979, Ritchie 1982).

The lack of a strong relation between root starch and subsequent root growth or seedling survival did not mean that starch reserves were unimportant. In contrast to our results, root starch concentration at lifting in slash pine (Pinus elliottii var. elliottii (Engelm.)) is significantly related ($r^2=0.59$) to first year growth (McNabb 1985). Starch in needles and fine roots can reach 25-30% of the dry weight in conifers (Ericsson 1979, Ericsson and Persson 1980), and may supplement spring root growth in sugar maple (Acer saccharum, Wargo 1979). The hydrolysis of starch to sugar may prepare cells for osmotic adjustment during water stress (McNabb 1985) and increased sugar content during the winter may be physiologically related to cold acclimation (Sakai and Yoshida 1968, Yamashita 1990).

Yet, weak quantitative relationships between starch and growth shows that the specific role of root carbohydrates in tree development are not completely

understood (Loescher 1990). Carbohydrate reserves, in general, are used in respiration associated with growth, but only a small fraction is actually mobilized and translocated to new growth (Glerum 1980). Carbohydrate reserves may be more critical when site conditions after planting (e.g., drought stress) prohibit photosynthesis (McNabb 1985, van den Driessche 1987).

Although of poor predictive ability, root starch was associated with new root activity in both the greenhouse test and in the field. Others have found that high root activity and starch accumulation are associated (Shiroya et al. 1966, Kreuger and Trappe 1967). Furthermore, starch accumulation patterns during the first growing season paralleled root initiation activity by seed source. SEP seedlings had poor root initiation and low starch up to the period before budbreak; NOV and MAR had high root activity and subsequently accumulated starch; OCT for Fremont accumulated starch (high root initiation) but remained low for Hood (low root initiation).

Accumulated starch after planting, therefore, indicated a functioning root system; a functioning root system led to increased water uptake, stomatal conductance (Chapter IV), and photosynthesis; these responses were indicative of functioning metabolic pathways that support photosynthesis and root respiration, providing the downward translocation of photosynthate for more root growth (Ritchie and Tanaka 1990).

High starch concentrations or content are not necessarily an indicator of seedling vigor. Starch accumulates when photosynthesis exceeds respiratory requirements. Factors that increase photosynthesis, decrease growth, or decrease maintenance respiration, lead to accumulation (Marshall 1985). For example, under slight moisture stress, growth is slowed relative to photosynthesis and starch concentration may increase.

Seasonal and partitioning patterns of starch accumulation

Seasonal patterns of starch were similar to what others have found for starch or nonstructural carbohydrates (Kreuger and Trappe 1967, van den Driessche 1978, Ritchie 1982, McNabb 1985, Cannell et al. 1990). Carbohydrate reserves, primarily starch, accumulate when photosynthate production exceeds demands by various sinks (Ericsson 1979). Starch concentration increases to a maximum in the spring before budbreak, and then declines during periods of high growth rate (Waring and Schlesinger 1985) or increased sink demand (e.g., in response to fertilization, Ericsson 1979).

The partitioning of starch among the various tissues was consistent throughout the growing season with roots having the greatest starch concentration and needles the least. This pattern was also observed before the seedlings were stored (Chapter III). The relative allocation between shoots and roots may be related to leaf

starch accumulation (Huber and Israel 1982). Low starch in needles reflects the transitory nature of chloroplast starch (Preiss 1982), the possible inhibitory effect of starch on photosynthesis (Nelson and Spollen 1987), and season. Low starch in needles during the fall may represent increased translocation of photosynthate to roots (Shiroya et al. 1966, Little 1970).

Seedlings exposed to extended periods of cool dark storage require a post-storage recovery time for the photosynthetic apparatus to reorganize (McCracken 1978). Both Fremont and Hood seedlings tended to accumulate starch before budbreak; however, Fremont appeared to have a quicker photosynthetic recovery as evidenced by a greater increase in starch accumulation after planting. The difference may have been due to poor root initiation in Hood seedlings and subsequent slow recovery of favorable water relations. Mattsson and Troeng (1986) attribute low photosynthetic rates after storage to water stress, as opposed to low carbon fixation capacity.

In summary, field performance potential was a good indicator of actual field performance when considering root initiation and changes in fresh weight. However, starch was not a very good predictor of subsequent root initiation or survival. Lifting seedlings early in the fall was detrimental, whereas lifting later in the fall was as good or better than spring lifting. October-lifted seedlings were somewhat intermediate. Fremont seedlings

performed better than Hood. These responses were probably associated with stress resistance at the time of lifting, root initiation, and the ability of seedlings to achieve a favorable water balance after planting.

Table V.1. Morphology of seedlings on day 0 and day 30, and days to root initiation for the greenhouse root initiation experiment. Seed source (n=4 replications x 4 lift dates=16) or lift date (n=4 replications x 2 seed sources=8) means down a column (s.e.=standard error of a seed source or lift date mean) with different letters are significantly different (p<0.05).

Day	Seed source	Root volume (cm^3)	Height (cm)	Stem diameter (mm)	Root dry weight		Days to root initiation
					New roots (mg)	Total (g)	
0	Fremont	4.9 a	18.1 b	5.5 a			
	Hood	4.4 b	28.5 a	5.7 a			
	s.e.	0.13	0.41	0.07			
	Lift date						
0	SEP	4.4 b	23.1 a	5.4 b			
	OCT	4.4 b	23.4 a	5.5 b			
	NOV	4.5 b	23.1 a	5.5 b			
	MAR	5.2 a	23.5 a	6.1 a			
	s.e.	0.18	0.58	0.11			
	Seed source						
30	Fremont	--	18.8 b	5.9 a	9.7 a	1.6 a	12.7 a
	Hood	--	28.8 a	5.8 a	3.4 a	1.4 b	12.5 a
	s.e.	--	0.41	0.40	1.8-3.8[1]	0.04	0.8-2.3[2]
	Lift date						
30	SEP	--	23.3 a	5.4 c	1.9 a	1.3 c	13.1 a
	OCT	--	23.7 a	5.8 b	7.4 a	1.5 b	14.1 a
	NOV	--	23.6 a	5.9 ab	9.1 a	1.5 b	11.2 a
	MAR	--	24.5 a	6.4 a	7.9 a	1.8 a	12.0 a
	s.e.	--	0.58	0.56	2.1-6.9[3]	0.06	1.1-4.5[4]

[1] Analysis of variance weighted by the number of seedlings (29-74) in each mean.
[2] Analysis of variance weighted by the number of seedlings (73-120) in each mean.
[3] Analysis of variance weighted by the number of seedlings (6-41) in each mean.
[4] Analysis of variance weighted by the number of seedlings (14-69) in each mean.

Table V.2. Stem starch concentration (% dry weight) and content (mg) at planting and 4, 10, and, 28 weeks from planting at the Bend site for Fremont and Hood seed sources and 4 nursery lifting dates. Lift date means (n=number of seedlings, s.e.=standard error of a lift date mean) down a column with different letters are significantly different ($p < 0.05$).

