AN ABSTRACT OF THE THESIS OF

Ursula K. Schuch for the degree of Master of Science

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Title: Frost Hardiness of Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] Seedlings Raised in Three Nurseries

Abstract approved: Signature redacted for privacy.

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Two-year-old Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] seedlings of two seed sources raised in three nurseries in Oregon and Washington were tested for differences in frost hardiness from September 1985 to March 1986. The objective of the study was to determine whether nursery location had an influence on seedling acclimation, deacclimation , budburst and first-year field performance.

Frost hardiness was determined five times from September to December in the nurseries. In January 1986 seedlings were lifted at the three nurseries. Dehardening of potted trees was observed under outdoor and growth chamber conditions. Budburst of the trees dehardening outdoors was recorded from March to June. Hardiness was determined with a whole plant freezing test. Seedlings were frozen at each sampling date to 4 test temperatures to evaluate needle, bud and stem tissue damage.

In general, trees raised in the highest elevation nursery or the most northern nursery had hardier tissue than seedlings raised in the coastal nursery. However, hardiness varied for

each tissue and among nurseries and seed sources. In February and March seedlings from the Cascade (975m) seed source were less hardy than seedlings from the coastal source (450m). A growth chamber experiment confirmed the outdoor dehardening pattern. With a 16 hour photoperiod a constant temperature of +5°C maintained cold hardiness, whereas +10°C and 15°C promoted rapid dehardening after 20 days.

To predict hardening, a regression equation with nursery weather data and elevation was calculated. Photoperiod, number of frost days, and elevation were the most important independent factors predicting hardening (R^2=0.29).

The environment of the three nurseries seemed to have a strong influence on budburst. Trees raised in the coastal nursery burst bud significantly earlier than trees from the other two nurseries. Mean terminal budburst of potted trees from both seed sources in each nursery occurred only 2 days apart. Frost hardiness in January and first-year field growth were not correlated.

A correlation between the electrolytic conductivity of shoot tips and the damage of needles, buds, and stems as determined by the whole plant freezing test was poor. The highest correlation coefficient (r) was 0.69. Different hardening rates of tissues and plant to plant variability may have contributed to the low correlation. The conductivity method as outlined in this study does not provide a satisfactory estimation of frost hardiness of two-year-old bareroot Douglas-fir seedlings.

Frost Hardiness of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] Seedlings Raised in Three Nurseries

by

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Frost Hardiness of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] Seedlings Raised in Three Nurseries

INTRODUCTION

Seedling quality is an important factor for regeneration success. Physiological characteristics, besides the morphological ones, are often evaluated in the nursery or during seedling cold storage to ensure vigorous performance in the field. Frost hardiness is one performance attribute on which much research has been focused, but which is still not entirely understood (Duryea and McClain, 1984). Frost hardiness or acclimation is a process that provides trees with the ability to survive cold temperatures without being damaged (Glerum, 1985).

Nurseries and foresters have different motivations for testing frost hardiness (Duryea, 1985). Nurseries are for example interested in a safe lifting window, whereas regeneration foresters might be looking for stock suited for a frost pocket.

At present it is common practice for timberland owners to distribute the seeds of one seed lot to several nurseries. However, nursery location can be remote and climatically different from the outplanting site. The influence of nursery location on frost hardiness of seedlings has not been addressed by many studies (Duryea and McClain 1984).

The goal of the research described in this thesis was to determine whether nursery location had an influence on frost hardiness, budburst, and first-year field performance of trees. The following studies compared frost hardiness of two-year-old

Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] seedlings of a coastal and a Cascade seed source that were raised in three nurseries.

The first chapter describes the acclimation process of seedlings in three nurseries and attempts a prediction of cold hardiness with nursery environmental data. The second chapter describes dehardening of seedlings under outdoor and growth chamber conditions. In addition, the effect of nursery location on budburst and first-year field performance of seedlings is determined.

The third chapter compares two methods of frost hardiness assessment. The goal of this study was to determine whether the electrical conductivity method as outlined by Colombo et al. (1984) correlated with visual damage ratings of the whole plant freezing test. The conductivity method would provide a faster method of screening trees for frost hardiness than the whole plant freezing test.

The research presented in this thesis could help nurseries and reforestation foresters to get a better understanding of the influence of nursery location on frost hardiness of seedlings. This could lead to improved decisions on the selection of nurseries for certain seed sources, and ultimately to better reforestation success.

CHAPTER I

HARDENING OF DOUGLAS-FIR SEEDLINGS RAISED IN THREE NURSERIES IN THE PACIFIC NORTHWEST

ABSTRACT

Two year old Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] seedlings of two seed sources raised in three nurseries in Oregon and Washington were tested for differences in frost hardiness between September 1985 and January 1986. The objective was to determine whether nursery location had a significant influence on frost hardiness. At six dates seedlings were tested by a whole plant freezing test, where seedlings were frozen to various temperatures, and evaluated for frost hardiness of needle, bud and stem tissue.

Acclimation of needles proceeded in the predicted pattern, with the highest elevation nursery seedlings having the hardiest needles, and the coastal nursery seedlings having the least hardy needles. Hardiness development of buds differed between seed sources only, but not among nurseries. Stem acclimation had the same pattern as needle hardening from late November to January. However, from September to early November seedlings from the coastal nursery had fewer damaged buds than those from the highest elevation nursery.

A regression equation was calculated to predict frost hardiness using the weather data and elevation of the nurseries. Elevation, photoperiod, and number of frost days were the most important independent factors predicting hardening (R^2=0.29).

INTRODUCTION

At present timberland owners distribute the seeds of one seed lot to several nurseries. This reduces the risk of losing the entire seed lot to either diseases or pests. Unfortunately, the location of these nurseries can be remote from the site where the seeds originated and where seedlings will be outplanted.

Unseasonal cold temperatures occurred in October and November 1985 in the Pacific Northwest, causing considerable damage to coniferous seedlings in some nurseries. The question of whether certain seed sources are suitable for seedling production at a range of nursery sites was raised again.

The influence of nursery climate on frost hardiness of Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] seedlings and subsequent field performance is not yet fully understood (Duryea and McClain 1984). Differences in frost hardiness of the seed sources raised in different nurseries are thought to be a result of cultural treatment and the specific nursery climate (Duryea and McClain 1984). The impact of cultural practices on seedling quality in general and frost hardiness in particular are summarized by Duryea and McClain (1984). For example, irrigation is reduced at the end of the growing period to produce a moderate moisture stress in the seedlings. This induces bud set and growth cessation, a prerequisite for hardiness development.

Rook et al. (1974) found that the frost tolerance of Pinus

radiata (D. Don) planting stock was associated with the nursery where the stock was raised. They found that the climatic conditions of a given nursery had a profound effect on frost hardiness and planting stock quality. Only marginal (0.5°C) differences in frost hardiness were due to cultural treatment (Menzies et al. 1981). In a similar study van den Driessche (1970b) found that the hardiness development of Douglas-fir provenances raised at 3 nurseries varied by nursery. However, it was not clear whether climate or other factors were responsible for these differences. Others have found that frost hardiness of Douglas-fir seedlings varied with seed source, elevation, and aspect (Larsen 1978a,b, Maronek and Flint 1974, Hattemer and Koenig 1975, Sakai and Weiser 1973).

According to Glerum (1976), temperature and light are the main environmental factors controlling hardiness development. These factors are believed to regulate three stages of frost hardiness development (Weiser,1970). The first stage is triggered by short days (SD) and warm temperatures, the second and third stage by low temperatures. Van den Driessche (1969a,1970a), McCreary et al. (1978), and Smit-Spinks et al. (1985) found that short days substantially increased frost hardiness in the first stage. In Douglas-fir seedlings, Tanaka (1974) reported that an 8 hr SD treatment accelerated the attainment of cold hardiness by 2-3 weeks. In a study on pine needles and apple bark, Bervaes et al. (1978) concluded that temperature or day length as single factors are of limited value,

but that short days should precede low temperatures to attain maximum hardiness. They found substantial hardiness reduction in pines when the sequence of events was reversed. In addition to short days, cool night temperature and low light intensity promoted hardiness development considerably (van den Driessche 1969a).

This study examines the process of cold hardiness acquisition of Douglas-fir seedlings that were raised in different nurseries in the Pacific Northwest. Members of the Nursery Technology Cooperative at Oregon State University decided that this topic was of importance to seedling producers and users. It was hypothesized that seedlings raised in a low elevation nursery close to the ocean would harden more slowly and would not become as frost hardy as seedlings raised in a higher elevation or a northern latitude nursery. Three nurseries, located in Oregon and Washington, were selected for the study. They each grew the same two seed sources and provided a chance to test the hypothesis that seedling hardiness depends on the nursery environment.

MATERIALS AND METHODS

Douglas-fir seedlings from two seed sources were each raised in three nurseries. The provenances were a coastal seed source (061 or seed source 1, 475m), from the Oregon Coast Range west of Mary's Peak, and a Cascade source (502 or seed source 2, 950m), from the west side of the Cascades in southern Oregon. Locations of the nurseries and outplanting sites are shown in Figure I.1. In the following discussion the nurseries will be identified by the numbers given in Figure I.1 (e.g., no.1 = the most southern nursery).

In July 1985 a bed from each seed source at each nursery was selected and divided into 4 blocks. Cultural practices and the seedbed density at the 3 nurseries differed as summarized in Table I.1, and followed standard operational procedures maintained in each facility.

Sampling Procedures in the Nurseries

Frost hardiness of seedlings in the nurseries was measured six times from September 1985 to January 1986. The sampling dates were September 10 and 11, October 7 and 8, October 28 and 29, November 18, December 8 and 9, 1985, and January 23, 1986. Samples from all three nurseries were harvested on two consecutive days, with the exception of one sampling date, November 19, 1985, when all samples were harvested the same day.

On each sampling date 16 seedlings per block were harvested.

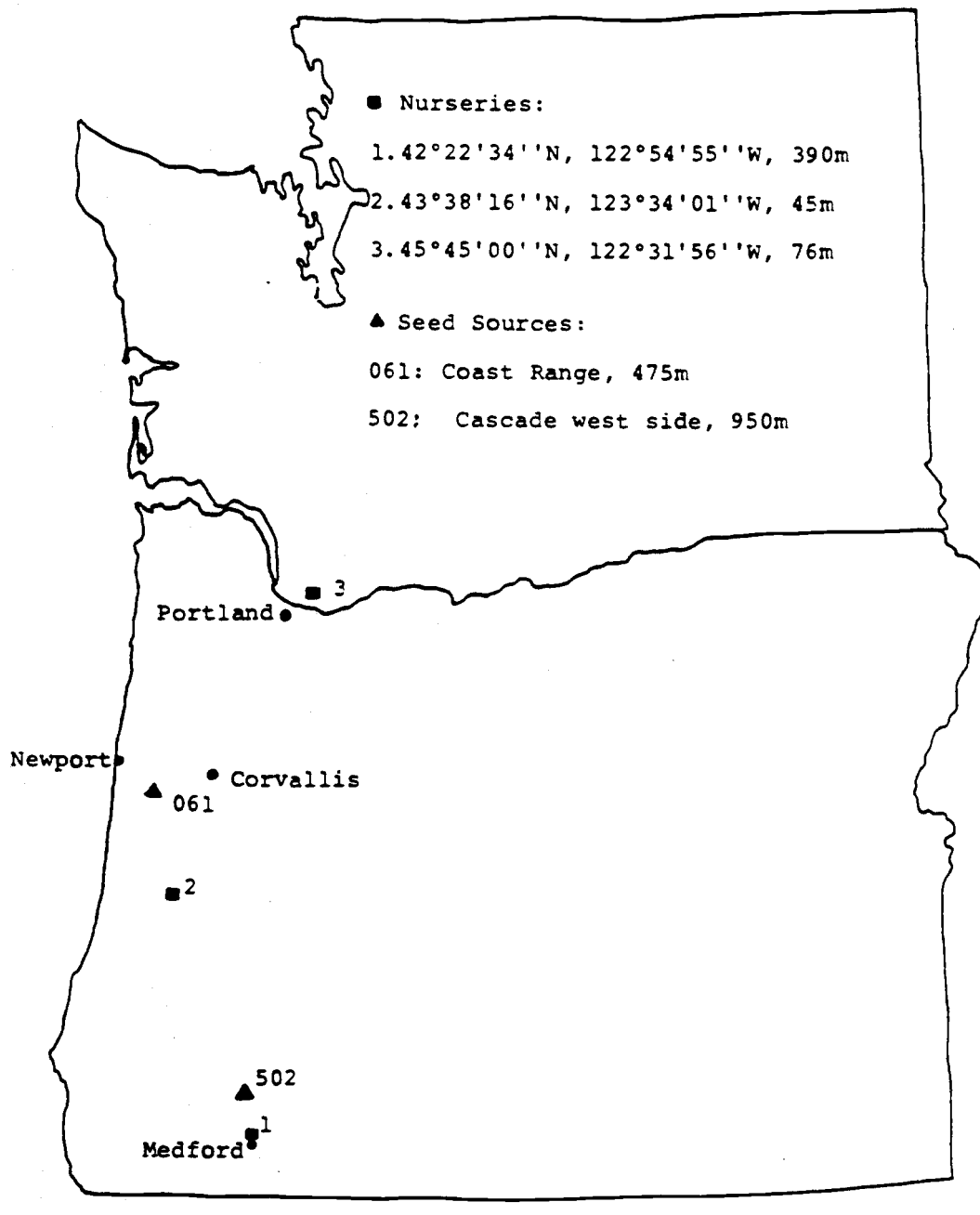


Figure 1.1: Location of nurseries ■ and outplanting sites ▲ in Oregon and Washington.

