AN ABSTRACT OF THE THESIS OF

Barbara Ellen Thompson   for the degree of Master of Science

in Forest Science   presented on November 26, 1979

Title: Root Regeneration Potential in Douglas-fir and Western Hemlock

Seedlings: The Role of Environmental Factors and Current Photosynthesis

Abstract Approved: Signature redacted for privacy.

Joe B. Zaerr

Three experiments were conducted on containerized seedlings to
examine the short-term and long-term effects of environment on the root
regeneration potential, and to examine the fate of the current photo-
synthate and its relationship to the root regeneration potential.

The short-term effects were studied first in a 3x3x2 factorial
experiment comprising photoperiod, air temperature, and soil type.
Treatments were repeated on two sample dates, each lasting 28 days with
root growth measurements both during and following the test period.
Measurements during the test period showed that sample date differences
in total length of new roots were clearly visible in five days and were
due to differences in average growth rate of individual roots. The roots
of dormant seedlings that received ten weeks of chilling grew faster than
those that received only five weeks. All the environmental factors
tested had a significant effect on root growth. Plants grown at
20°C produced more roots than 15° or 25° C. Root growth increased with

increasing photoperiod (16 hr > 12 hr > 8 hr) and was greater in the sandy loam than in the perlite for the Douglas-fir while the hemlock roots grew significantly better in the perlite. These results supported the initial hypothesis that futher standardization of the RRP evaluation environment was warranted.

In the second experiment, the hypothesis that long-term enviornmental treatments cause changes in the RRP in direct proportion either to the amount of chilling or the dormancy status of the bud was examined. A series of 11 treatments was employed incorporating three photoperiods, three air temperatures regimes and six dates of chilling initiation. Seedlings were sampled from these treatments at one or two-month intervals from June 1977 to March 1978, and at each sampling, RRP and bud dormancy were evaluated.

The results showed a definite seasonal pattern of root growth for the three parameters measured; total length, number and average growth rate of new roots. Bud dormancy was only weakly correlated to RRP. Chilling when the buds were in a dormant state led to a consistent increase in the number of new roots regenerated. The occurrence of a seasonal pattern of number of new roots in the continuously warm environment and of the elongation rate of the roots under all treatment environments suggested the existence of some endogenous controls.

The possibility of using current photosynthate translocated to the root as a rapid measure of RRP was explored by incubating seedlings with $^{14}CO_2$ and measuring the distribution of radioactive marker after 24 hours. The results indicate that although the percent ^{14}C-photosynthate translocated to the root was not predictive of the

RRP, it was significantly correlated with both the dormancy status of the bud and the elongation rate of the roots.

Comparison of the results of the two experiments indicate that seedlings age is an important factor in determining how the seedlings will respond to environmental factors. In general, younger seedlings are more dependent on current photosynthate for root growth than older ones.

Root Regeneration Potential in Douglas Fir
and Western Hemlock Seedlings: The
Role of Environmental Factors and Current Photosynthesis

by

Barbara Ellen Thompson

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

November 26, 1979

Commencement June 1980

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Root Regeneration Potential in Douglas Fir
and Western Hemlock Seedlings:
Role of Environmental Factors and Current Photosynthesis

I. INTRODUCTION

Forestry is an important source of revenue in the Pacific North-
west. Each year thousands of acres of forest land are clear-cut. It
becomes important both economically and aesthetically to replant the
cutover areas as soon as possible with seedlings that have the ability
to become rapidly established and grow.

Many major advances have been made in the past 20 years in
reforestation. Each increase in field survival has been based on
management practices derived from field and laboratory research.
Seedlings produced by many nurseries in the region are able to survive
on sites with average or favorable soil and moisture characteris-
tics. There is, however, still a great deal of plantation failure on
droughty and high elevation sites.