	Weeks from planting							
	0^{1}				4^{1}			
	Concentra-tion (%)		Content (mg)		Concentra-tion (%)		Content (mg)	
Lift date	Fremont	Hood	Fremont	Hood	Fremont	Hood	Fremont	Hood
SEP	0.04 b	0.03 b	0.70 b	0.67 b	--	--	--	--
OCT	0.05 b	0.04 b	0.93 b	0.99 b	--	--	--	--
NOV	0.06 b	0.04 b	1.07 b	0.78 b	0.13 b	0.08 b	2.21 b	2.14 a
MAR	0.51 a	0.34 a	10.28 a	9.97 a	0.85 a	0.22 a	14.29 a	4.79 a
n	20	20	20	20	12	9-12	12	9-12
s.e.	--	--	--	--	--	--	--	--

	Weeks from planting			
	10		28	
	Concentra-tion (%)	Content (mg)	Concentra-tion (%)	Content (mg)
Lift date	Fremont	Fremont	Fremont	Fremont
SEP	--	--	--	--
OCT	--	--	--	--
NOV	6.23 a	138.28 a	0.30 a	33.10 a
MAR	7.13 a	174.48 a	0.17 a	11.31 a
n	8	8	8	8
s.e.	0.54	18.41	0.04	8.44

[1]Means retransformed from the log.

Table V.3. Needle starch concentration (% dry weight) and content (μg/fascicle or mg/needles) at planting and 4, 10, and, 28 weeks from planting at the Bend site for Fremont and Hood seed sources and 4 nursery lifting dates. Lift date means (n=number of seedlings, s.e.=standard error of a lift date mean) down a column with different letters are significantly different (p<0.05).

	Weeks from planting								
	0						4		
	Concentration (%)		Content				Concentration (%)	Content	
			Fascicle (μg)		Needles (mg)			Fascicle (μg)	Needles (mg)
Lift date	Fremont	Hood	Fremont	Hood	Fremont	Hood	Fremont	Fremont	Fremont
SEP	0.04 b	0.03 b	22 b	13 b	1.12 b	0.88 b	--	--	--
OCT	0.03 b	0.03 b	17 b	15 b	0.97 b	0.93 b	--	--	--
NOV	0.03 b	0.03 b	21 b	14 b	1.24 a	0.78 b	0.05 a	28 a	1.65 a
MAR	0.07 a	0.07 a	50 a	43 a	2.67 a	2.50 a	0.11 a	64 a	3.24 a
n	20	20	20	20	20	20	12	12	12
s.e.	--[1]	--[1]	5	5	--[1]	--[1]	0.03	15	0.69

	Weeks from planting					
	10			28		
	Concentration (%)	Content		Concentration (%)	Content	
		Fascicle (μg)	Needles (mg)		Fascicle (μg)	Needles (mg)
Lift date	Fremont	Fremont	Fremont	Fremont	Fremont	Fremont
SEP	--	--	--	--	--	--
OCT	--	--	--	--	--	--
NOV	2.40 a	1830 a	90.78 a	0.08 a	101 a	13.07 a
MAR	2.63 a	1837 a	107.51 a	0.07 a	95 a	9.57 a
n	8	8	8	8	8	8
s.e.	1.04	648	37.26	0.01	16	1.81

[1]Means retransformed from the log.

Table V.4. Starch concentration (% dry weight) and content (mg or μg) for seedlings with or without new roots in roots, stems, and needles for 3 dates after planting. Means denoted with ** are significantly different at p<0.01; *=p<0.05, ns=not significant, p>0.05 (number of seedlings in parentheses).

Weeks from planting	Roots					
	Concentration (%)			Content (mg)		
	Presence of new roots					
	no		yes	no		yes
4	0.18 (57)	**	0.70 (39)	2.97 (57)	**	11.51 (39)
10	0.09 (22)	**	6.02 (41)	1.21 (22)	**	150.09 (41)
28	1.36 (8)	ns	1.93 (51)	151.56 (8)	ns	161.60 (51)

	Stems					
4[1]	0.46 (19)	ns	0.55 (26)	9.98 (19)	ns	9.74 (26)
10[2]	--		6.68 (16)	--		156.38 (16)
28[2]	0.28 (4)	ns	0.22 (12)	27.87 (4)	ns	20.31 (12)

	Needles								
	Concentration (%)			Fascicle content (μg)			Needle content (mg)		
	Presence of new roots								
	no		yes	no		yes	no		yes
4[2]	0.06 (11)	ns	0.09 (13)	38 (11)	ns	53 (13)	2.21 (11)	ns	2.64 (13)
10[2]	--		2.51 (16)	--		1833 (16)	--		99.14 (16)
28[2]	0.10 (4)	ns	0.07 (12)	124 (4)	ns	89 (12)	15.53 (4)	*	9.91 (12)

[1] Data for Fremont and Hood, NOV and MAR lift dates only.
[2] Data for Fremont MAR lift date only. All Fremont MAR seedlings had new roots 10 weeks from planting.

Table V.5. Dry matter partitioning between seed sources
at the time of planting, and 10 and 28 weeks from
planting, at the Bend site. Seed source means (n=number
of seedlings, s.e.=standard error) within a column with
different letters are significantly different (p<0.05).

Weight variable	Seed source	Weeks from planting		
		0	10	28
Stem (g)	Fremont	1.70 b	1.92 b	6.21 b
	Hood	2.53 a	2.63 a	8.41 a
	n	159-160	31-32	27-32
	s.e.	0.09	0.18	0.55-0.67
Root (g)	Fremont	1.79 a	2.32 a	9.27[1]
	Hood	1.54 b	1.53 b	8.28[1]
	n	159-160	31-32	27-32
	s.e.	0.05	0.12-0.13	0.58-0.71
Fascicle (mg)	Fremont	59 a	68 a	104 a
	Hood	45 b	55 b	108 a
	n	80	31-32	27-32
	s.e.	2	3	6-7
Total needles (g)	Fremont	3.34 a	3.43 a	11.19 a
	Hood	3.00 a	2.90 a	10.96 a
	n	80	31-32	27-32
	s.e.	0.14	0.21	0.85-1.03
Shoot (g)	Fremont	5.17 a	5.36 a	17.40 a
	Hood	5.47 a	5.54 a	19.37 a
	n	80	31-32	27-32
	s.e.	0.23	0.36-0.37	1.35-1.63
Root:shoot ratio	Fremont	0.38[1]	0.45 a	0.56 a
	Hood	0.27[1]	0.28 b	0.44 b
	n	80	31-32	27-32
	s.e.	0.01	0.02	0.01-0.02

[1]Significant seed source x lift date interaction.