Table I.1: Cultural practices and initial seedbed density (number of seedlings per linear meter seedbed) in the nurseries in 1985.

nursery	seed source	top mowing	horizontal root pruning	vertical root pruning	root wrenching	initial seedbed density
		number of times				per m seedbed
1	1	3	1	2	-	36
	2	1	1	2	-	30
2	1	-	1	1	-	46
	2	-	1	1	-	40
3	1	1	2	1	1	58
	2	1	2	1	1	58

Excess soil was removed by first shaking the trees, and then rinsing the roots for a few minutes in a bucket of water. The seedlings were placed in polyethylene bags and then into an insulated cooler with ice for transport to the lab. They were stored in a cold room at +2°C for 1 day until the freezing tests started.

Outdoor temperatures in the nurseries were measured from September 1985 to January 1986 with thermographs. Environmental data of each nursery are summarized in Table I.2.

Freezing Procedures with the Whole Plant Freezing Test

Frost hardiness of the seedlings was determined with the whole plant freezing test and visual injury estimation (Glerum 1985) from September 1985 to January 1986.

The methods in these studies were adapted with slight modifications from those used operationally by International Paper Company, Lebanon, Oregon. The roots of each seedling were pruned 8 cm below the root collar. The seedlings were then placed individually in vials containing 2 cm of water with the root collar placed well above the vial rim. Plant positions were randomized in a test tube rack. A programmable freezer reduced the temperature at a rate of 5°C/hr. The samples were kept at a predetermined freezing temperature for 2 hours and then thawed at a rate of 10°C/hr. The sensors for the programmable freezer were thermocouples placed in the foliage of the trees.

At each sampling date 384 seedlings (16 trees x 3 nurseries x 2 seed sources x 4 test temperatures) were frozen to various

Table I.2: Environmental data for the three nurseries from September 1985 to January 1986.

Date	Nursery	Min. Temp. (°C)	Max. Temp. (°C)	#Frost Days	Photo-period (hr:min)
Sept.10 -	1	+ 1.1	+32.2	-	12:06
Oct.7	2	- 1.1	+28.9	1	12:06
	3	- 1.1	+27.8	1	12:07
Oct.8 -	1	- 4.4	+24.4	8	10:59
Oct.28	2	- 3.9	+23.3	2	10:57
	3	- 1.1	+20.6	5	10:52
Oct.29 -	1	- 8.9	+18.9	13	10:06
Nov.18	2	- 5.6	+19.4	5	9:57
	3	-10.0	+16.7	12	9:53
Nov.19 -	1	- 6.7	+13.3	17	9:32
Dec.8	2	- 5.6	+11.1	10	9:16
	3	-12.2	+ 7.8	20	9:09
Dec.9-Jan.15	1	- 7.8	+14.4	30	9:19
Dec.9-Jan.21	2	- 5.0	+17.8	21	9:15
Dec.9-Jan.13	3	- 8.9	+14.4	30	8:58

temperatures, spaced 3°C apart. Test temperatures ranged from -1 to -10°C in September and early October, -4 to -13°C in late October, -10 to -19°C in November, and -16 to -25°C in December and January. These ranges were chosen to find the T50, the temperature where 50% of the tissue is damaged.

Frost Damage Rating System

After freezing the seedlings were placed in beakers and kept well watered in a greenhouse at +23°C (+/-5°C) and 16 hour photoperiod for 7-10 days to allow the browning symptoms to develop. The following rating system was used to evaluate the damage to the needles, buds, and stem tissue:

Needles:
0 = 0% damage, entire foliage green
1 = 1 - 25% of foliage discolored (red/brown)
2 = 26 - 50% " " "
3 = 51 - 75% " " "
4 = 76 - 99% " " "
5 = 100% " " "

Buds:
Rating is based on 10 sliced buds per seedling.
0 = 0% damage
1 = 1 - 25% dead buds
2 = 26 - 50% " "
3 = 51 - 75% " "
4 = 76 - 99% " "
5 = 100% " "

Stem tissue:
0 = no visible damage (0% damage).
1 = light tan discoloration, not considered to have serious effect on survival potential (1-25% damage).
2 = darker brown discoloration to approximately 26-50% of the cambium of the seedling stem.
3 = darker brown discoloration of the cambium, spread more extensively over 51-75% of the stem.
4 = very dark brown discoloration of the stem of the cambium layer (76-99% damage).
5 = very dark discoloration throughout phloem, cambium, and into the wood. (100% damage).

For some of the results, hardiness differences between

nurseries were calculated as T50, the temperature at which 50% of the tissue was damaged. The T50 value corresponded with the temperature at which tissue damage was rated as 2.5 with the rating system described above. This was a relative value, chosen as a reference only. It was not part of this study to determine whether it was the lethal temperature for 50% of the tested population.

Statistical Analysis

Two approaches were taken to evaluate differences in hardiness between nurseries and seed sources. A full regression model with intercepts and slopes for each seed source by nursery combination with time as a continuous variable was calculated for each tissue/ organ. Subsequently, attempts to reduce the full model were analyzed. F-values were considered significant at a 5% level. A model that could be reduced to a common slope or intercept indicated that there were no significant differences in damage between seed sources, nurseries, or both. As time progressed, the development of hardiness had a curved rather than a linear relationship. Therefore, adding a quadratic term for days elapsed to each of the reduced models was also tested.

In the second approach a split-plot analysis of variance was done for each tissue and date with nursery and seed source as the whole plot, and temperature as the split plot. The regression compares the damage evaluation over the period from September 1985 to January 1986, whereas the ANOVA compares nursery and seed source effects at each sampling date.

To determine whether T50 could be predicted from nursery environmental data, regressions with T50 as dependent and nursery environmental data (Table I.2) and elevation as independent variables were calculated.

RESULTS

Acclimation of Needles

Hardiness development, calculated as T50, differs for needles, buds, and stems (Figs. I.2, I.3, and I.4). The "best" regression equations (Fig. I.2) show that there were significant ($p=0.05$) differences in needle acclimation among nurseries, but not between seed sources within a nursery, as the collapsed curves imply. The results for needles should be interpreted with caution, because the regression shows that the calculated T50 values for December and January were lower than -25°C, the lowest test temperature. Thus, the needles were either hardier than the lowest test temperature, or the time in the greenhouse was not long enough to allow the development of needle browning. Regardless of this, the graph (Fig. I.2) indicates fastest needle hardening in nursery 1, followed by nursery 3, and nursery 2 respectively.

The mean tissue damage (Table I.3 and I.4) shows no significant differences ($p=0.05$) between needle damage of nurseries or seed sources at each sampling date. However, as hardening progresses, nursery 1, the highest elevation nursery, consistently had the lowest damage rating.

Acclimation of Buds

The hardiness development of buds as calculated by the overall regressions differed significantly between seed sources (Fig. I.3). The Cascade source (502) was almost 1°C hardier at all

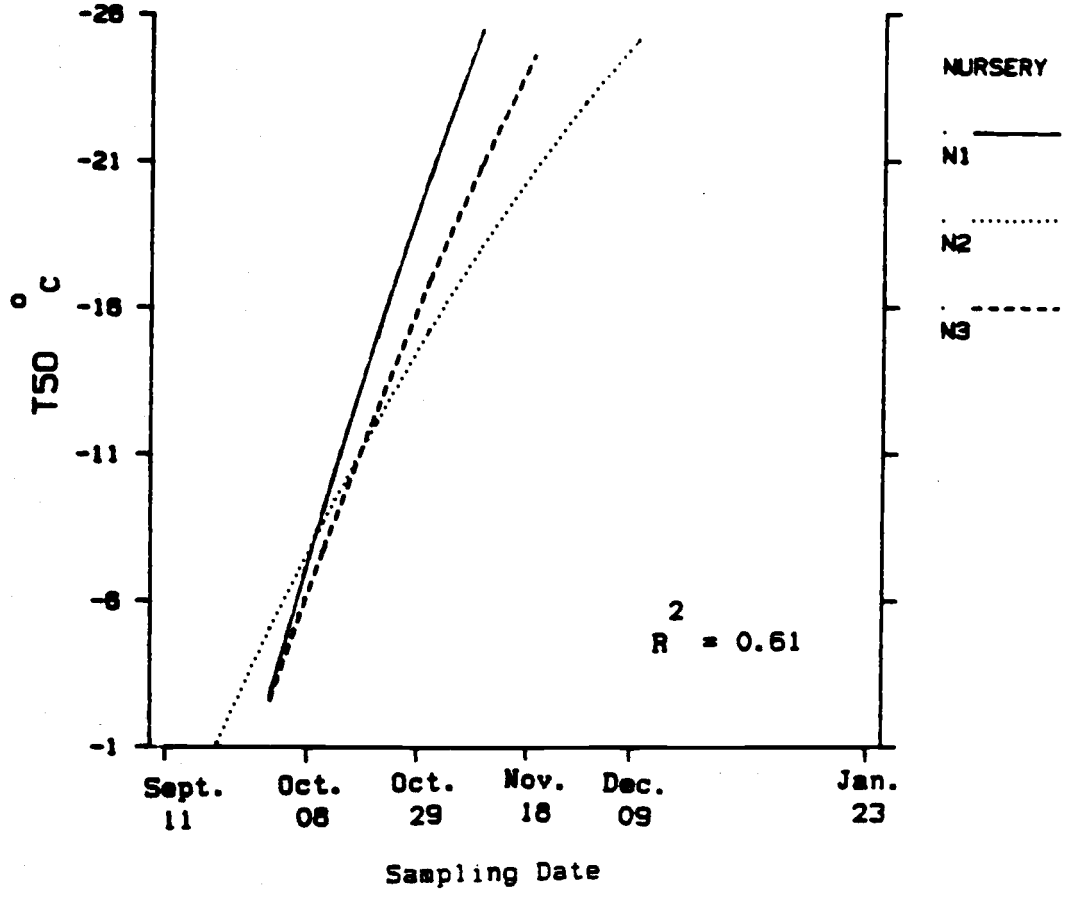


Figure I.2: T50 for hardening needles of Douglas-fir seedlings growing in 3 nurseries.

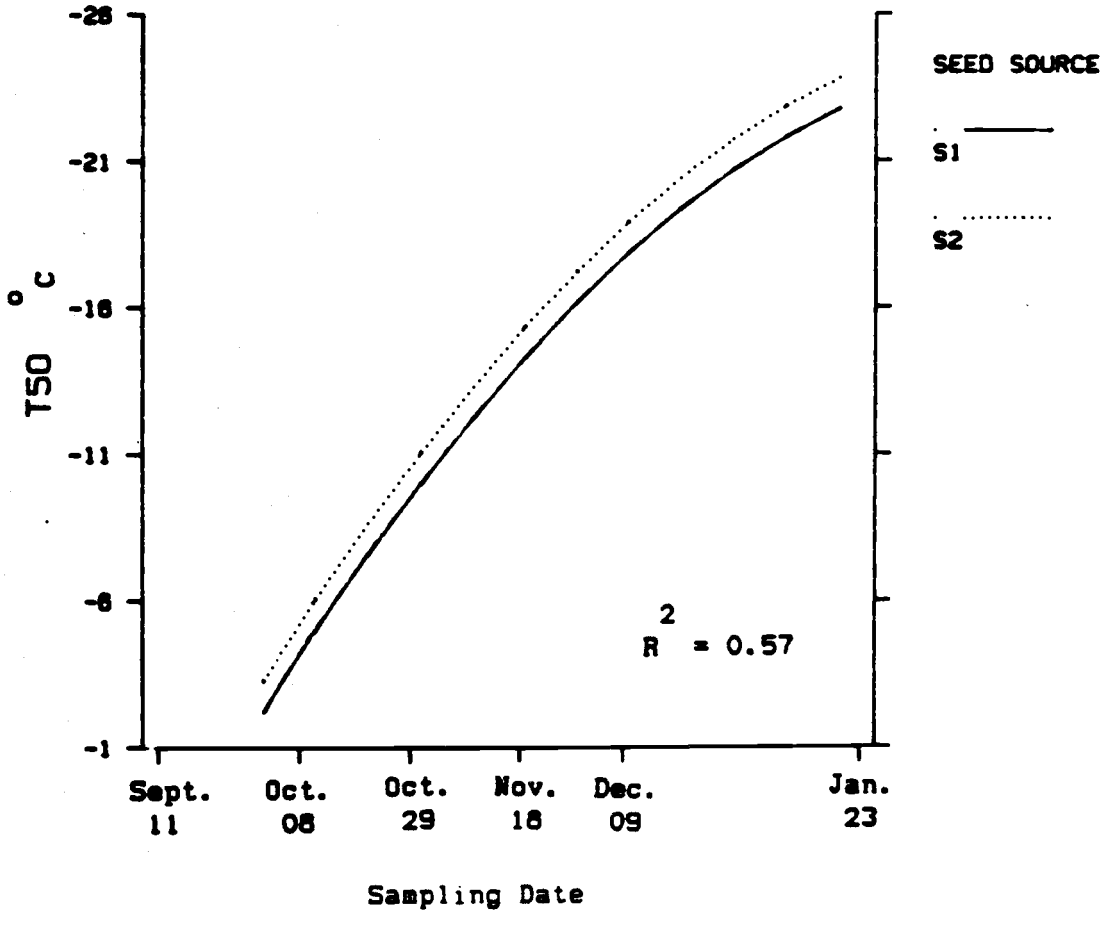


Figure 1.3: T50 for hardening buds of Douglas-fir seedlings growing in 3 nurseries.