The rapid regeneration of new roots by the transplanted tree seed-
lings is an important factor in successful reforestation. While much
of the seasonal patterns of root growth observed in the field (Reed
1939, Morrow 1950, Stevens 1931, Hoffmann 1972) can be explained by
changes in soil temperature and water availability (Lyr and Hoffmann
1967, Bilan 1967), there are cases where roots do not regenerate even
under favorable conditions (Morrow 1950). This inhibition of growth
under favorable conditions is attributed to an endogenous root growth

pattern in the plant that can be described by a measurement termed the root regeneration potential (RRP). In general, the RRP is defined as a measure of the plants potential to regenerate new roots when placed in an environment favorable for root growth (Stone 1955).

The ability to regenerate roots (RRP) is especially important in reforesting marginally moist or early drying sites. A loss of up to 50 percent of the root volume on lifting in the nursery (Hoffmann 1969 as cited in Lüpke 1976) leaves predominantly non-fibrous, suberized roots (Burdett 1976) which are less able to take up water (Chung and Kramer 1956, Kramer and Bullock 1966). Seedlings with these severely restricted root systems when placed on a dry site must rapidly regenerate roots to survive (Lavender 1964). RRP has been directly correlated with survival in Pseudotsuga menziesii (Stone et al. 1962, Lavender 1964), Pinus ponderosa (Stone and Schubert 1959), Pinus taeda (Rhea 1977), Pinus contorta (Burdett 1976), Abies concolor (Stone and Norberg 1979) and several broadleaf species (Webb 1977).

Short Term Effect of Environment on RRP Measurement

RRP is determined by lifting the seedling, removing all the white root tips, repotting into a standard soil and placing in an optimum environment for root growth for 28-days. At the end of the 28-day evaluation period, the seedlings are lifted, washed clean of soil and the number and length of new white roots recorded (Stone 1955).

The evaluation environment in which the seedlings are kept during the 28-day period varies greatly from investigator to investigator.

Stone and Schubert (1959) used a greenhouse as the standard evaluation environment with an air temperature that varied between 20-35°C, natural photoperiod and water baths to control soil temperature. Most other investigators have used modifications of this system. There is, however, a large body of literature on air temperature, soil temperature, photoperiod and soil media effects on short term root growth (Lyr and Hoffmann 1967) that may warrant further standardization of the evaluation environment.

The direct or short term effects of environment on root growth have been reported mainly with respect to growth rate of roots already initiated. Effects of soil temperature and moisture have been extensively studied (Stone and Schubert 1959, Hellmers 1963a, Larson and Whitemore 1970, Larson 1970, Cooper 1973, Heninger and White 1974, Day and MacGillivray 1975, Kaufmann 1977) with the result that RRP is generally evaluated in soil that is adequately watered and maintained at 20°C. But the short-term effect of lighting and temperature in the shoot environment and the nature of soil medium have received relatively little attention.

One study by Stahel (1972) illustrated the importance of a long daily duration of light for promoting rapid root growth in _Picea sitchensis_ seedlings. But only in Douglas-fir cuttings (Bhella and Roberts 1972) and transplants (Lavender and Waring 1972) was this effect demonstrated to be truly photoperiodic. In general, light energy levels have been found to change root growth, more or less in proportion to their effect on photosynthesis in many species (Barney 1951, Richardson 1953a, 1953b, Brix 1970, Fairbairn and Neustein 1970,

Webb 1976a). In fact, root growth on cloudy days in a greenhouse evaluation environment may be only one quarter of that on sunny days (Barney 1951). Furthermore the lighting may have significant though less pronounced short-term effects on the number of roots initiated (Carlson 1976).

Air temperature, likewise, probably exerts its influence on expression of RRP through photosynthesis (Richardson 1953a), since root growth is retarded at temperatures both above and below the photosynthetic optimum (Richardson 1953a, Hellmers 1963a, 1963b, Good and Good 1976). There is conflicting evidence regarding the importance of diurnal fluctuations in air temperature. Diurnal fluctuations were seen to cause a dramatic increase in root growth of Pinus radiata seedlings if night temperature was around 5°C (Hellmers and Rook 1973), whereas Brix (1971) concluded that in most species the total heat sum satisfactorily accounted for variations in root dry matter production under various fluctuating air temperature regimes.