Table V.6. Dry matter partitioning among lift dates at the time of planting, and 10 and 28 weeks from planting, at the Bend site. Lift date means (n=number of seedlings, s.e.=standard error) within a column with different letters are significantly different (p<0.05).

Weight variable	Lift date	Weeks from planting		
		0	10	28
Stem (g)	SEP	1.86 b	1.79 a	5.61 b
	OCT	2.13 ab	2.24 a	6.55 b
	NOV	2.03 b	2.31 a	9.16 a
	MAR	2.44 a	2.77 a	7.91 ab
	n	79-80	15-16	11-16
	s.e.	0.12	0.26	0.78-1.08
				Fremont[1] Hood
Root (g)	SEP	1.50 b	1.29 b	5.09 c 9.10 a
	OCT	1.69 b	1.90 a	8.68 b 7.77 a
	NOV	1.56 b	2.18 a	13.90 a 8.09 a
	MAR	1.92 a	2.32 a	9.39. b 8.16 a
	n	79-80	15-16	8 3-8
	s.e.	0.07	0.18	1.17 1.17-1.98
Fascicle (mg)	SEP	44 c	47 b	82 b
	OCT	52 b	63 a	102 ab
	NOV	52 b	66 a	120 a
	MAR	61 a	69 a	120 a
	n	40	15-16	11-16
	s.e.	2	5	8-12
Total needles (g)	SEP	2.70 b	1.78 b	7.16 c
	OCT	3.21 ab	3.45 a	10.61 bc
	NOV	3.13 ab	3.58 a	15.44 a
	MAR	3.65 a	3.86 a	11.09 bc
	n	40	15-16	11-16
	s.e.	0.20	0.30	1.20-1.67
Shoot (g)	SEP	4.66 b	3.58 a	12.77 b
	OCT	5.38 ab	5.69 a	17.16 b
	NOV	5.09 b	5.89 a	24.60 a
	MAR	6.15 a	6.64 a	19.00 a
	n	40	15-16	11-16
	s.e.	0.32	0.52-0.53	1.91-2.65
		Fremont[1] Hood		
Root:shoot ratio	SEP	0.42 a 0.29 a	0.39 a	0.57 a
	OCT	0.42 a 0.25 a	0.35 a	0.50 ab
	NOV	0.34 b 0.27 a	0.38 a	0.45 b
	MAR	0.35 b 0.28 a	0.34 a	0.47 b
	n	20 20	15-16	11-16
	s.e.	0.02 0.02	0.03	0.02-0.03

[1]Significant seed source x lift date interaction.

Table V.7. Percentage of seedlings with new roots (number of seedlings in parentheses) for two seed sources and four nursery lifting dates at the Bend site 4, 10, and 28 weeks after planting, and chi-square test of mutual independence (df=10) for testing the hypothesis that there is no association among presence of new roots, seed source, and lift date (**=p<0.01, ns=not significant p>0.05).

	Weeks from planting					
	4		10		28	
Lift date	Fremont	Hood	Fremont	Hood	Fremont	Hood
SEP	8.3 (12)	8.3 (12)	37.5 (8)	14.3 (7)	87.5 (8)	100 (3)
OCT	58.3 (12)	8.3 (12)	87.5 (8)	12.5 (8)	87.5 (8)	100 (8)
NOV	66.7 (12)	75.0 (12)	100 (8)	87.5 (8)	50.0 (8)	100 (8)
MAR	41.7 (12)	58.3 (12)	100 (8)	75.0 (8)	100 (8)	75.0 (8)
chi-square:	27.94 **		32.62 **		15.35 ns	

Table V.8. Survival and growth responses between seed sources at the time of planting (initial), at the end of the first growing season, and at the end of the second growing season, at the Bend site. Seed source means (n=number of seedlings, s.e.=standard error) within a column with different letters are significantly different (p<0.05).

Seed source	Initial height (cm)	First-year survival (%)	First-year height (cm)	First-year growth (cm)	Second-year survival (%)	Second-year height (cm)	Second-year growth (cm)
		First growing season			**Second growing season**		
Fremont	19.30 b	78.33 a	22.93 b	3.81[1]	74.52 a	33.48 b	10.43 b
Hood	27.81 a	48.92 b	32.08 a	4.26[1]	41.94 b	47.01 a	16.21 a
n	308-315	240	112-181	112-180	208-210	83-155	80-149
s.e.	0.38-0.39	4.02	0.49-0.72	0.17-0.26	4.55-4.57	0.78-1.19	0.47-0.75

	Initial[2] stem diameter (mm)	First-year stem diameter (mm)	First-year fascicle length (mm)	Second-year stem diameter (mm)	Second-year fascicle length (mm)
Fremont	5.36 a	9.46[1]	107.96 a	18.93 a	130.79 b
Hood	5.41 a	9.48[1]	113.67 a	19.93 a	151.50 a
n	159-160	27-32	105-181	83-155	83-155
s.e.	0.09	0.24-0.29	2.07-3.13	0.38-0.59	1.86-2.84

[1]Significant seed source x lift date interaction.

[2]Stem diameter in the first year was measured on a subsample of trees used for starch analysis.

Table V.9. Survival and growth responses among nursery lift dates at the time of planting (initial), at the end of the first growing season, and at the end of the second growing season, at the Bend site. Lift date means (n=number of seedlings, s.e.=standard error) within a column with different letters are significantly different (p<0.05).

	First growing season					Second growing season		
Lift date	Initial height (cm)	First-year survival (%)	First-year height (cm)	First-year[1] growth (cm) Fremont	Hood	Second-year survival (%)	Second-year height (cm)	Second-year growth (cm)
SEP	23.08 a	24.07 c	25.80 b	1.03 c	3.95 ab	16.92 c	34.55 c	10.66 b
OCT	23.39 a	66.27 b	26.65 b	4.42 b	3.88 b	59.05 b	40.47 b	13.70 ab
NOV	23.50 a	84.17 a	29.17 a	5.38 a	5.46 a	80.03 a	44.52 a	15.30 a
MAR	24.25 a	80.00 ab	28.41 ab	4.39 b	3.76 b	76.92 ab	41.43 b	13.61 b
n	143-160	119-121	27-100	19-59	7-41	102-109	18-82	16-81
s.e.	0.54-0.57	5.67-5.72	0.60-1.34	.27-.49	.33-.82	6.32-6.53	0.95-2.27	0.56-1.45

	Initial[2] stem diameter (mm)	First-year[1] stem diameter (mm) Fremont	Hood	First-year fascicle length (mm)	Second-year stem diameter (mm)	Second-year fascicle length (mm)
SEP	5.25 a	7.38 c	9.40 a	89.20 b	15.99 c	131.50 b
OCT	5.41 a	9.07 b	9.24 a	113.03 a	20.21 b	148.18 a
NOV	5.26 a	11.41 a	9.49 a	121.42 a	21.79 a	146.76 a
MAR	5.60 a	9.98 b	9.79 a	119.61 a	19.72 b	138.13 b
n	79-80	8	3-8	27-99	18-82	18-82
s.e.	0.12	0.48	.48-.82	2.60-5.72	0.46-1.12	2.25-5.42

[1]Significant seed source x lift date interaction.