Table I.3: Mean tissue damage rating of seedlings in the three nurseries at each sampling date, seed sources combined. (Means within the same vertical cell for each date and tissue were not significantly different (p=0.05)).

date	nursery	mean test temp. (°C)	needles	buds	stems
09/11/85	1	-5.5	3.0	4.0	3.3
	2		2.9	3.6	3.2
	3		2.7	4.6	3.2
10/08/85	1	-5.5	2.4	2.8	2.5
	2		2.1	2.5	2.2
	3		2.5	2.2	2.5
10/29/85	1	-8.5	1.5	1.5	2.3
	2		1.9	1.9	2.4
	3		1.6	1.2	1.9
11/18/85	1	-14.5	0.8	1.7	1.7
	2		1.6	2.0	2.5
	3		1.2	1.9	1.9
12/09/85	1	-20.5	1.3	2.8	2.1
	2		2.0	3.1	2.7
	3		1.4	3.1	2.4
01/23/86	1	-19.0	0.8	1.8	1.3
	2		1.0	1.9	1.6
	3		1.2	2.1	1.5

Table I.4: Mean tissue damage rating of seed sources at each sampling date, all nurseries combined.
(Means within the same vertical cell for each date and tissue with different letters are significantly different (p=0.05)).

date	seed source	mean test temp. (°C)	needles	buds	stems
09/11/85	coastal	-5.5	3.0 A	4.0 A	3.2 A
	Cascade		2.7 A	3.6 A	3.2 A
10/08/85	coastal	-5.5	2.4 A	2.7 A	2.5 A
	Cascade		2.3 A	2.3 A	2.3 A
10/29/85	coastal	-8.5	1.6 A	1.5 A	2.1 A
	Cascade		1.7 A	1.6 A	2.2 A
11/18/85	coastal	-14.5	1.1 A	1.9 A	2.0 A
	Cascade		1.3 A	1.8 A	2.0 A
12/09/85	coastal	-20.5	1.5 A	3.1 A	2.2 A
	Cascade		1.6 A	2.8 A	2.6 A
01/23/86	coastal	-19.0	1.0 A	2.0 A	1.3 A
	Cascade		1.0 A	1.9 B	1.5 B

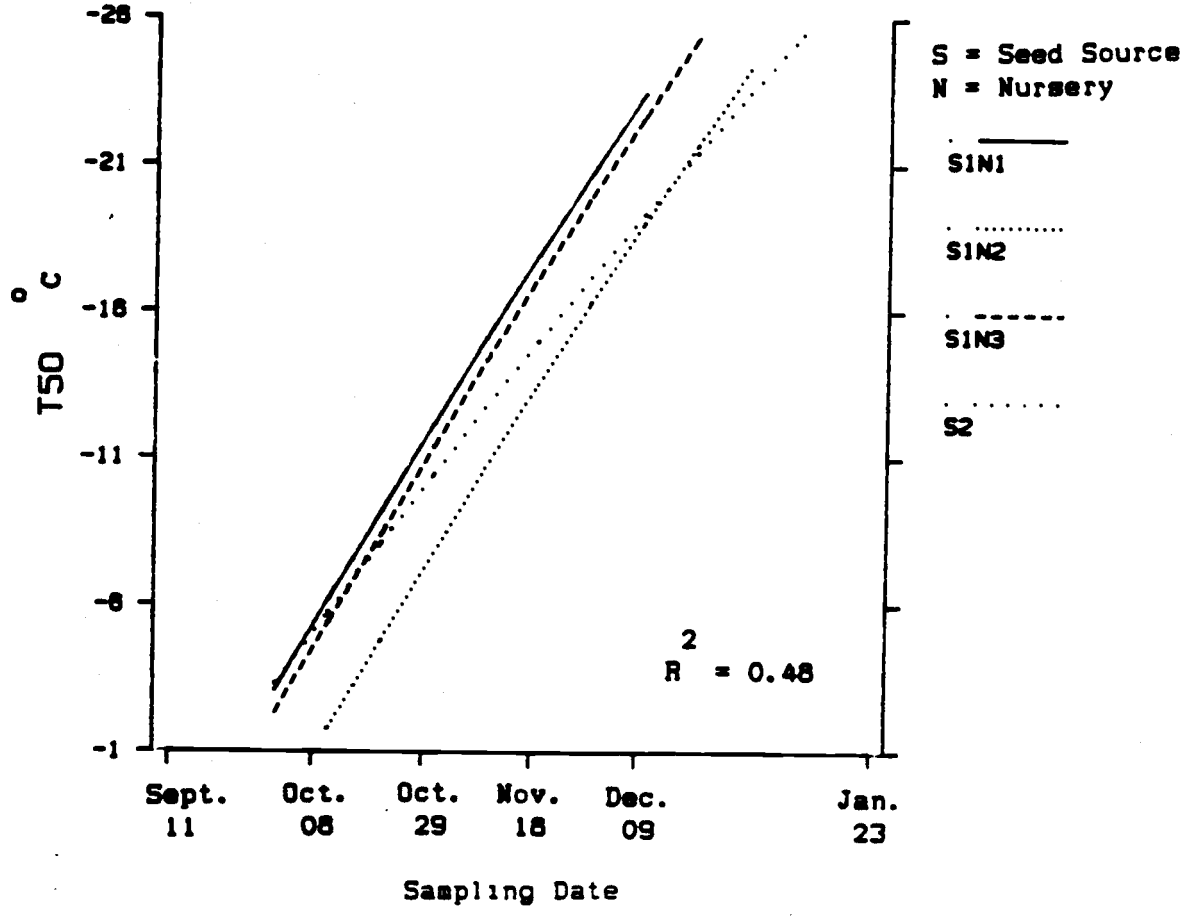


Figure I.4: T50 for hardening stems of Douglas-fir seedlings growing in 3 nurseries.

sampling dates. This is also demonstrated by the mean tissue damage rating across seed sources (Table I.4), with one exception in November. Ranking of the mean damage rating among nurseries is variable and shows no definite pattern.

Acclimation of Stems

The hardiness development of the coastal seed source was significantly different ($p=0.05$) at the three nurseries throughout the sampling period (Fig. I.4), with the hardiest stems occurring at nursery 1, followed by nursery 3 and then 2. Seedlings from nursery 2 were consistently about $4^{o}C$ less hardy than trees from nursery 1.

The mean tissue damage rating of seedlings from each nursery (Table I.3) confirms that the hardiest stem tissue was found in nursery 1 from late November to January, but from September to early November seedlings from either nursery 3 or 2 had the hardiest stem tissue. The comparison of the seed sources shows significant differences ($p=0.05$) at the January sampling date, with the coastal source having less damage than the Cascade source (Table I.4).

Predicting T50 with Environmental Data

A multiple regression analysis with T50 as dependent and nursery environmental data (Table I.2) and elevation as independent variables was calculated. The significant equation ($p=0.01$) was:

T50 = -56.86 - 0.03 ELEVATION + 4.57 PHOTOPERIOD - 0.47 #FROST DAYS

The equation had an R^2 value of 0.29. Photoperiod was the most important factor predicting T50, followed closely by number of frost days and elevation, if the importance of each factor was determined by the standardized parameter estimation for each variable. The standardized estimates for photoperiod, number of frost days and elevation are 0.27, -0.24, and -0.23, respectively. The standardized parameter estimates are independent of units, whereas parameter estimates in the equation are not corrected for units.

DISCUSSION

This study shows evidence that the location of the nursery in which Douglas-fir seedlings are produced may have an influence on their frost hardiness. The results of needle and stem acclimation T50's, where significant differences among nurseries occur, support the hypothesis, but are not demonstrated as significant differences between mean tissue damage ratings. The ranking of the tissue damage ratings (Table I.3) suggests that trees in the coastal nursery (2) and the most northern nursery (3) start the hardening process earlier than trees in nursery 1, the highest elevation nursery. This could be due to the shorter photoperiod in nurseries 2 and 3. During the first stage of acclimation, photoperiod in conjunction with warm temperatures is the most important environmental trigger for acclimation (Weiser 1970, Smit-Spinks et al. 1985). As acclimation proceeded, the exposure to colder temperatures stimulated greater hardiness in trees growing at the highest elevation nursery (1) and the most northern nursery (3). The bud acclimation data shows the trend of the hardier Cascade source. These results are in contrast with van den Driessche's (1970b), where nursery but not provenance differences were reported in acclimating Douglas-fir seedlings raised in three nurseries.

With respect to the nursery climate effect on frost hardiness development, photoperiod turned out to be the most important factor, followed by number of frost days and elevation.

This supports the overall observation that trees in nursery 1 and nursery 3 were generally hardier than the seedlings in nursery 2. The minimum temperature and total number of frost days in nursery 1 and 3 was considerably greater than in nursery 2 (Tab. I.2). These findings are in agreement with the generally accepted fact that cold temperatures during the second and third stage of acclimation promote frost hardiness (McGuire and Flint 1962; van den Driessche 1969a,1970a; Kobayashi et al. 1983; Smit-Spinks et al. 1985). Menzies et al. (1981) found that elevation, mean temperature, number of frost days and latitude were at various times of the year significant factors to predict frost hardiness of Pinus radiata.

However, in our study it has to be kept in mind that the regression explains only 29% of the variation. Other factors like nursery distance from the ocean, minimum and maximum temperature as well as fluctuations in the diurnal temperatures are likely to have significant effects on frost hardiness. The goal of the regression was to predict frost hardiness with as few parameters as possible, and to consider only variables that are independent of each other. By including minimum temperature, latitude, distance from the ocean etc., more variation could be explained, but the parameters would be strongly correlated, and interpretation of the equation more confusing. Another factor limiting the inference from the results is that only three nurseries in only one year were used for this study.

It is possible that some of the differences in frost

hardiness are due to the cultural practices at the nurseries. Rook et al. (1974) found that Pinus radiata responded with only 0.5°C difference in frost hardiness to various cultural treatments.

The study was conducted during an unusually cold year. This might account for the small differences in hardiness that were found among nurseries. Observations during the winter of 1986/87 show that the coastal nursery had only a single frost day. Operational frost hardiness tests (Johnson 1987, personal communication) have confirmed, that during a mild or normal year such as 1986/87, larger differences in frost hardiness between nurseries might be detected. In any case, the nursery site seems to influence acclimation.

CHAPTER II

DEHARDENING, BUDBURST, AND FIRST-YEAR FIELD PERFORMANCE OF DOUGLAS-FIR SEEDLINGS RAISED IN THREE NURSERIES IN THE PACIFIC NORTHWEST

ABSTRACT

This study was undertaken to test whether nursery location has an influence on loss of frost hardiness after lifting, bud burst, and first-year field growth and survival. Frost hardiness of Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] seedlings raised at three nurseries in Oregon and Washington was measured with a whole plant freezing test in January, February, and March 1986. In general, seedlings raised at a high elevation nursery and in a few cases trees from the most northern nursery were the hardiest. Trees from the coastal nursery were often the least frost resistant. Significant differences ($p=0.05$) in dehardening occurred among nurseries as well as between seed sources. In February and March, seedlings from the high elevation (975 m) seed source were less hardy than seedlings from the coastal source (450 m).

A growth chamber experiment confirmed the dehardening studies. A constant temperature of $+5^{\circ}C$ and 16 hr photoperiod maintained cold hardiness, whereas $+10^{\circ}$ and $+15^{\circ}C$ with 16 hr photoperiod promoted rapid dehardening after 20 days.

The nursery environment influenced bud burst. Trees raised in the coastal nursery flushed significantly earlier than trees

from the other two nurseries. Trees of both provenances from each nursery burst bud only 2 days apart.

No correlation between first year mean growth in the field and frost hardiness in January was found.