Soil type is the third general factor which has received little study or standardization as part of the RRP evaluation environment. Lüpke (1976) found significant differences in short-term root growth of Douglas-fir seedlings among five soils, these differences being unrelated to pH or nutrient status. He concluded that aeration was the controlling factor, but bulk density or penetrability differences may have been responsible, as found in this and other species by Minore et al. (1969).

The measurement parameter used to report root regeneration potential also varies among investigators with the number of new roots or

the total length of new roots being the most common. RRP measurements
have the most physiological significance and the best correlation with
survival when they are expressed in units that reflect the absorptive
surface for both water and nutrient uptake. The obvious choice of a
parameter to measure RRP is, therefore, total length of new roots.
The length of new roots, however, is a composite parameter that is the
result of both the number of new roots and their elongation rate during
the evaluation period. Both initiation and elongation of new root may
be under the control of different environmental factors (Carlson 1976).
RRP, may, therefore, be best understood from a physiological standpoint
by examining the effect of environment on both elongation and
initiation.

Control of Root Regeneration Potential

Evidence indicates that the long-term environmental control of
root initiation and elongation is achieved through variation in the
levels of translocatable factors in the shoot. In seedlings of decidu-
ous species the stimulus for vigorous root growth is localized within
the bud, and debudding greatly lowers this growth (Farmer 1975).
Furthermore, the bud must be fully formed and non-dormant in order to
elicit the greatest root growth response (e.g. Richardson 1958, Farmer
1975, Taylor and Dumbroff 1975, Webb 1976b, 1977).

In conifers, however, experiments involving either or both
debudding and defoliation (Gilmore 1965, Lavender and Hermann 1970,
Carlson 1977, Brown 1976) have indicated that both the buds and mature

foliage are sources of translocatable substances controlling root
growth. This makes the identification of environmental and physiolog-
ical controlling factors more complex, and to date neither these
factors nor the chemical nature of the substance(s) they affect, have
been fully identified. And only in the case of root initiation from
cuttings has a correlation with bud dormancy similar to that in
deciduous species been reported (Roberts and Fuchigami 1973). Much
evidence, however, has shown that improved RRP in conifers is associ-
ted with the cumulative effects of chilling and therefore at least
indirectly with bud dormancy which is released by chilling.

Krugman and Stone (1966) found that dormant ponderosa pine seed-
lings in controlled environments needed at least 90 consecutive cold
(6°C) nights to significantly increase their RRP. The general relation-
ship with chilling has been confirmed in nursery studies (Schubert and
Baron 1965, Stone and Jenkinson 1971), but is not a precise one and
varies greatly between provenances and nurseries and depends upon
the timing of chilling. Stone and Norberg (1976) found that one-month
chilling of Abies concolor became progressively more effective in
increasing RRP the later it was begun between October and January.

There is also evidence for an endogenous rhythm of RRP that is
not controlled by chilling. Stone and Norberg's study showed that
seedlings grown in a continuously warm environment had two RRP peaks,
one of which corresponded to the peak found in the chilled seedlings.
The RRP's were always higher for the chilled seedlings. Merritt
(1968), also reported a significant periodicity of RRP in Pinus
resinosa seedlings grown in a continuously warm environment.

He concluded that "the gross pattern of root activity is endogenously controlled, although environmental variation may induce significant quantitative changes in activity and influence the timing of events."

Rapid Prediction of RRP

The present method of measuring RRP requires one month to complete. Thus, by the time the results are available, the seedlings' RRP has changed substantially. This delay reduces the usefulness of this method for determining the best time to lift seedlings in the nursery.

Using results of the RRP seasonal periodicity from past years for the prediction of the best lifting date is also risky because large differences in the timing of the RRP peaks occur from year to year (Stone and Jenkinson 1971, Jenkinson 1975). Some investigators (Krugman and Stone 1966, Stone and Jenkinson 1971) have related the timing of RRP peaks to the nursery environment, particularly the number of cold nights. This method may not prove to be practical, however, because different seed sources respond differently to cold treatment.