[2]Stem diameter in the first year was measured on a subsample of trees used for starch analysis.

Table V.10 Survival and growth responses among nursery lift dates at the time of planting (initial), at the end of first growing season, and at the end of the second growing season, at the Mt Hood site for Hood seedlings. Lift date means (n=number of seedlings, s.e.=standard error) within a column with different letters are significantly different (p<0.05).

Lift date	Initial height (cm)	First growing season			Second growing season		
		First-year survival (%)	First-year height (cm)	First-year growth (cm)	Second-year survival (%)	Second-year height (cm)	Second-year growth (cm)
SEP	26.51 a	30.00 c	26.84 a	1.72 c	25.53 c	29.59 a	2.64 a
OCT	27.84 a	83.33 b	30.48 a	2.49 bc	75.15 b	32.82 a	2.81 a
NOV	26.82 a	90.00 ab	29.93 a	3.15 a	80.78 ab	33.55 a	2.78 a
MAR	27.18 a	93.33 a	30.22 a	2.58 ab	87.60 a	33.65 a	3.64 a
n	60	60	18-51	16-50	54-59	13-43	13-38
s.e.	0.81	2.66	1.05-1.78	0.18-0.32	3.58-3.76	0.93-1.71	0.41-0.71

	First-year fascicle length (mm)	Second-year stem diameter (mm)	Second-year fascicle length (mm)
SEP	32.61 c	8.44 a	108.34 a
OCT	40.14 bc	9.46 a	101.16 a
NOV	44.17 ab	9.53 a	107.15 a
MAR	51.20 a	10.18 a	120.60 a
n	17-50	13-43	13-43
s.e.	2.47-4.26	0.28-0.51	6.62-12.13
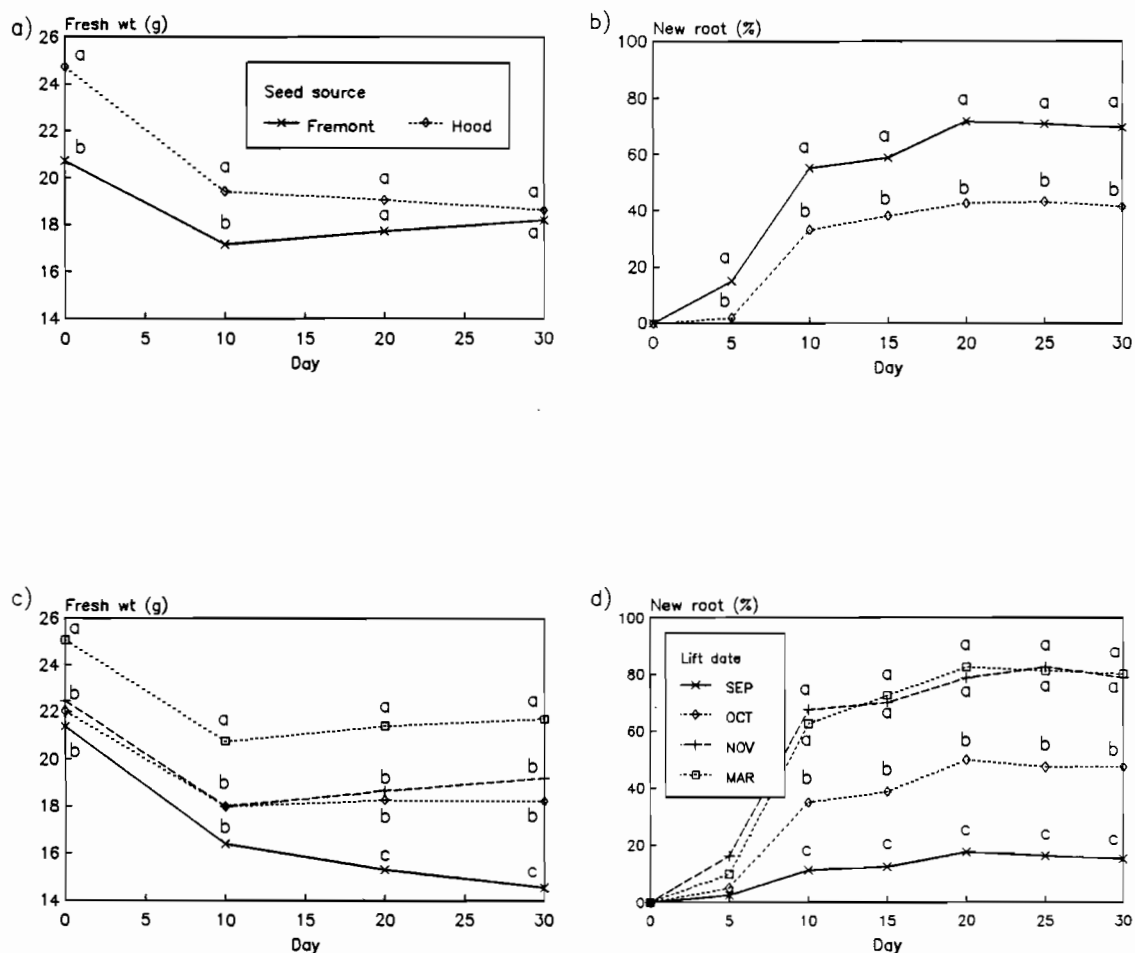
Figure V.1. Seed source comparisons for a) fresh weight and b) percent of seedlings with new roots, and lift date comparisons for c) fresh weight and d) percent of seedlings with new roots. Seed source or lift date means for a given day with different letters are significantly different (p<0.05).
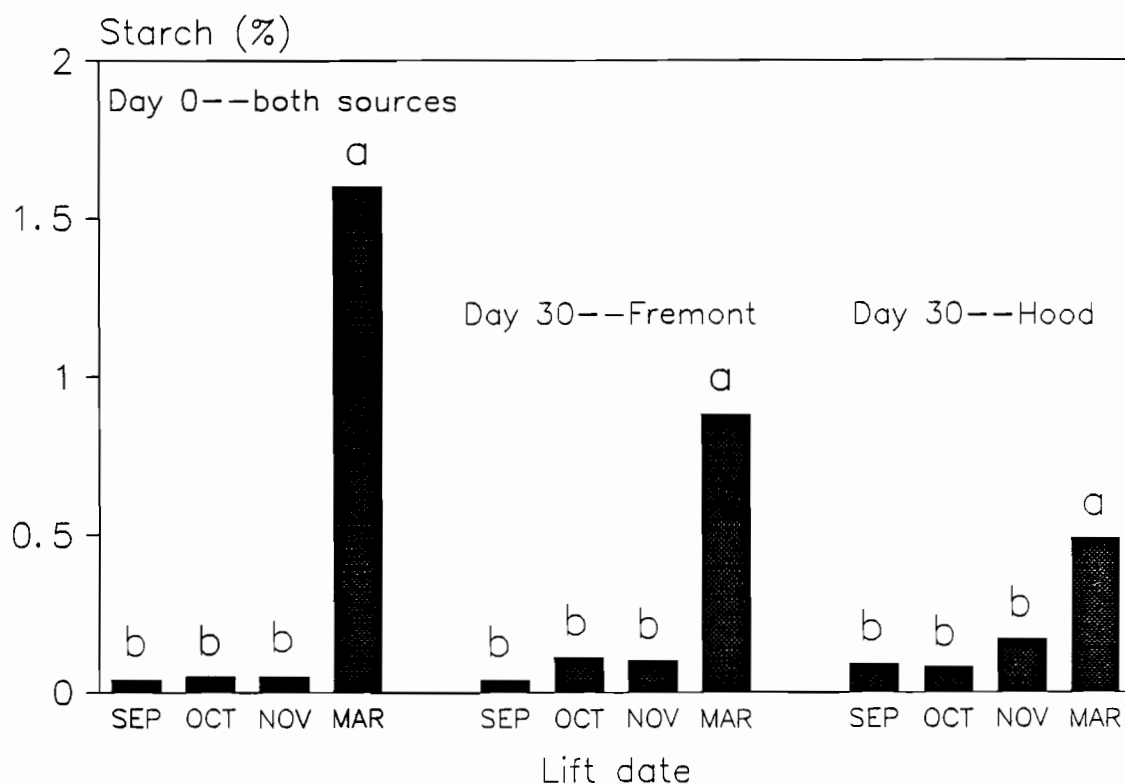
Figure V.2. Root starch concentration (% dry weight) on day 0 (both seed sources combined) and on day 30 (by seed source) for four lift dates. Lift date means with different letters are significantly different (p<0.05) on day 0 or by seed source for day 30.
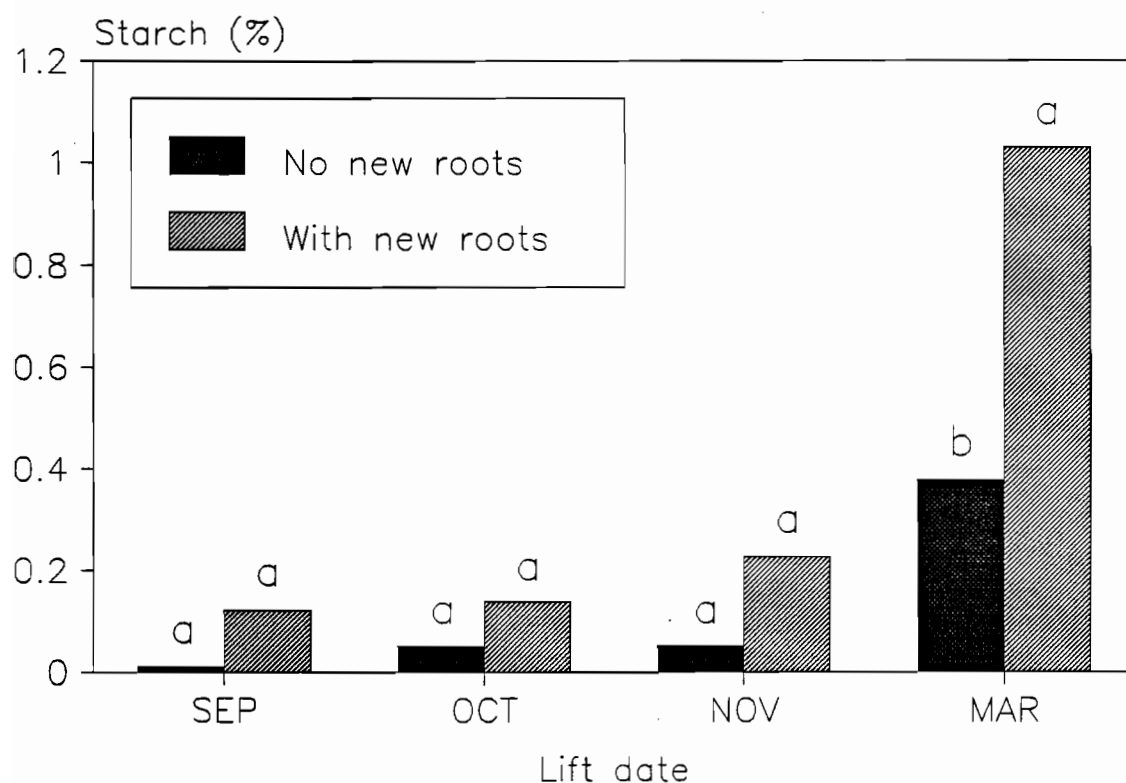