INTRODUCTION

The major cause of winter injury to conifer seedlings are early fall and late spring frosts (Duryea and McClain 1984). Therefore, to reduce freezing injury, the time and rate of fall acclimation and spring deacclimation are important. Dehardening, or deacclimation, is the seasonal transition when plants change from a frost resistant condition to a condition where they become susceptible to frost (Weiser et al. 1979).

Differences in cold hardiness related to seed source are thought to be an adaptation mechanism of plants (Larsen 1978a). Campbell and Sorensen (1973) found Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] seedlings from northern latitude provenances to be less damaged by frost than seedlings from southern provenances in the Pacific Northwest. Larsen (1978a) found that low elevation sources (350-500m) of Douglas-fir were highly resistant to spring frost, whereas provenances from higher altitudes (500-750m) had lower resistance to spring frost. In contrast to this, van den Driessche (1970b) studied low, medium, and high elevation Douglas-fir sources and found no significant provenance differences. However, the high elevation provenance deacclimated somewhat earlier in spring.

Bud burst is used as a phenological measure of complete loss of hardiness (Fuchigami et al. 1982). Bud burst of various Douglas-fir provenances was studied and seemed to depend on latitude and elevation of the seed source (Campbell and Sugano 1979, Hermann and Lavender 1968). A significant trend of delay in

bud burst was found with increasing elevation and latitude of the provenance. However, Kaya's (1987) results caution against generalizing, as he found larger differences in bud burst of two year old Douglas-fir seedlings among populations and families than between inland and coastal sources.

There is some controversy in the literature concerning the relationship between bud dormancy and cold hardiness. Irving and Lanphear (1976b) as well as Timmis and Worrall (1975) stated that bud dormancy and cold hardiness development are distinctly separate and independent. Seibel and Fuchigami (1978) reported that the onset of acclimation coincides with the onset of dormancy, and Kobayashi et al. (1983) found that the rate of acclimation and deacclimation are dependent on the growth stage. According to Ritchie (1986), however, there is a direct relationship between cold hardiness and bud dormancy.

Dehardening is stimulated by environmental signals and the physiological condition of the plant. Aronsson (1975) reported that dehardening of spruce and pine was influenced mainly by temperature. Smithberg and Weiser (1968) found the same relationship for deacclimating red-osier dogwood. Alden (1971) found that for Douglas-fir seedlings with fully water saturated tissues exposed to 25°C days, the loss of hardiness was delayed by cool (2°C) night temperatures. In March, when temperatures favored deacclimation, increased moisture stress resulted in hardier twigs. Van den Driessche (1969a) concluded that loss of frost hardiness in Douglas-fir seedlings is only affected by

temperature.

The effect of temperature on deacclimation is dependent on the growth stage (Kobayashi et al. 1983). Irving and Lanphear (1967a) found that dormancy helped maintain cold hardiness when Acer and Viburnum plants were exposed to one week of 21°C. In non-dormant plants dehardening occured readily. Hamilton (1973) concluded that for deacclimation, a few days at warm temperatures are only important when the plant is not dormant, whereas extended periods of warm temperatures are important, regardless of the dormancy status. Kobayashi et al. (1983) found that at the end of rest, 5°C temperatures increased hardiness, whereas 10°, 15° and 20°C temperatures decreased hardiness with increasing rates at the higher temperatures. With progressively later stages of development (during quiescence), all temperatures caused deacclimation. Kobayashi et al. (1983) found the same relationship between rehardening and growth stage. At later stages of dormancy the temperature required to reharden tissues decreased and the rate of rehardening decreased.

It is suspected that the nursery where seedlings are raised has an influence on frost hardiness and dormancy. This could affect first year field performance if, for example, trees could not harden properly at a certain nursery location, and are not frost hardy enough when lifted. Damage to the trees could result from exposure to frost during storage or in the field after outplanting (Duryea and McClain 1984). Menzies et al. (1981) found hardiness differences of 4° during the spring between Pinus

radiata (D. Don) seedlings grown at different nursery locations in New Zealand. Seedlings raised in the higher elevation nursery were hardier. In Douglas-fir, van den Driessche (1970b) reported hardiness differences between seedlings grown at three different nurseries in British Columbia.

More information on the nursery effect on frost hardiness and dormancy will be beneficial for regeneration foresters and nursery managers. Regeneration might be more successful if the regeneration forester could select the optimum nursery location, if there is any, to grow a given seed source. The following study investigates the effect of nursery location on dehardening, budburst and first-year field survival and growth of Douglas-fir seedlings raised at three nurseries.

MATERIALS AND METHODS

Two year old Douglas-fir seedlings from two seed sources that were raised at three nurseries in Oregon and Washington (described in Chapter I) were used for this study. On January 13, 15, and 21, 1986 the seedlings were lifted in nursery 3, 1 and 2, respectively, graded, packed, and transported to the laboratory in Corvallis. Seedlings were stored in a cold room at +2°C until the trees from all nurseries could be potted for the growth chamber and outdoor dehardening study.

Outdoor Dehardening Study

On January 24, 1986 seedlings were potted in forest soil in 96 25cm diameter pots, 12 trees per pot, and placed outdoors in a completely randomized design at the Forest Research Laboratory in Corvallis, Oregon. A total of 1152 trees was used for this study (16 seedlings x 4 test temperature x 3 nurseries x 2 seed sources x 3 dates). The pots were buried to the rim with sawdust to protect the roots from frost damage. A thermograph without shelter, placed 20 cm above the ground between the rows of pots recorded the ambient temperature.

Frost hardiness was determined as described in Chapter I. Hardiness determinations were taken at 0 (January 24), 25 (February 17) and 50 (March 13, 1986) days after potting. At each sampling date 16 seedlings from each seed source and nursery were frozen to four test temperatures. The remaining seedlings (64 per seed source and nursery) were examined for lateral and

terminal bud burst twice a week, starting March 22, 1986. The evaluation of bud break terminated on June 6.

Growth Chamber Study

Seedlings from the coastal seed source (061) from the three nurseries were potted in forest soil in 48 25cm diameter pots on January 24, 1986. A total of (8 trees x 4 test temperatures x 3 nurseries x 2 dates x 3 growth chamber regimes) 576 seedlings was used. They were placed in three growth chambers set to +5°, +10° and +15°C, and 16 hour photoperiod at 19,375 lumen m^{-2}. Frost hardiness of 32 plants per nursery and growth chamber was tested after 10 and 20 days. The whole plant freezing test as described in Chapter I was used to measure hardiness. The plants were frozen to four test temperatures, spaced 3°C apart. Due to a freezer malfuction on the second sampling date, the trees were frozen to three temperatures spaced 6°C apart.

Field Performance

Seedlings of each seed source from all three nurseries were planted in their original seed zone (061 and 502 from the Oregon Coast Range and the Cascades, respectively - see Fig. I.1) to determine the interaction between nursery location and seed source on first-year seedling growth and survival. The seedlings were planted in a completely randomized design at the Coast Range site on January 30, and at the Cascade site on February 26, 1986. At each location four blocks per nursery were planted at each of two sites. Each block contained 14 trees from each nursery. Therefore each nursery is represented by 112 trees (14 x 4 blocks

x 2 sites) from each seed zone. First-year height growth and survival were measured in September 1986.

Statistical Analysis

The outdoor dehardening data was analyzed in the same way as the hardening data in Chapter I. Regressions for each nursery and seed source combination were calculated to evaluate differences between nurseries and seed sources over the entire sampling period (January to March, 1986). If the models could be reduced, e.g. nurseries collapsed for one seed source, then no significant differences ($p=0.05$) existed among nurseries. Analysis of variance (ANOVA) was used to determine the nursery and seed source effect on mean tissue damage rating for each sampling date. A split plot model with nursery and seed source as main plot, and temperature as split plot was used. Differences between means were tested for significance with Tukey's studentized range test.

The budburst data was analyzed by ANOVA. A General Linear Model (GLM) procedure was used to account for the missing observations (dead trees).

The growth chamber experiment was analyzed by ANOVA as a split-split plot design for each sampling date. Nursery and growth chamber temperature were the main effects, with test temperature and block as the splits. Each treatment was tested for significance with the appropriate error term.

First year field growth was analyzed by ANOVA, with the GLM procedure to account for the unequal number of living trees in

each block. At each site the effect of seed source and nursery was analyzed separately. This was done because initial analyses showed significant nursery x seed source interactions. To determine a possible relationship between first year growth and frost hardiness in January, a regression was calculated.

RESULTS

Outdoor Dehardening

Needles

Dehardening of needles as determined by the overall regression differed significantly ($p=0.05$) between nurseries (Fig. II.1). Seedlings from nurseries 1 and 3 had hardier needles than trees raised at nursery 2, which were about 1.5°C less hardy between February and March. The fact that the regression curves of seed sources within a nursery could be collapsed to one curve in Fig. II.1 shows that no significant differences between seed source existed.

Tables II.1 and II.2 summarize the mean needle damage rating for each sampling date across nurseries and seed sources. No significant differences in needle damage ($p=0.05$) among nurseries for each sampling date were found. However, seedlings from nursery 2 have the highest damage rating at each sampling date.

Buds

Dehardening of buds of the coastal seed source was significantly different ($p=0.05$) among nurseries. Buds from nursery 1 were hardiest, followed by buds from nurseries 2 and 3 respectively (Fig. II.2). Buds from the Cascade source dehardened at the same rate in all nurseries.

The mean damage rating for buds showed no significant ($p=0.05$) differences between seed sources and nurseries (Table

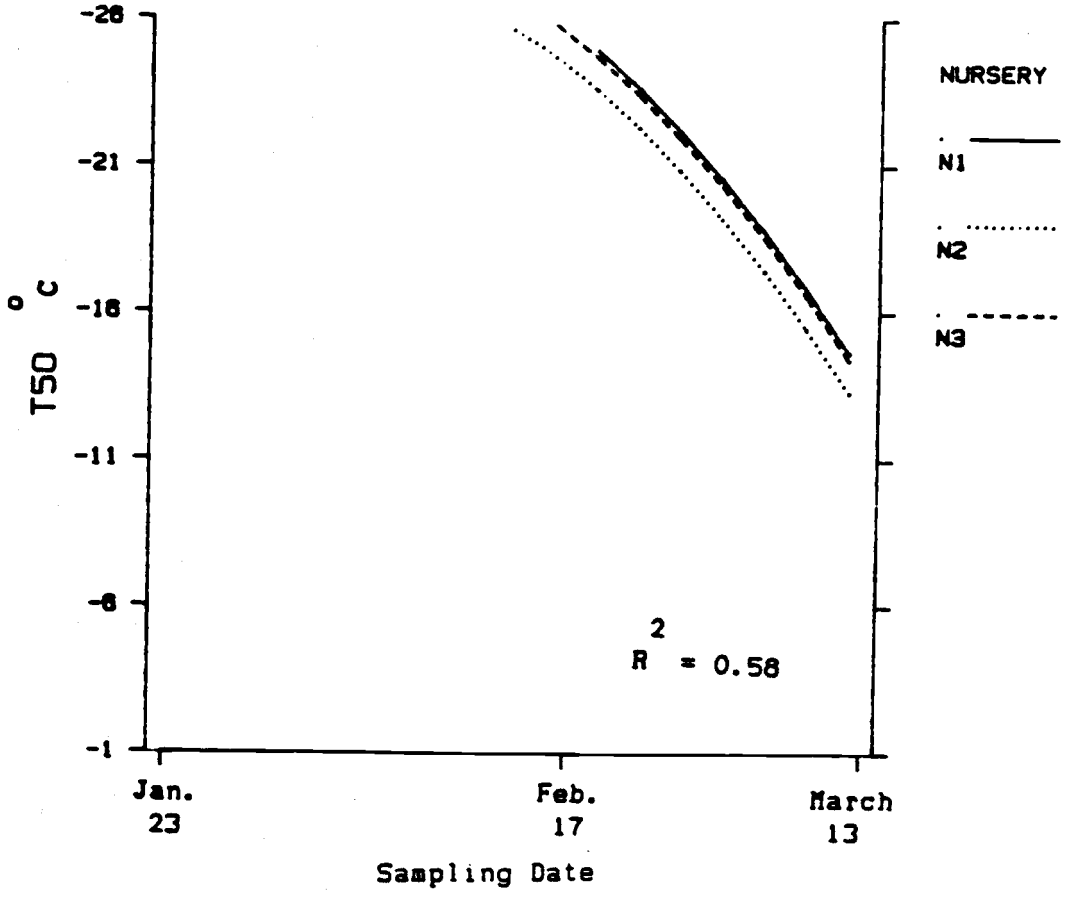


Fig. II.1: T50 for needles of Douglas-fir seedlings produced at 3 nurseries and growing outdoors at the Forest Research Laboratory in Corvallis.