It has been reported in a large number of species that new root production requires current photosynthate (Ursino, et al. 1968, Eliason 1971, Lavender and Hermann 1970, Carlson 1976, and Webb and Dumbroff 1978). However, Krueger and Trappe (1967) suggest that the requirement of current photosynthate for root growth may depend on the season the root growth in initiated. For example, in the fall

and winter, Douglas-fir root growth appeared independent of current photosynthate while spring root growth was maintained by sugars transported from the shoot.

Although no direct correlation between RRP and the distribution of current photosynthate has been reported in the literature, most of the evidence suggests that such a correlation may be useful in predicting RRP and hence seedling survival.

II. EXPERIMENT I - THE SHORT TERM EFFECTS OF AIR
TEMPERATURE AND PHOTOPERIOD ON RRP MEASUREMENTS

Successful reforestation depends on the survival of seedlings transplanted into the field. Seedling survival, especially in marginally moist or early drying sites depends to a great extent on the seedling's ability to regenerate roots (Lavender 1964). Up to 50 percent of the seedling root volume may be lost during nursery lifting operations (Hoffman 1966 as cited by Lupke 1976) leaving mostly non-fibrous suberized roots (Burdett 1976) which are less able to absorb water (Chung and Kramer 1975). If the seedling is to survive, roots must be rapidly regenerated in order to explore new soil volumes and obtain water.

The current method for measuring a seedling's root regeneration potential (RRP) was developed by Stone (1955). The method consists of placing transplanted seedlings into a standard growth environment for 28-days and then measuring the length and number of new roots. In Douglas-fir (Stone et al. 1962, Lavender 1964) and in many other species, this RRP measurement has been closely correlated with field survival (Webb 1977, Rhea 1977).

Comparing RRP patterns reported in the literature for similar studies done by different investigators is difficult because of the wide variety of standard environments employed (Table 1). Root temperature is usually controlled at about 20°C (Stone and Schubert 1959, Stone et al. 1963) but other environmental parameters employed during the 28-day evaluation period vary widely.

Table 1. Some examples of environments under which different investigators have evaluated root regeneration potential.

Investigator	Species	Root Temperature (°C)	Air Temperature (°C)	Photoperiod (hr)	Soil Type	Root Parameter Measured
Burdett (1976)	Pinus contorta	24 ± 4	24 ± 4	16	unspecified	volume
Krugman, Stone and Bega (1965)	Pinus radiata	20	15-30	natural	sandy loam	number
Krugman and Stone (1966)	Pinus ponderosa	20	20-35	natural	sandy loam	length
Larson (1970)	Quercus rubra	day 13, 18 24 or 29 night 13, 18, 24, or 29	20-39	12		number
Lavender (1964)	Pseudotsuga menziesii	day 20 night 10	day 25 night 5	16	unspecified	number
Lüpke (1976)	Picea abies Pseudotsuga menziesii Pinus sylvestris	20	day 24 night 16 or 20-35	natural or 14	sandy loam loam-peat mix sand-peat mix sand-loam mix peat-compost mix	number
Rhea (1977)	Platanus occidentalis Liquidambar styraciflua Liriodendron tulipifera Pinus taeda	23	23	14	sand	length and number

Table 1. Cont.

Investigator	Species	Root Temperature (°C)	Air Temperature (°C)	Photoperiod (hr)	Soil Type	Root Parameter Measured
Schubert and Baron (1965)	Pinus ponderosa	20	20-35	natural	unspecified	length and number
Stone (1955)	Pinus jeffreyi Abies concolor Pinus ponderosa Abies magnifica Pseudotsuga menziesii	20-35	20-35	natural	sandy loam	length and number
Stone and Schubert (1959)	Pinus ponderosa	10, 15 20, 25	20-35	natural	sand or sponge rock	length and number
Stone et al. (1962)	Pseudotsuga menziesii	10, 15 20, 25	20-35	natural	perlite	length and number
Stone et al. (1963)	Pinus ponderosa	20	20-35	natural	sandy loam	number
Stone and Norberg (1976)	Abies concolor	20	day 25 night 20	12	clay loam	length
Webb (1976b)	Acer saccharum		day 28 night 18	16	soil:sand:peat (2:1:2 v/v)	number
Winjum (1963)	Pseudotsuga menziesii Abies procera	15	day 22 night 12	16	hydroponic	number