Figure V.3. Root starch concentration (% dry weight) by lift date for seedlings with and without new roots on day 30. For a given lift date, means with different letters are significantly different (p<0.05).
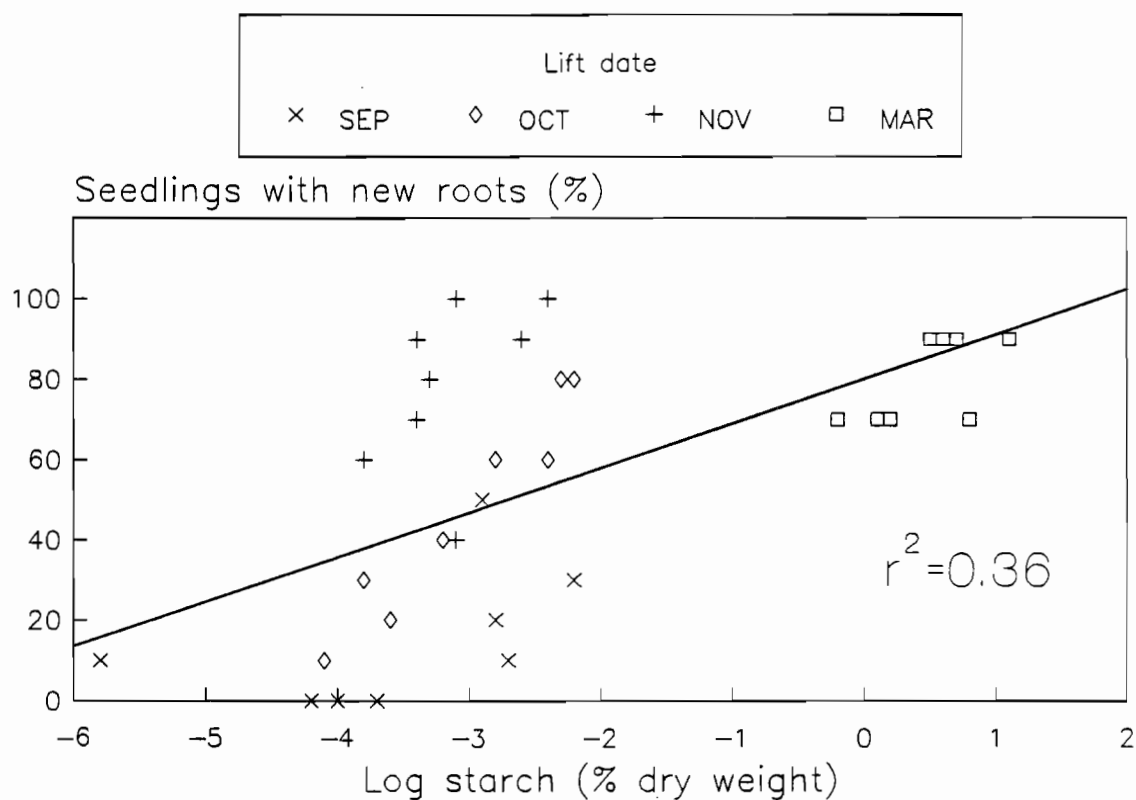
Figure V.4. Relationship between initial root starch concentration (log % dry weight) and percent of seedlings with new roots on day 30.
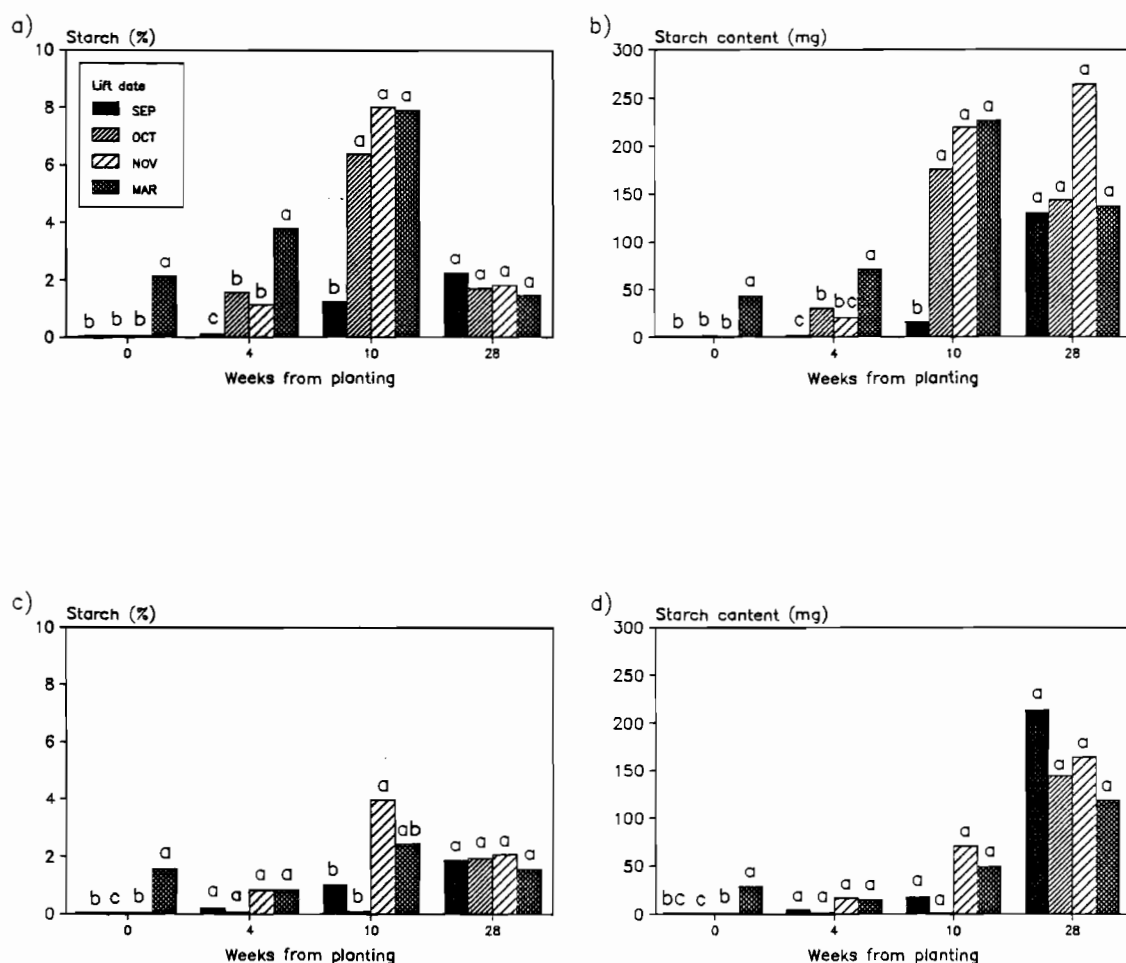
Figure V.5 Root starch concentration (% dry weight) and root starch content (mg) at the Bend site at planting, and 4, 10, and 28 weeks from planting, for a), b) Fremont and c), d) Hood seed sources. For a given measurement date, lift date means with different letters are significantly different (p<0.05).
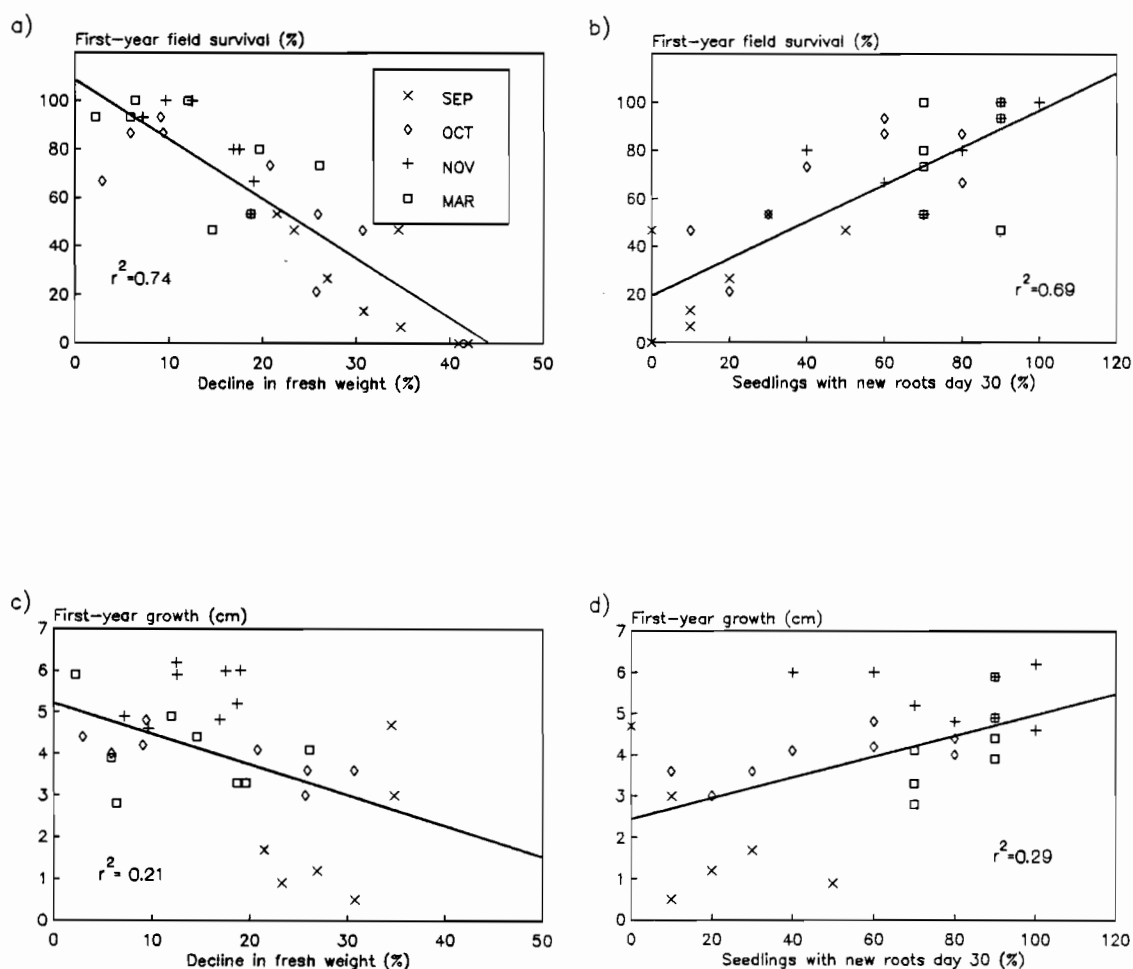
Figure V.6. Relationship between first-year field survival (%) and a) decline in fresh weight (%) or b) seedlings with new roots on day 30 (%), and first-year growth (cm) and c) decline in fresh weight (%) or d) seedlings with new roots on day 30 (%).