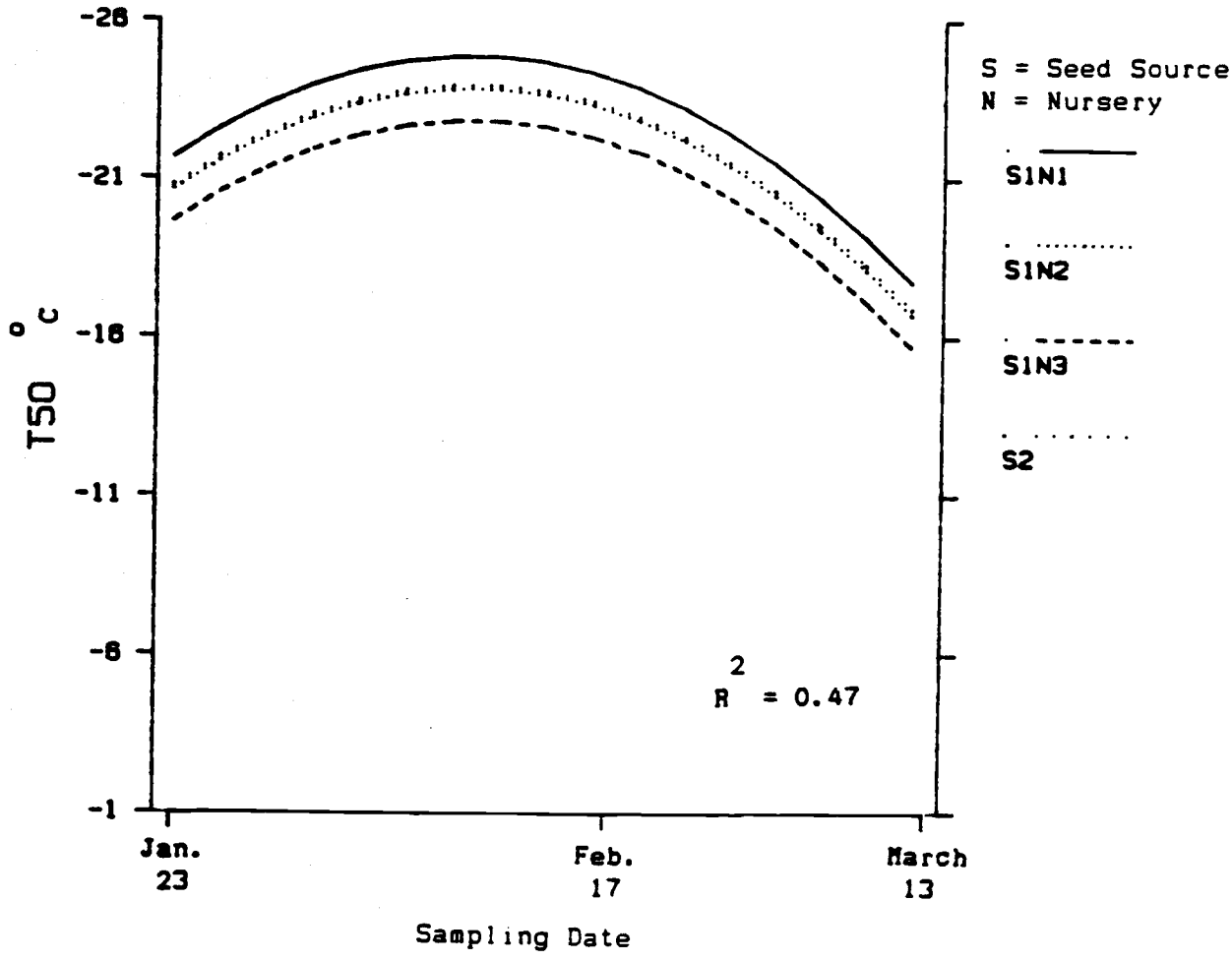


Fig. II.2: T50 for buds of Douglas-fir seedlings produced at 3 nurseries and growing outdoors at the Forest Research Laboratory in Corvallis.

Table II.1: Mean tissue damage rating of seedlings in the three nurseries at each sampling date, seed sources combined. (Means within the same vertical cell at each date were not significantly different (p=0.05)).

date	nursery	mean test temp. (°C)	needles	buds	stems
01/23/86	1	-19	0.8	1.8	1.3
	2		1.0	1.9	1.5
	3		1.2	2.1	1.5
02/17/86	1	-21.5	1.6	1.7	2.3
	2		1.9	1.8	2.3
	3		1.7	1.8	2.2
03/14/86	1	-8.5	1.8	0.3	2.5
	2		2.0	0.6	2.6
	3		1.5	0.8	1.8

Table II.2: Mean tissue damage rating of seed sources at each sampling date, nurseries combined.
(Means followed by the same letter within the same vertical cell at each date are not significantly different (p=0.05)

date	seed source	mean test temp. (°C)	needles	buds	stems
01/23/86	coastal	-19.0	1.0 A	2.0 A	1.3 A
	Cascade		1.0 A	1.9 A	1.5 B
02/17/86	coastal	-21.5	1.7 A	1.7 A	2.1 A
	Cascade		1.8 A	1.8 A	2.4 A
03/14/86	coastal	-8.5	1.9 A	0.7 A	2.2 A
	Cascade		1.6 A	0.5 A	2.4 A

II.1 and II.2). Nursery 1 had the lowest damage rating at all three sampling dates.

Stems

Deacclimation of stems as determined by the overall regression shows significant differences ($p=0.05$) between seed sources. Stems of the coastal source (061) were consistently hardier than stems of the Cascade source (502) (Fig. II.3).

Significant differences ($p=0.05$) for stem deacclimation between seed sources were found in January (Table II.2). At this sampling date nursery 1 had the hardiest stems, whereas in February and March nursery 3 had the hardiest stems.

Bud Burst

Table II.3 summarizes the mean number of days to 100% lateral and terminal bud burst of the potted trees at the Forest Research Laboratory. The seedlings from nursery 2, the coastal nursery, burst bud significantly ($p=0.05$) earlier than the trees from the other two nurseries. Seedlings from nursery 1 flushed last. Mean date of budburst within each seed source differed by only two days within each specific nursery. Figure II.4 shows the ambient air temperature at the Forest Research Laboratory, recorded by the thermograph.

Growth Chamber Study

Needles

The growth chamber temperatures did not alter needle hardiness after 10 days. After 20 days, however, +15°C and +10°C

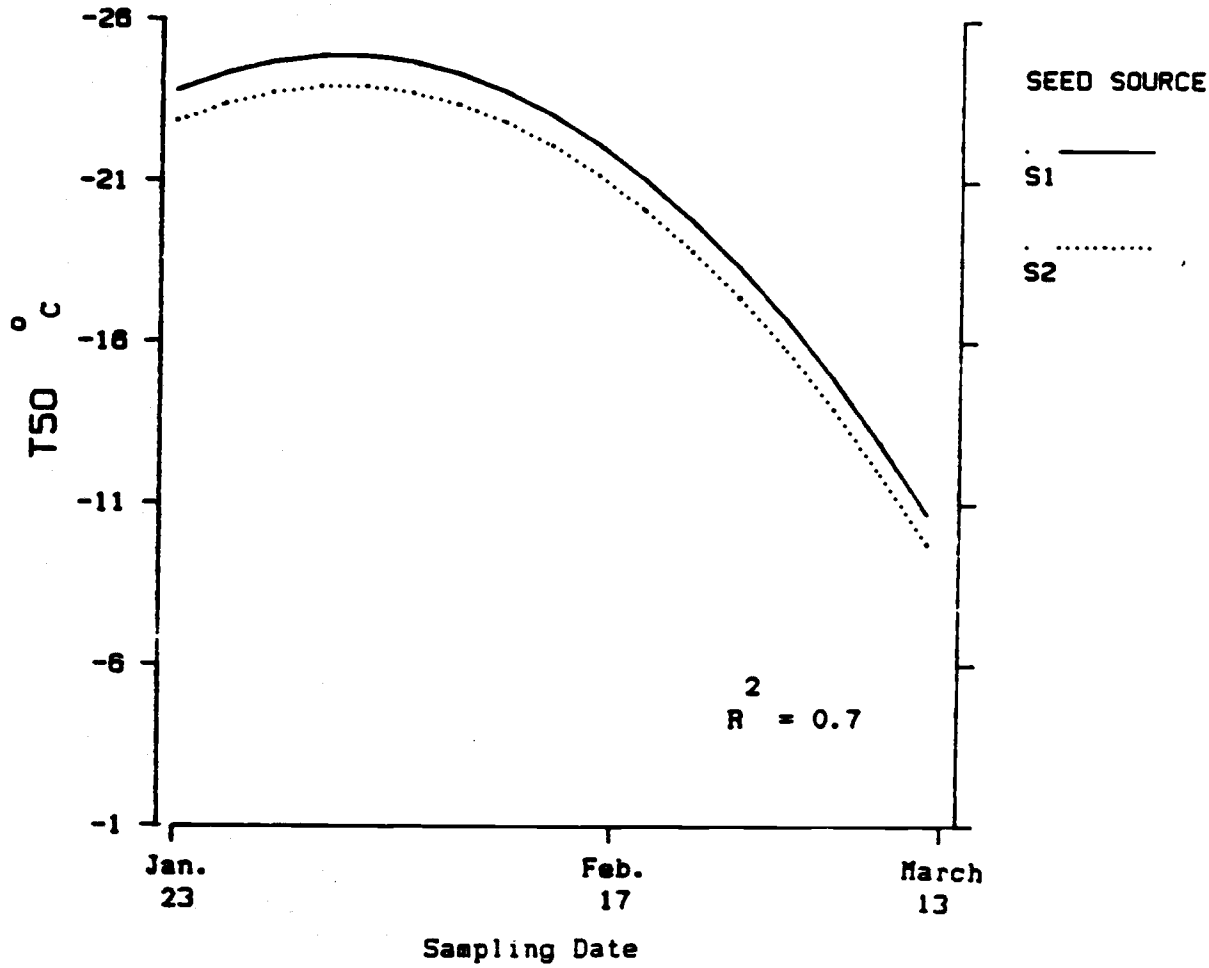
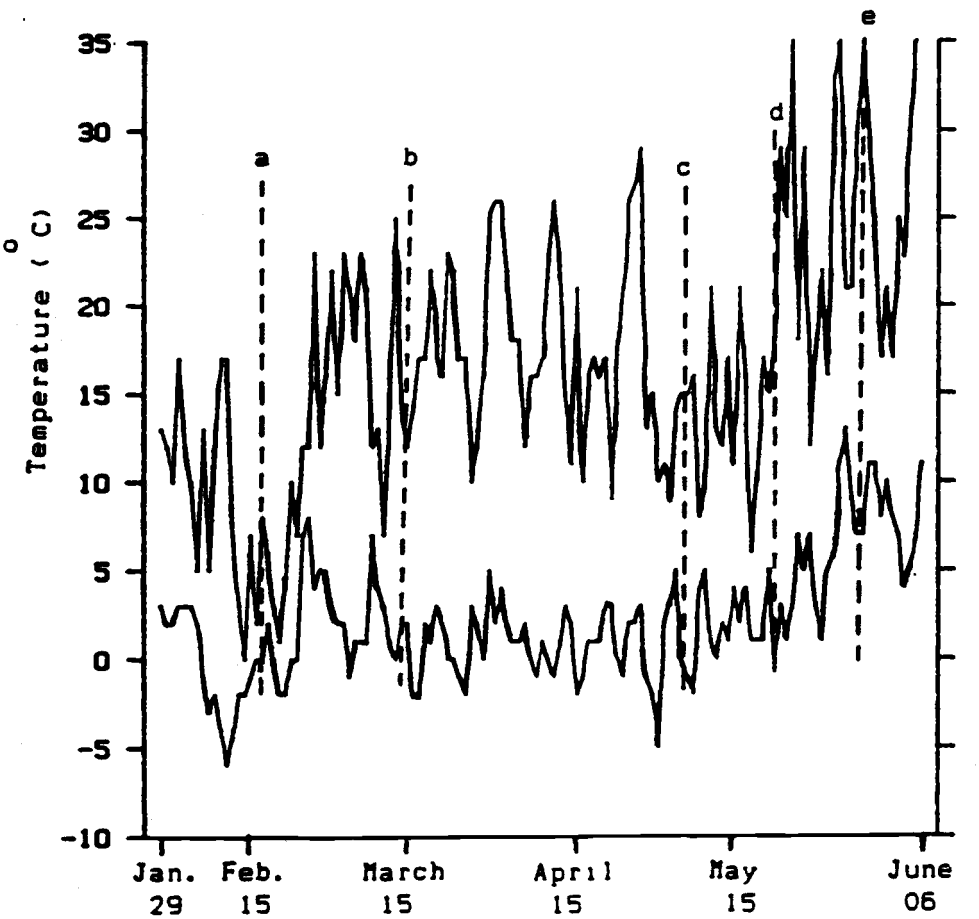


Fig. II.3: T50 for stems of Douglas-fir seedlings produced at 3 nurseries and growing outdoors at the Forest Research Laboratory in Corvallis.



a = sampling on Feb. 18. 1986
b = sampling on March 13. 1986
c = last frost (-2.2oC) on April 30. 1986
d = mean terminal bud burst nursery 2. seed source 502
e = mean terminal bud burst nursery 1. seed source 502

Fig. II.4: Maximum and minimum temperature from Jan. 29 to June 6. 1986 at the Forest Research Laboratory in Corvallis.