11

A large body of literature exists demonstrating the short term effects of air temperature, photoperiod and soil type on root growth. For example, air temperature has been shown to influence root growth through its effect on photosynthesis, with root growth retarded both above and below the photosynthetic optimum (Richardson 1953a, Helmers 1963a, 1963b, Good and Good 1976). Photoperiod may have a more complex effect on root growth with both a hormonal photoperiodic effect (Bhella and Roberts 1972) and a photosynthetic effect related to available light (Brix 1970, Fairbairn and Neustein 1970, Webb 1976). Soil type has received less attention but can be expected to affect root growth in proportion to aeration and bulk density (Lüpke 1976).

This study was designed to test the hypothesis that air temp-perature, photoperiod and soil type used during the evaluation period have a significant effect on the magnitude of the RRP measured for Douglas-fir and western hemlock seedlings.

Methods

Douglas-fir seed collected at an elevation of 1065 m near Elbe, Washington and western hemlock seed collected at an elevation 458 m near Snoqualmie, Washington were sown in 65 ml cylindrical plastic containers containing a 1:1, peat, vermiculite, planting medium at a density of 1080 m^{-2} during March 1976, grown in a greenhouse for the summer and placed outside at Rochester, Washington over winter. In March 1977, the seedlings were returned to the 15-20°C greenhouse where they soon broke bud and grew for eight weeks, after which the sequence of events as indicated in Figure 1 was followed: a dormancy
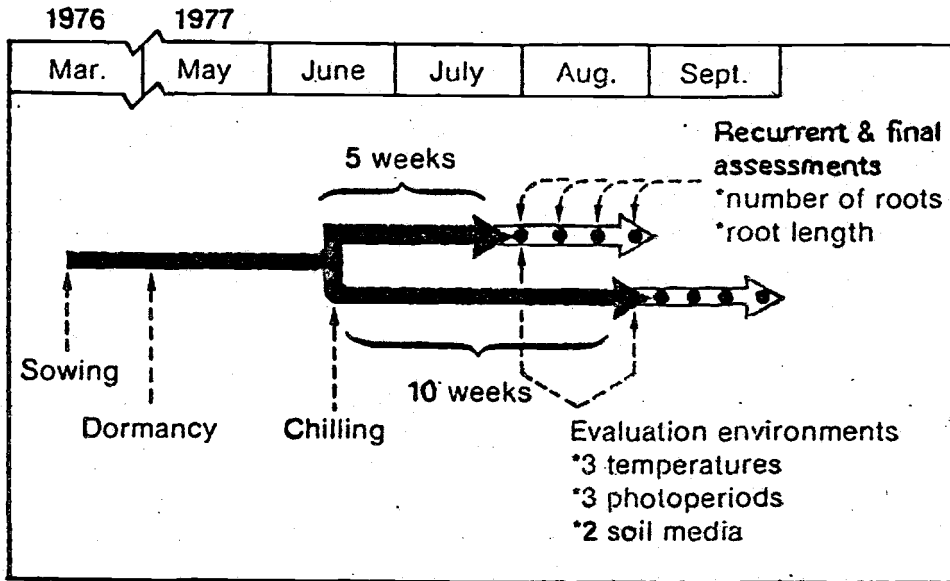
Figure 1. Sequence of treatments and measurements. Open lines represent the two RRP evaluation periods in each of which a factorial combination of 18 environments were tested. Solid circles show approximate times of new root measurement through transparent container walls, and correspond with points in Figures 2 and 3.

inducing treatment, a fulfillment or part or all of the chilling requirement, a series of evaluation environments, and various measurements of root growth.