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APPENDICES

APPENDIX A

ANALYSIS OF VARIANCE MODELS FOR CHAPTERS I AND II

Appendix AI.1. Analysis of variance model for determining the effect of seed source and lift date-storage treatment on budbreak in the greenhouse.

	Degrees of freedom				
Source of variation	Budbreak (%)	Percent with foliated length<10 mm	Days to budbreak	Foliated shoot length (mm)	Percent with bud abortion
Seed source (SS)	1	1	1	1	1
Block in SS	6	6	6	6	6
Lift date and storage (LDS)	9	8	8	5	9
SS x LDS	9	8	8	5	9
Error	54	47	47	30	54
Total	79	70	70	47	79

Appendix AI.2. Factorial analysis of variance model for determining the effect of seed source, lift date, and storage on budbreak in the greenhouse.

Source of variation	Degrees of freedom
Seed source (SS)	1
Block in SS	6
Lift date (LD)	2
SS x LD	2
(Block in SS) x LD	12
Storage (ST)	2
SS x ST	2
LD x ST	4
SS x LD x ST	4
Error	36
Total	71

Appendix AI.3. Analysis of variance model for determining the effect of seed source and lift date on percent flushing, percent of seedlings with foliated shoot length less than 10 mm, and foliated shoot length in the field study at Bend.

Source of variation	Degrees of freedom
Block	3
Seed source (SS)	1
Lift date (LD)	3
SS x LD	3
Error	21
Total	31

Appendix AI.4. Analysis of variance model for determining the effect of lift date on foliated shoot length and percent of seedlings with foliated shoot length less than 10 mm, for Hood seedlings at Mt Hood.

Source of variation	Degrees of freedom
Block	3
Lift date (LD)	3
Error	9
Total	15

Appendix AII.1. Analysis of variance to test the effect of sample and enzyme on starch concentration.

	Degrees of freedom	
Source of variation	Experiment 1	Experiment 2
Sample (S)	3	2
Replication in S	8	6
Enzyme (E)	2	2
S x E	6	4
Error	16	12
Total	35	26

APPENDIX B

QUANTIFYING STARCH USING A COMBINATION OF α-AMYLASE AND
AMYLOGLUCOSIDASE VS AMYLOGLUCOSIDASE ALONE

INTRODUCTION

A combination of α-amylase and amyloglucosidase enzymes was used in the enzymatic digest method (Chapters II, III, and V) because of its efficiency in degrading starch at both the α-1,4 and α-1,6 glucosidic linkages of amylose and amylopectin (Greub and Wedin 1969). While α-amylase is necessary for initiating the degradation in living plant systems, several hydrolases (including glucosidases) are capable of initiating hydrolysis of starch in vitro (Goodwin and Mercer 1983). Several researchers use amyloglucosidase alone for the enzymatic determination of starch (references, Reid 1986, Philipson 1988). The objective of the experiment described below was to compare the precision of quantifying starch using a combination of α-amylase and amyloglucosidase versus amyloglucosidase alone.

MATERIAL AND METHODS

Root tissue from ponderosa pine was oven-dried at 70°C, ground in a Wiley Mill (420μ screen), and then stored at -17°C until analysis. Starch was quantified using the enzymatic digest procedure described in Rose et al. (1990).

The two enzyme digest solutions being compared were: 1) a combination of α-amylase (Sigma no. A-2643) and amyloglucosidase (Sigma no. A-3514) (digest solution used

throughout this thesis), and 2) amyloglucosidase (Sigma no. A-3514). The enzymes were suspended in 0.05M sodium acetate buffer, pH 5.1, and the activities of the enzymes in the final solutions were 1900 and 5.2 μM units ml^{-1} for α-amylase and amyloglucosidase, respectively.

Four samples of root tissue were compared (#8-11). These were chosen because they were known to contain a range of starch, based on preliminary investigations. Each sample was measured three times, with each replication sub-sampled twice (aliquots).

Statistical analysis

To determine if differences among samples varied by enzyme solution, the starch concentrations were analyzed as a split-plot analysis of variance with sample as the main plot, enzyme solution (both enzymes versus amyloglucosidase alone) as the sub-plot, and three replications. The analysis of variance had the form:

Source of variation	df
Sample (S)	3
Rep within S	8
Enzyme (E)	1
S x E	3
Error	8
Total	23

A log transformation of starch percent was necessary to help normalize the error. Because of heterogeneous variance among enzyme solutions (see below), mean separation was based on a rank transformation of the data using Fisher's protected LSD.

To determine if aliquot:aliquot values varied significantly by enzyme solution, the precision of aliquot errors were compared using the F-test of variances (Dixon and Massey 1951). Aliquot error was estimated as the mean square error after accounting for variation due to samples and replication.

Replication error was determined by averaging aliquots over each replication and then estimated by the mean square error after accounting for variation due to samples.