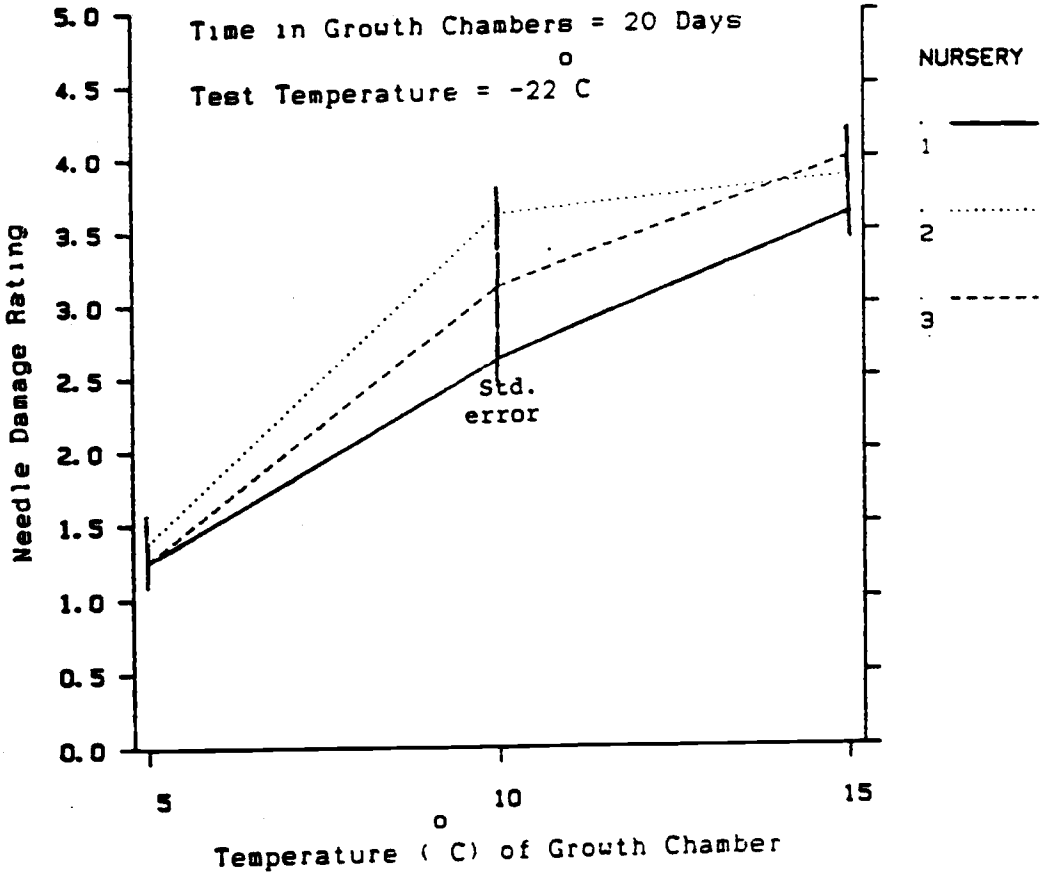


Fig. II.5: Needle damage rating of Douglas-fir seedlings produced at 3 nurseries and grown under 3 constant temperature regimes (+5°, +10° and +15°C) from Jan. 24 to Feb. 13, 1986 in growth chambers.

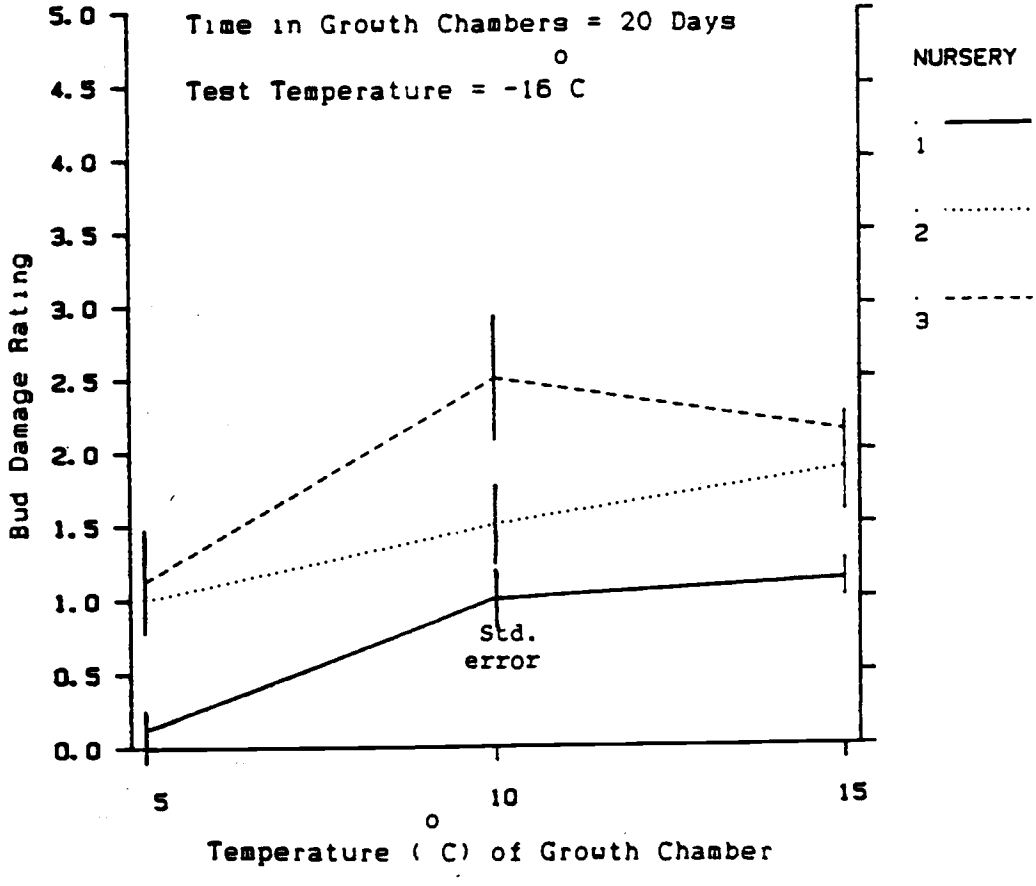


Fig. II.6: Bud damage rating of Douglas-fir seedlings produced at 3 nurseries and grown under 3 constant temperature regimes (+5o, +10o and +15oC) from Jan. 24 to Feb. 13, 1986 in growth chambers.

treatments triggered rapid dehardening of stems (Fig. II.5). A significant ($p=0.01$) nursery x test temperature interaction was found for the second sampling (Appendix A).

Buds

Dehardening of buds was stimulated after seedlings stayed for 20 days in the growth chambers at $+10^{\circ}$ and $+15^{\circ}C$. The analysis of variance shows significant nursery ($p=0.05$) and growth chamber temperature ($p=0.01$) effects and, a significant nursery x test temperature interaction (Appendix B). Figure II.6 shows the increasing damage rating for trees from nurseries 1, 2, and 3, respectively.

Stems

Stem dehardening occured faster than needle and bud dehardening. The analysis of variance shows that nursery and growth chamber treatment were highly significant ($p=0.01$) after 10 and 20 days exposures in the growth chambers (Appendix C). Growth chamber temperatures of $+10^{\circ}$ and $+15^{\circ}C$ stimulated dehardening. In general, trees from nursery 1 had the lowest damage rating (Fig. II.7).

Field Performance

First-year field performance, expressed as growth and survival, is summarized in Table II.4. The seed sources, planted in their original seed zone, were represented with two sites in the Coast Range and the Cascades. These sites were kept separate in the analysis due to different competing vegetation. The

Table II.3: Mean days to lateral (DLBB) and terminal (DTBB) bud burst of trees dehardening outdoors at the Forest Research Laboratory, Corvallis, Oregon from January to June 1986. (Means followed by the same letter within the same vertical cell at each site are not significantly different (p=0.05)).

seed source	nursery	DLBB	DTBB
coastal (061)	1	89 A	116 A
"	2	76 BC	108 BC
"	3	93 A	114 A
Cascade (502)	1	92 AC	117 A
"	2	78 B D	106 BC
"	3	85 CD	112 A

Table II.4: Mean first year growth (cm) and survival percent of seedlings planted in the field in their original seed zone. (Means in each vertical cell at each site followed by the same letter are not significantly different (p=0.05)).

nursery	site	Coast Range seed source 061 growth (cm)	Coast Range seed source 061 survival (%)	Cascades seed source 502 growth (cm)	Cascades seed source 502 survival (%)
1	1	10.2 AB	96 A	4.5 B	84 A
2	1	11.7 A	100 A	7.6 A	87 A
3	1	8.3 B	91 A	4.6 B	59 B
1	2	18.5 A	98 A	7.5 A	89 A
2	2	14.2 A	100 A	8.6 A	91 A
3	2	11.2 A	89 A	4.8 B	91 A

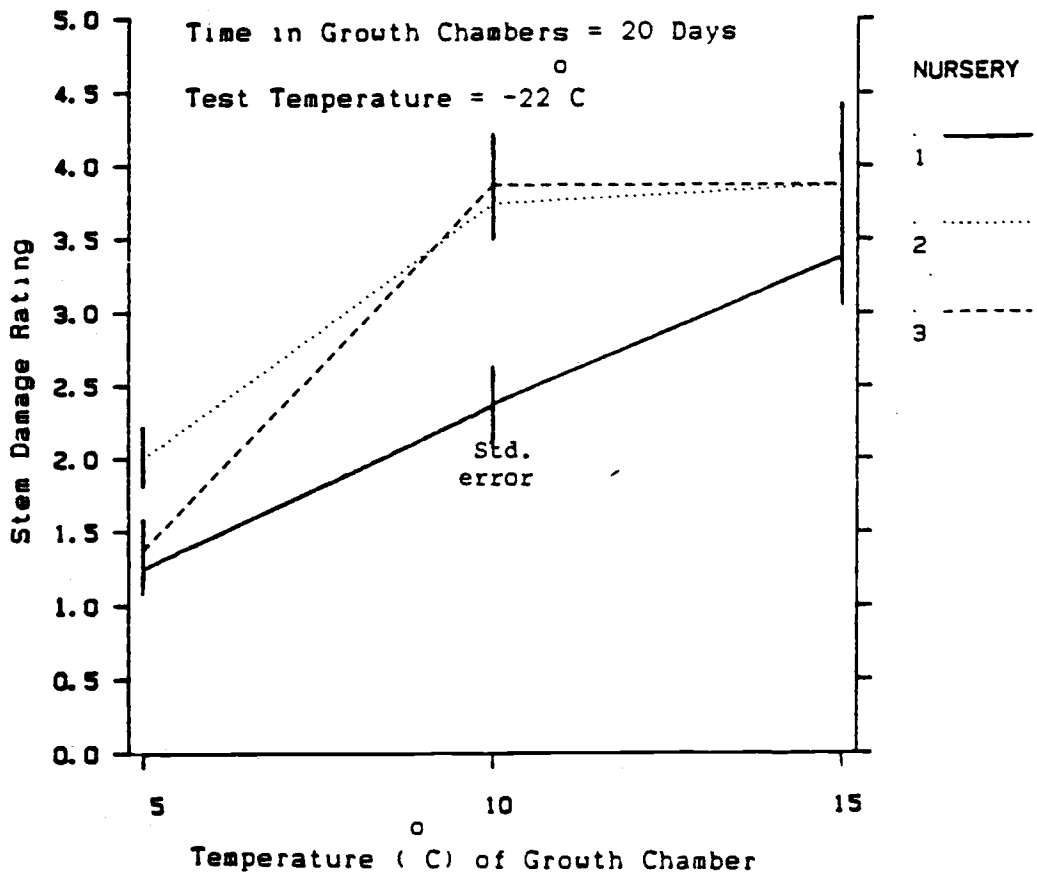


Fig. II.7: Stem damage rating of Douglas-fir seedlings produced at 3 nurseries and grown under 3 constant temperature regimes (+5°, +10° and +15°C) from Jan. 24 to Feb. 13, 1986 in growth chambers.

seedlings of site 1 in the Coast Range and the Cascades had considerable competition from salal, fern, blackberries, and in the Cascades also from poison oak. Seedlings in the Cascades planted at 950m grew, on the average, less and had slightly lower survival than trees planted in the Coast Range at 475m. Other than differences in the environment, the plantation in the Coast Range was sprayed twice in 1986 to control grass and brush, whereas the Cascade plantation did not receive any release treatments after planting.

Differences in mean growth among nurseries were apparent. Seedlings from nurseries 2 and 1 grew, in general, more than seedlings from nursery 3. It is important to mention that the trees from nursery 3 were not as large at the time of planting as the trees from the other two nurseries. Grading standards had to be lowered, especially for the Cascade source. This might partially explain the low survival percent of this stock.