All seedlings were watered and fertilized to maintain a vigorous growth throughout this study unless otherwise stated. The light intensities in all the growth rooms were 200-300 μEinsteins m^{-2} (high output, cool white, fluorescent lamps). A more detailed description of this procedure follows.

To induce dormancy, seedlings were placed in a growth chamber at a temperature of 16 ± 2°C and a 9-hr photoperiod with a minimal amount of watering (Lavender 1968).

After seven weeks all seedlings had set buds and were transferred to a growth room at 5 ± 1°C and an 8-hr photoperiod. This environment simulated the conditions known to most rapidly satisfy the chilling requirement of Douglas-fir buds (Campbell and Sugano 1975). Half of the 360 seedlings of each species were removed from the cold room five weeks later (early August), transplanted into watertight tanks and transferred to the test environments described below. The rest remained in the growth room at 5° ± 1°C for an additional five weeks before being transferred.

The test environments comprised a factorial combination of three air temperatures (15°, 20° and 25°C), three photoperiods (8, 12 and 16 hrs) and two soil types (sandy loam and standard horticultural grade perlite). These environments received ten seedlings of each species per environment on two dates (i.e. after five or ten weeks of

chilling, respectively). This resulted in 18 treatment combinations each containing 40 seedlings, 20 Douglas-fir and 20 western hemlock.

The perlite and sandy loam soil were placed in two slightly different containers. The sandy loam was placed directly into nine watertight metal containers (8 x 57 x 30 cm deep) with 20 seedlings in each; the perlite was placed into 18 transparent acrylic trays (7 x 27 x 25 cm deep) with ten seedlings in each. These transparent trays, designed to allow growth measurements of individual roots during the 28-day evaluation period, were in turn placed inside nine watertight metal trays, two per tray. The acrylic containers were enclosed in black plastic to eliminate any light that might inhibit root growth.

The 18 watertight metal trays were divided into three equal groups and placed into thermostatically controlled water baths maintained at $20 \pm 1°C$ in three growth rooms. The growth rooms were maintained at a constant temperature of $15 \pm 2°$, $20 \pm 2°$ or $25 \pm 2°C$ and a 16-hr photoperiod. In each room, the 8- and 12-hr photoperiods were obtained by placing blackout cloths over the appropriate seedlings after 8 or 12 hours of light. These covers allowed sufficient air circulation to prevent overheating.

Roots that reached and grew in the perlite along the transparent container wall were measured weekly. At the end of the 28-day test period, all the seedlings were removed from the tanks, the potting medium was washed off, and the number and length of new white roots over 0.5 cm in length were recorded. Average growth rate per day was calculated by dividing the total length of new roots of a seedling by the number of new roots and finally dividing that by the days (28) in the evaluation environment.

An analysis of variance procedure was used to determine the significance of the various treatments. The significance of differences between means was tested using Scheffe's method (Neter and Wasserman 1974).

Results

The pattern of root growth as observed through the transparent boxes differed significantly between sample dates for both the Douglas-fir and western hemlock seedlings (Figure 2). After five weeks of chilling, the daily growth rate was nearly constant throughout the evaluation period with the Douglas-fir maintaining an average growth rate of 1.95 ± .18 mm/day and the western hemlock of 1.44 ± .10 mm/day. Both species grew faster during the first two weeks in the evaluation environment after ten weeks of chilling than after five weeks. The maximum rate of growth for Douglas-fir was 4.3 ± .4 mm/day and for western hemlock was 3.3 ± .23 mm/day. However, after two weeks the growth rate in both species of seedlings chilled ten weeks slowed to approximately one-half of the maxima and was similar to that found for plants chilled for five weeks.