RESULTS AND DISCUSSION

Enzyme solution x sample interaction was not significant, indicating that the differences among samples were similar for both solutions (Appendix B.1). Additionally, the absolute concentration of starch for both enzyme solutions for any given sample were nearly identical. For example, the mean starch concentration for samples #9, 10, and 11 were 7.6%, 5.4%, and 1.3%, respectively, for the digest solution containing both enzymes; and 7.6%, 5.2%, and 1.2%, respectively, for the digest solution containing amyloglucosidase alone.

The digest solutions did differ, however, in aliquot:aliquot or replication:replication variability (Appendix B.2). In both cases, the solution containing amyloglucosidase alone had significantly ($p<0.05$) greater error than the combination.

A preliminary conclusion from this experiment was that a digest solution containing amyloglucosidase alone may be just as efficient as a combined solution in detecting differences in starch among samples. The greater error found in using amyloglucosidase alone may be due to a real difference in variability between the two solutions, or could just be chance. Further testing would be worthwhile because using amyloglucosidase alone would reduce cost and time (necessary for assaying activity) associated with the enzyme digest method. This testing should include analysis of samples with known concentration of starch, as well as determination of percent recovery of starch.

Appendix B.1. Starch (% dry weight) in ponderosa pine root tissue, by enzyme digest solution (α-amylase and amyloglucosidase versus amyloglucosidase) and sample. Means within an enzyme or sample column with different letters are significantly different ($p<0.05$).

Enzyme solution	n	Starch (%)
α-amylase and amyloglucosidase	12	5.6 a
amyloglucosidase	12	5.5 a
Sample		
8	6	8.0 a
9	6	7.6 b
10	6	5.3 c
11	6	1.2 d

Appendix B.2. F-test of aliquot and replication errors for two enzyme digest solutions.

Enzyme solution	df	Aliquot error	F	df	Replication error	F
α-amylase and amyloglucosidase	12	2.3×10^{-5}	2.8^*	8	0.0012	3.5^*
amyloglucosidase	12	6.5×10^{-5}		8	0.0042	

*Significant at $p < 0.05$.

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APPENDIX C

ANALYSIS OF VARIANCE MODELS FOR CHAPTERS III, IV, AND V

Appendix CIII.1. Analysis of variance model for morphology during the second growing season.

Source of variation	Degrees of freedom
Seed source (SS)	1
Block in SS	6
Lift date and storage (LDS)	15
SS x LDS	15
<u>Error</u>	<u>90</u>
Total	127

Appendix CIII.2. Analysis of variance model for morphology after fall lifting and storage.

Source of variation	Degrees of freedom
Seed source (SS)	1
Block in SS	6
Lift date (LD)	2
Storage (ST)	3
LD x ST	6
SS x LD	2
SS x ST	3
SS x LD x ST	6
<u>Error</u>	<u>66</u>
Total	95

Appendix CIII.3. Analysis of variance model for allocation coefficients and relative growth rates in the nursery.

Source of variation	Degrees of freedom
Seed source (SS)	1
Error	6
Total	7

Appendix CIII.4. Analysis of variance model for starch concentration and content at lifting for roots, stems, and needles.

	Degrees of freedom		
Source of variation	Roots	Stems	Needles
Seed source (SS)	1	1	1
Block in SS	6	6	6
Lift date and storage (LDS)	12	8^1	8
SS x LDS	12	8^1	8
Error	72	48^1	48
Total	103	71^1	71

[1]For content, degrees of freedom as follows: LDS=7, SS x LDS=7, Error=42, Total=63.

Appendix CIII.5. Analysis of variance model for starch concentration and content after fall lifting and storage.

	Degrees of freedom		
Source of variation	Roots	Stems	Needles
Seed source (SS)	1	1	1
Block in SS	6	6	6
Lift date (LD)	2	2	2
Storage (ST)	3	1	1
LD x ST	6	2	2
SS x LD	2	2	2
SS x ST	3	1	1
SS x LD x ST	6	2	2
Error	66	30	30
Total	95	47	47

Appendix CIV.1. Analysis of variance model for determining differences among lift date and store treatments for the water relations study.

Source of variation	Degrees of freedom
Lift date and store (LDS)	4
Error	20
Total	24

Appendix CIV.2. Factorial analysis of variance model for determining seed source and lift date effects in the water relations study.

Source of variation	Degrees of freedom
Seed source (SS)	1
Lift date (LD)	1
SS x LD	1
Error	16
Total	19

Appendix CV.1. Analysis of variance model for variables associated with the greenhouse root initiation experiment.

	Degrees of freedom					
Source of variation	Morph-ology	Percent with new roots	Dry weight of new roots	Days to root initiation	Root starch Day 0 (%)	Fresh weight by day (g)
Seed source (SS)	1	1	1	1	1	1
Lift date (LD)	3	3	3	3	3	3
SS × LD	3	3	3	3	3	3
Error	24	24	19	21	24	24
Total	31	31	26	28	31	31

Appendix CV.2. Analysis of variance model for comparing fresh weight (g) on day 0 versus day 10 for the greenhouse root initiation test.

Source of variation	Degrees of freedom
Seed source (SS)	1
Lift date (LD)	3
SS × LD	3
Rep within (SS × LD)	24
Day (D)	1
D × SS	1
D × LD	3
D × SS × LD	3
Error	24
Total	63

Appendix CV.3. Analysis of variance model for comparing root starch concentration (% dry weight) and content (mg) between seedlings with new roots vs seedlings without new roots on day 30 of the greenhouse root initiation test.

Source of variation	Degrees of freedom
Seed source (SS)	1
Lift date (LD)	3
SS × LD	3
Rep within (SS × LD)	24
With new roots vs without (WW)	1
WW × SS	1
WW × LD	3
WW × SS × LD	3
Error	19
Total	58

Appendix CV.4. Analysis of variance model for starch concentration and content after planting for roots, stems, and needles.

	Degrees of freedom											
	Weeks from planting											
	0			4			10			28		
Source of variation	Roots	Stems	Needles	Roots	Stems	Needles	Roots	Stems	Needles	Roots	Stems	Needles
Block	3	3	3	3	3	3	3	3	3	3	3	3
Seed source (SS)	1	1	1	1	1	--	1	--	--	1	--	--
Lift date (LD)	3	3	3	3	1	1	3	1	1	3	1	1
SS × LD	3	3	3	3	1	--	3	--	--	3	--	--
Error	24	24	21	21	8	3	21	3	3	19	3	3
Total	31	31	31	31	14	7	31	7	7	29	7	7

Appendix CV.5. Analysis of variance model for morphology at planting and subsequent harvests, and for first and second-year survival and growth at Bend.

Source of variation	Degrees of freedom
Block	3
Seed source (SS)	1
Lift date (LD)	3
SS x LD	3
Error	24
Total	31

Appendix CV.6. Analysis of variance model for first and second-year survival and growth at Mt Hood, for Hood seedlings.

Source of variation	Degrees of freedom
Block	3
Lift date (LD)	3
Error	9
Total	15