A regression with first year mean growth and frost hardiness of the seedlings in January was calculated, but no correlation was found between the variables.

DISCUSSION

The main objective of this study was to test the influence of nursery location on deacclimation, budburst, and first-year field performance of 2+0 Douglas-fir seedlings. One hypothesis of this study was that seedlings from the higher elevation nursery will be more frost hardy than seedlings from a low elevation nursery. Another hypothesis was that seedlings raised in a nursery close to the ocean will be less frost hardy than plants raised in a nursery at a greater distance from the ocean.

The outdoor dehardening study shows no strong evidence to support the two hypothseses. Generally, dehardening of the needles followed a predictable pattern. Bud dehardening of the coastal seed source (Fig. II.1) confirms the assumption that the higher elevation nursery (1) produces the hardiest seedlings, as reported by Menzies et al. (1981). The dehardening results for the stem tissue, as calculated by the regression and analysis of variance, reject the hypotheses, because significant differences ($p=0.05$) are found only between seed sources. This would confirm van den Driessche's findings (1970b), that the high elevation provenance deacclimated slightly earlier in spring. The ranking of the mean damage values (Table II.2) confirms that the high elevation source is hardier in January, though significantly only for the stems, but dehardens faster in February and March than the coastal seed source.

From January to February, hardening in the needle and bud

tissue proceeded, whereas the stem tissue had started the dehardening process already. From February to March dehardening continued rapidly in all tissues (Fig. II.1, II.2, and II.3). Looking at the ambient temperature under which the seedlings dehardened (Fig. II.4), there is evidence that prolonged minimum temperatures below zero degree Celsius in January and February promote hardening as found by Sakai (1966). From February 18 to March 13, 1986 minimum temperatures rose only twice above +5°C, but the maximum temperatures stayed almost all of March well above +20°C. Alden (1971) found the same slow dehardening with cool night (2°C) and warm day temperatures (25°C). Ketchie and Beeman (1973) demonstrated a better relation between the maximum temperature preceeding sampling for 7 days and cold hardiness than between the minimum temperature prior to sampling and frost resistance.

Results of the bud burst study strongly suggest that the nursery environment in which seedlings were raised had an influence on the flushing pattern. Trees raised in the lower elevation coastal nursery burst their terminal buds first, whereas trees from higher elevation or more inland nurseries flushed last. This is in agreement with Hermann and Lavender (1968), who found that seedlings in a nursery with warmer climate burst bud six days earlier than trees in the nursery with colder environmental conditions. These facts are supported by Campbell and Sugano's (1979) suggestions, that the date of bud burst is mainly a function of spring temperatures.

Results of the growth chamber study support the hypothesis that dehardening is influenced by the previous environmental conditions that seedlings experienced. The higher elevation nursery (1) had trees with the hardiest tissue, especially at the higher growth chamber temperatures. The most northern situated nursery (3) and trees from the coastal nursery (2) had less resistance to frost. This is in agreement with Menzies' et al. results for radiata pine (1981).

Needles and buds did not respond strongly to the temperature regimes after 10 days, but showed well defined nursery and treatment differences after 20 days. Dehardening of the stems started already after 10 days at 10°C and 15°C and resulted in significant nursery differences (Fig. II.6). These differences were reinforced after 20 days, with an even larger treatment effect than at the first sampling date (Appendix C). The dehardening of the different tissues under the growth chamber and outdoor dehardening conditions correspond, as stems consistently deacclimated more readily than needles and buds. The results suggest that a period of more than 10 days above 10°C is necessary to induce dehardening of sufficiently chilled Douglas-fir seedlings. Hamilton (1973) stated that extended periods of warm temperatures are important for plant deacclimation. The response of plants to the growth chamber regimes correspond with the results of other research that 5°C retains or increases hardiness, whereas 10°C and 15°C promotes increasing deacclimation (Smithberg and Weiser 1968, Hamilton 1973,

Kobayashi et al. 1983).

First-year growth was independent of seedling frost hardiness in January. Differences in growth and survival are apparent, but cannot be attributed to nursery location.

CHAPTER III

COMPARING ELECTRICAL CONDUCTIVITY AND THE WHOLE PLANT FREEZING TEST AS VIABILTY TESTS FOR DETERMINING FROST HARDINESS OF DOUGLAS-FIR SEEDLINGS

ABSTRACT

Viability of Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] seedlings following freezing tests was determined at five sampling dates by whole plant freezing and electrical conductivity methods. Conductivity of shoot tips, expressed as index of injury (IT) and relative conductivity, was measured and correlated with the visual viability (browning) in needles, buds, and stems of seedlings subjected to the whole plant freezing test. Correlations between the conductivity and whole plant freezing test for determining injury were poor. The highest correlation coefficient (r) was 0.69. It is suspected that the different hardening rates of tissues and the plant to plant variability contributed to the low correlation coefficients. The electrical conductivity viability test did not provide a satisfactory estimation of frost hardiness of two-year-old bareroot Douglas-fir seedlings.

INTRODUCTION

Monitoring frost hardiness is useful in protecting nursery plants from freeze damage (Glerum 1985, Colombo et al. 1984). In container nurseries it can be used by managers to alter conditions for maximum seedling hardiness, and to determine the time when stock can be moved outdoors. In bareroot nurseries it can be important to determine the need for frost protection (Burr et al. 1986).

Many tests are used to evaluate the viability of frozen plant material. The whole plant freezing test, electrolytic conductivity, electrical impedance, TTC reduction, differential thermal analysis (DTA), and ethylene and ethane production are the most widely used screening techniques (Colombo et al. 1984, Glerum 1985, Burr et al. 1986, Harber and Fuchigami 1986). Often more than one test is used to calibrate the visual viability ratings with continuous measurements or regrowth ability.

The conductivity method is based on the fact that when tissue is injured the site of injury is the cell membrane, which loses its selective permeability. Upon injury the electrolytes in the aqueous cellular cytoplasm have a greater tendency to diffuse out of the tissue when it is placed in water. The extent of the injury is proportionate to the amount of electrolytes that diffuse out of the tissue and is estimated by comparing the conductivity of uninjured tissue diffusate with that of injured tissue (Wilner 1962, Dexter 1932).

According to Glerum (1985), only the whole plant freeze test and the electrolytic conductivity method are operational frost hardiness tests for conifer seedlings. Both of these methods have their advantages and disadvantages. The whole plant freezing test gives a satisfactory accuracy of frost hardiness, but takes 7-14 days until the results are available, and involves destructive sampling of the plants. The electrolytic conductivity test has to be calibrated to the whole plant freezing test or to another regrowth test to predict hardiness (Burr et al. 1986). Most of the time only one tissue or organ like needles, buds, or stems is used. Since tissues harden at different rates (Alden 1981), it is possible that the tissue hardiness test over- or underestimates the hardiness of the whole plant. The big advantage of the use of tissues/organs is the availability of results after 2 days, the great amount of material available, the ease of handling the materials, and the survival of the plant.

Burr et al. (1986) compared the whole plant freeze test, electrolyte leakage, and other tests on three western conifer species. The predictive ability of the whole plant freeze test and the electrolyte leakage was rated excellent and good, respectively. Harber and Fuchigami (1986) found visual damage rating (browning) and conductivity of rhododendron leaf disks to be highly correlated. Ketchie et al. (1972) measured the electrolytic conductance of apple, pear, and citrus seedlings and found a close correlation between conductance and survival.

Timmis (1973) found significant correlations between the browning of Douglas-fir needles attached to a twig and electrolytic conductivity of excised needles. However, the electrolyte leakage provided a less sensitive injury estimation. Van den Driessche (1976) attempted to predict cold hardiness of Douglas-fir seedlings by index of injury and conductivity methods. He found that conductivity percentage could predict lethal temperature for whole plants reasonably well, but was not able to establish critical values that corresponded to the lethal temperature for 50% of the whole plants.

The purpose of this study was to compare electrolytic conductivity and the whole plant freeze test as hardiness estimators of Douglas-fir seedlings. The electrolytic conductivity procedures were adapted with slight modifications from Colombo et al. (1984) method to measure frost hardiness for extended greenhouse operations. This study also evaluates the applicability of Colombo's method, which was designed for one-year old container stock, to estimate frost hardiness of two-year-old bareroot seedlings.

MATERIALS AND METHODS

Two-year-old Douglas-fir seedlings from the coastal seed source (061) were used for this study. Seedlings were taken simultaneously for the conductivity and the whole plant freezing test at five sampling dates from September to December 1985 as described in Chapter I.

Shoot tips (2-3cm length), including needles, buds, and stem, from the two upper most lateral branches were excised from each seedling. One shoot tip was used for the freezing treatment, and the other was used as a control. At each sampling date 24 seedlings for the electrolytic conductivity and 48 seedlings for the whole plant freezing method were tested at each of 4 temperatures. Shoots were washed with tap water, rinsed with distilled water and placed in test tubes. The control shoot from each seedling was left in the cooler at +2°C. The shoot tip to be frozen was put in the freezer and subjected to the same freezing regime as the seedlings of the whole plant freezing test (Chapter I). After the freezing treatment the test tubes with the control and frozen shoot tips were filled with 30 ml of distilled water each and shaken for 7 hours at 23°C (+/-2°C). Conductivity was measured with an ALTEX conductivity bridge (model RC16C, conductivity cell constant 1.0). Test tubes were put for 5 minutes in a boiling water bath to kill the shoot tissue, shaken for two hours at 23°C (+/-2°C), and final conductivity determined as before. The index of injury (IT) was calculated according to the method of Flint et al. 1967 and Colombo et al. 1984):

$$IT = \frac{\text{RC frozen} - \text{RC control}}{1 - (\text{RC control}/100)}$$

RC frozen = (EC frozen/EC frozen killed) * 100
RC control= (ECcontrol/EC control killed) * 100

IT is a relative expression of the amount of injury caused by freezing. The lower the IT, the more frost hardy the seedlings.

Relative conductivity (RC frozen) was also used in the analysis to compare damage from the whole plant freeze test and relative conductivity of the frozen sample only.

Correlations were calculated for each date and freezing temperature between the mean tissue damage from the whole plant freezing test (as determined in Chapter I) of needles, buds, and stems, and the electrolytic conductivity, expressed as IT and as relative conductivity. Negative IT values were set to zero for the correlation, because they would be meaningless for the interpretation of the results. Ketchie (pers. comm. 1987) suggested that negative values result from freezing temperatures that harden the tissue artificially.

RESULTS AND DISCUSSION

The correlation coefficients (Tab.III.1 and III.2) that compare IT and relative conductivity with the mean tissue damage of needles, buds, and stems were all relatively low. The highest values were -0.69 and -0.68 for relative conductivity and IT respectively.

One reason for the poor relationship between the whole plant freeze test and the electrolytic conductivity may be that the conductivity is a sum of membrane leakage of needle, bud, and stem tissue. Alden (1971) found that these tissues harden at different rates. Figure III.1 shows the IT, relative conductivity, and the mean damage of needles, buds, and stems of seedlings that were frozen to -7 and -13°C at different sampling dates. In September and early October buds were least hardy, acclimated rapidly, and were even hardier than stems and needles at the end of October. By December 9, the hardiness of all three tissues were similar. A shoot tip of two-year-old bareroot Douglas-fir seedlings for the electrical conductivity method provided results that were not sensitive enough to estimate frost hardiness of the whole plant. Colombo et al.'s (1984) method was designed for one year old container seedlings grown in a greenhouse. The controlled greenhouse environment and the younger plants may have been morphologically and physiologically more uniform. Seedlings grown in a bareroot nursery are exposed to a wider variety of environmental conditions and nursery cultural practices, which promote larger plant to plant

Table III.1: Correlation coefficients of index of injury (IT) and mean damage rating of needles, buds, and stems of Douglas-fir seedlings.

date	test temp. (C)	needles	buds	stems
09/11/85	-10	-0.38	0.17	0.33
	- 7	-0.31	-0.18	0.12
	- 1	-0.18	-0.17	-0.58 **
10/08/85	-10	-0.31	0.17	-0.01
	- 7	-0.14	-0.14	0.29
	- 4	0.26	0.21	0.17
	- 1	-0.59 **	-0.68 ***	-0.59 **
10/29/85	-13	0.45 *	0.20	0.23
	- 7	0.14	0.31	-0.02
	- 4	0.18	0.22	-0.18
11/18/85	-19	0.08	-0.18	0.01
	-16	-0.55 *	-0.48 *	-0.37
	-13	0.30	-0.38	-0.31
	-10	-0.41	-0.01	-0.10
12/09/85	-25	0.13	0.15	0.28
	-22	0.44	-0.21	0.36
	-19	0.29	-0.22	-0.11
	-16	0.14	-0.14	-0.25

* ** *** are significance levels of 10, 5, and 1% respectively.

Table III.2: Correlation coefficients of relative conductivity and mean damage of needles, buds and stems of Douglas-fir seedlings.

date	test temp. (C)	needles	buds	stem
09/11/85	-10	0.49 *	-0.19	-0.14
	- 7	-0.28	-0.08	0.02
	- 1	0.22	-0.10	-0.31
10/08/85	-10	-0.69 ***	0.43	0.09
	- 7	-0.10	-0.03	0.12
	- 4	-0.04	0.57 **	0.25
	- 1	0.09	0.38	-0.14
10/29/85	-13	-0.19	-0.51 *	0.37
	-10	0.23	0.45	-0.08
	- 4	0.36	0.40	-0.39
11/18/85	-19	-0.38	-0.46	-0.50 *
	-16	-0.62 **	-0.44	-0.64 **
	-13	0.32	-0.31	-0.25
	-10	-0.27	-0.25	-0.14
12/23/85	-25	-0.54 **	-0.18	-0.54
	-22	0.11	-0.04	-0.31
	-19	0.04	-0.41	-0.28
	-16	0.39	-0.16	-0.21

* ** *** are significance levels of 10, 5, and 1% respectively.

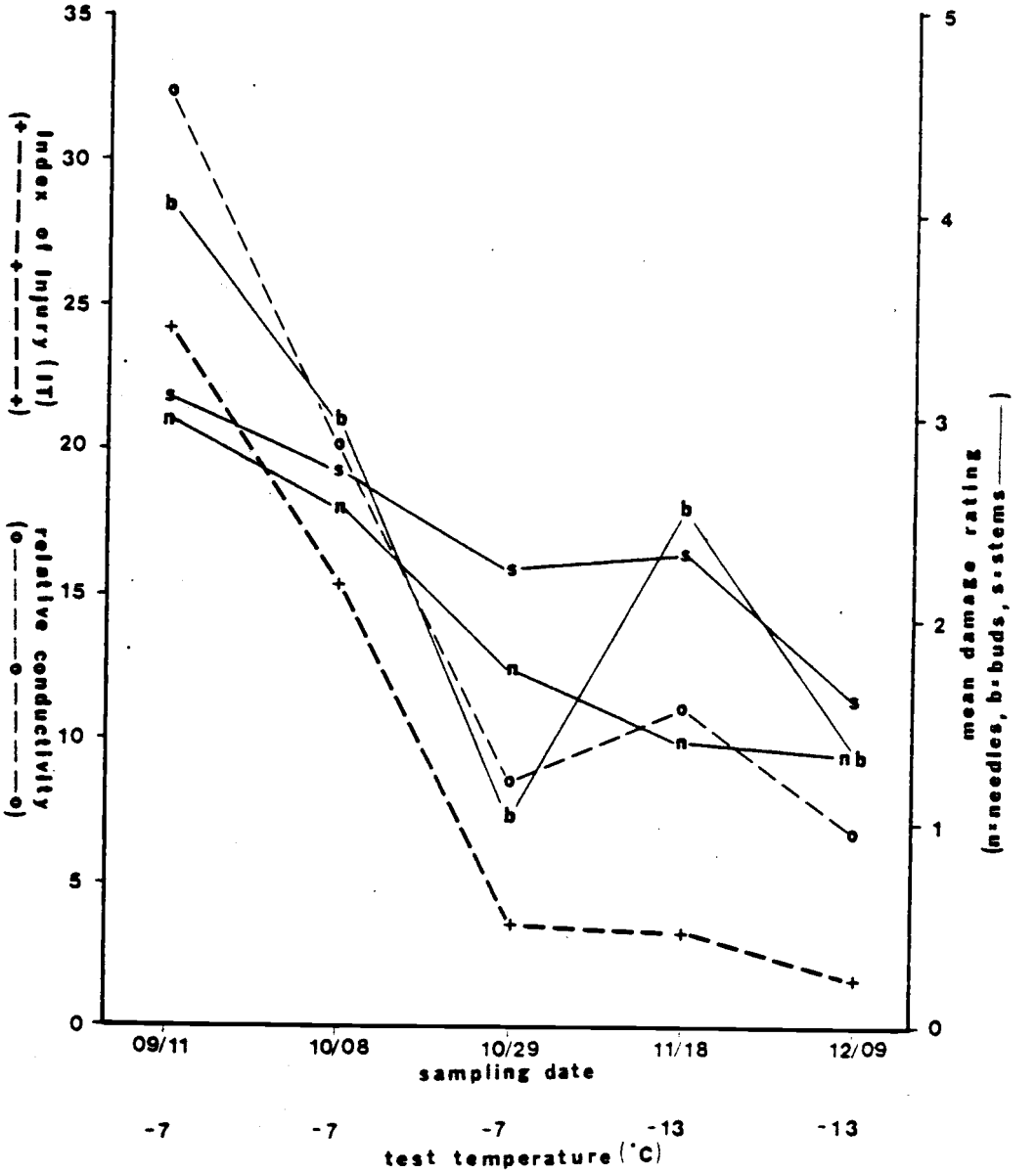


Fig. III.1: Index of injury (IT), relative conductivity, and mean damage rating of needles, buds, and stems of Douglas-fir seedlings frozen at several sampling dates to various test temperatures.

variability. Frost hardiness of trees from a bareroot nursery might not be as uniform as container seedlings that are under less variable environmental conditions. Because of the variable acclimation states of seedlings plus the different hardiness of tissues it might not be too surprising that the results of the conductivity method do not correlate well with the evaluations of the whole plant freeze test.

The results of other researchers show that a satisfactory correlation between electrolytic conductivity and visual damage evaluation (browning) exists if the same tissues or organs are compared (Burr et al. 1986, Harber and Fuchigami 1986). However, Timmis (1973) found that the conductivity method was a less sensitive injury estimation for hardiness of Douglas-fir needles than the visual estimation of browning.

Another source of variation is the methodology used for the electrical conductivity. Preparation of samples, freezing protocol, amount of liquid added to samples, time elapsed between conductivity measurements are just some of the factors that influence the final result. Some authors suggest, for example, to add 5 ml of distilled water for each gram of the sample weight. (Ketchie et al. 1972).

Recent research suggests a different protocol for the conductivity method. Measurements of conductivity after 1 and 18 hours are suggested after the sample has been exposed to freezing stress. The differential conductivity seemed to estimate T50, the temperature where 50% of the cells are injured, fairly well

(Zhang and Willison 1987). This method was used in cell suspension cultures and is far from being operationally usable. However, it might have a potential if the methodology is tested on whole plants. This approach would have the advantage that the samples do not have to be killed, as they were in this study, and results would be available in an even shorter time.

For operational testing of frost hardiness the electrolytic conductivity method as proposed by Colombo et al. (1984) and modified in this study needs some refinement before it can be used as a reliable technique. Calibration with the whole plant freeze test or a regrowth test seem to be essential in any case. Even though it is more time consuming, the whole plant freezing test with visual viability estimation is a reliable frost hardiness test for seedlings.

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APPENDIX

Appendix A

SUMMARY ANOVA TABLE FIRST GROWTH CHAMBER SAMPLING

VARIABLE NEEDLES

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	44	25.37847222	0.57678346	4.09	0.0001
Error	99	13.94791667	0.14088805		
Corrected Total	143	39.32638889			

R-Square	C.V.	Root MSE	ND Mean
0.645329	41.259910	0.37535057	0.90972222

Source	DF	Anova SS	Mean Square	F Value	Pr > F
NURS	2	1.14930556	0.57465278	2.07	n.s.
TREAT	2	2.94097222	1.47048611	5.29	n.s.
NURS*TREAT	4	1.11111111	0.27777778	1.10	-
TEMP	3	12.57638889	4.19212963	7.26	***
TREAT*TEMP	6	2.10069444	0.35011574	0.61	n.s.
NURS*TREAT*TEMP	12	1.47222222	0.12268519	0.84	n.s.
NURS*TEMP	6	2.72569444	0.45428241	3.14	n.s.
BLOCK(NURS)	9	1.30208333	0.14467593	1.03	-

SUMMARY ANOVA TABLE SECOND GROWTH CHAMBER SAMPLING

VARIABLE NEEDLES

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	35	138.47685185	3.95648148	25.54	0.0001
Error	72	11.15277778	0.15489969		
Corrected Total	107	149.62962963			

R-Square	C.V.	Root MSE	ND Mean
0.925464	21.252941	0.39357298	1.85185185

Source	DF	Anova SS	Mean Square	F Value	Pr > F
NURS	2	2.67129630	1.33564815	1.61	n.s.
TREAT	2	51.81018519	25.90509259	31.26	***
NURS*TREAT	4	3.31481481	0.82870370	0.89	-
TEMP	2	57.78240741	28.89120370	25.97	***
TREAT*TEMP	4	16.57870370	4.14467593	3.72	*
NURS*TREAT*TEMP	8	1.04629630	0.13078704	0.87	n.s.
NURS*TEMP	4	3.92592593	0.98148148	6.56	***
BLOCK(NURS)	9	1.34722222	0.14969136	0.97	-

Appendix B

SUMMARY ANOVA TABLE FIRST GROWTH CHAMBER SAMPLING

VARIABLE BUD:

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	44	88.77083333	2.01751894	7.84	0.0001
Error	99	25.46354167	0.25720749		
Corrected Total	143	114.23437500			

R-Square	C.V.	Root MSE	BUD Mean
0.777094	61.629117	0.50715628	0.82291667

Source	DF	Anova SS	Mean Square	F Value	Pr > F
NURS	2	3.57291667	1.78645833	0.98	n.s.
TREAT	2	4.90625000	2.45312500	1.35	n.s.
NURS*TREAT	4	7.27083333	1.81770833	2.49	-
TEMP	3	59.50520833	19.83506944	22.66	***
TREAT*TEMP	6	2.46875000	0.41145833	0.47	n.s.
NURS*TREAT*TEMP	12	6.14583333	0.51215278	1.69	n.s.
NURS*TEMP	6	2.17708333	0.36284722	1.19	n.s.
BLOCK(NURS)	9	2.72395833	0.30266204	1.18	-

SUMMARY ANOVA TABLE SECOND GROWTH CHAMBER SAMPLING

VARIABLE BUD:

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	35	230.40046296	6.58287037	35.51	0.0001
Error	72	13.34722222	0.18537809		
Corrected Total	107	243.74768519			

R-Square	C.V.	Root MSE	BUD Mean
0.945242	21.577726	0.43055556	1.99537037

Source	DF	Anova SS	Mean Square	F Value	Pr > F
NURS	2	6.35185185	3.17592593	9.98	*
TREAT	2	10.01851852	5.00925926	15.74	***
NURS*TREAT	4	1.27314815	0.31828704	0.14	-
TEMP	2	204.46296296	102.23148148	87.28	***
TREAT*TEMP	4	1.37037037	0.34259259	0.29	n.s.
NURS*TREAT*TEMP	8	0.79629630	0.09953704	0.49	n.s.
NURS*TEMP	4	4.28703704	1.07175926	5.24	*
BLOCK(NURS)	9	1.84027778	0.20447531	1.10	-

Appendix C

SUMMARY ANOVA TABLE FIRST GROWTH CHAMBER SAMPLING

VARIABLE STEM:

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	44	86.10763889	1.95699179	7.03	0.0001
Error	99	27.55729167	0.27835648		
Corrected Total	143	113.66493056			

R-Square	C.V.	Root MSE	STEM Mean
0.757557	54.073793	0.52759500	0.97569444

Source	DF	Anova SS	Mean Square	F Value	Pr > F
NURS	2	11.01388889	5.50694444	33.74	***
TREAT	2	16.26388889	8.13194444	49.83	***
NURS*TREAT	4	0.65277778	0.16319444	0.26	-
TEMP	3	38.36631944	12.78877315	14.25	***
TREAT*TEMP	6	3.13888889	0.52314815	0.58	n.s.
NURS*TREAT*TEMP	12	9.56944444	0.79745370	1.10	n.s.
NURS*TEMP	6	0.59722222	0.09953704	0.14	n.s.
BLOCK(NURS)	9	6.50520833	0.72280093	2.60	-

SUMMARY ANOVA TABLE SECOND GROWTH CHAMBER SAMPLING

VARIABLE STEM:

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	35	111.35185185	3.18148148	11.11	0.0001
Error	72	20.61111111	0.28626543		
Corrected Total	107	131.96296296			

R-Square	C.V.	Root MSE	STEM Mean
0.843811	26.506459	0.53503779	2.01851852

Source	DF	Anova SS	Mean Square	F Value	Pr > F
NURS	2	7.03240741	3.51620370	22.67	***
TREAT	2	35.01851852	17.50925926	112.90	***
NURS*TREAT	4	0.62037037	0.15509259	0.17	-
TEMP	2	47.01851852	23.50925926	20.59	***
TREAT*TEMP	4	10.55092593	2.63773148	2.31	n.s.
NURS*TREAT*TEMP	8	5.56018519	0.69502315	1.66	n.s.
NURS*TEMP	4	1.78703704	0.44675926	1.07	n.s.
BLOCK(NURS)	9	3.76388889	0.41820988	1.46	-