The growth rate effect was reflected in the total length of new roots observed through the transparent boxes (Figure 3). Total length after ten weeks of chilling was 1.6 times that after five weeks. This difference was established during the first two weeks of growth in the evaluation environment and then maintained during the remaining two weeks.
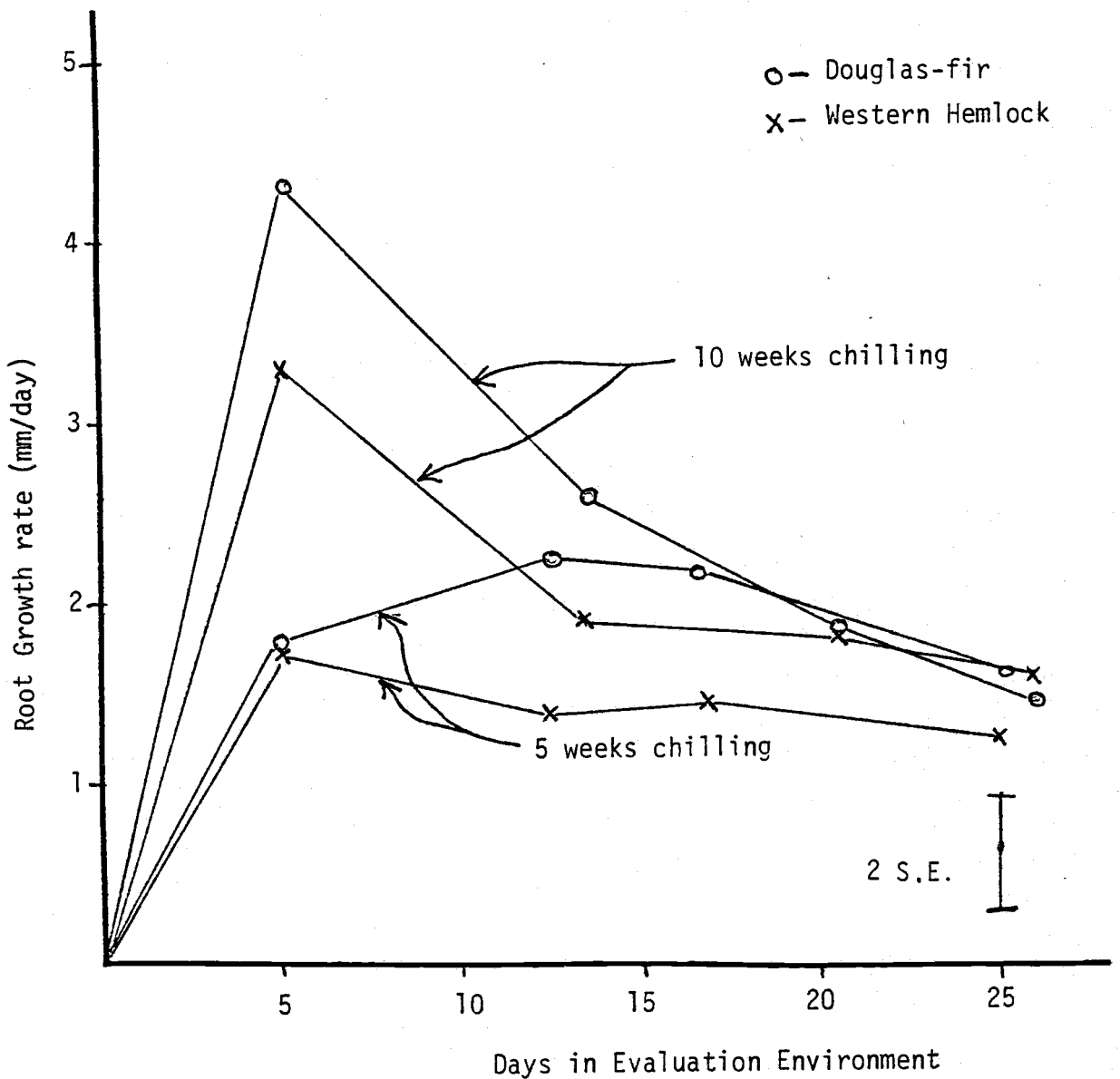
Figure 2. The average elongation rate of individual roots growing in perlite along the transparent container wall during the evaluation period. Each point is the mean of 90 seedlings. Average standard error of the mean for all curves is equal to 0.31 mm/day. The difference after five days between the averages of the seedlings chilled five weeks and those chilled ten weeks is significant at the p < .01 level.
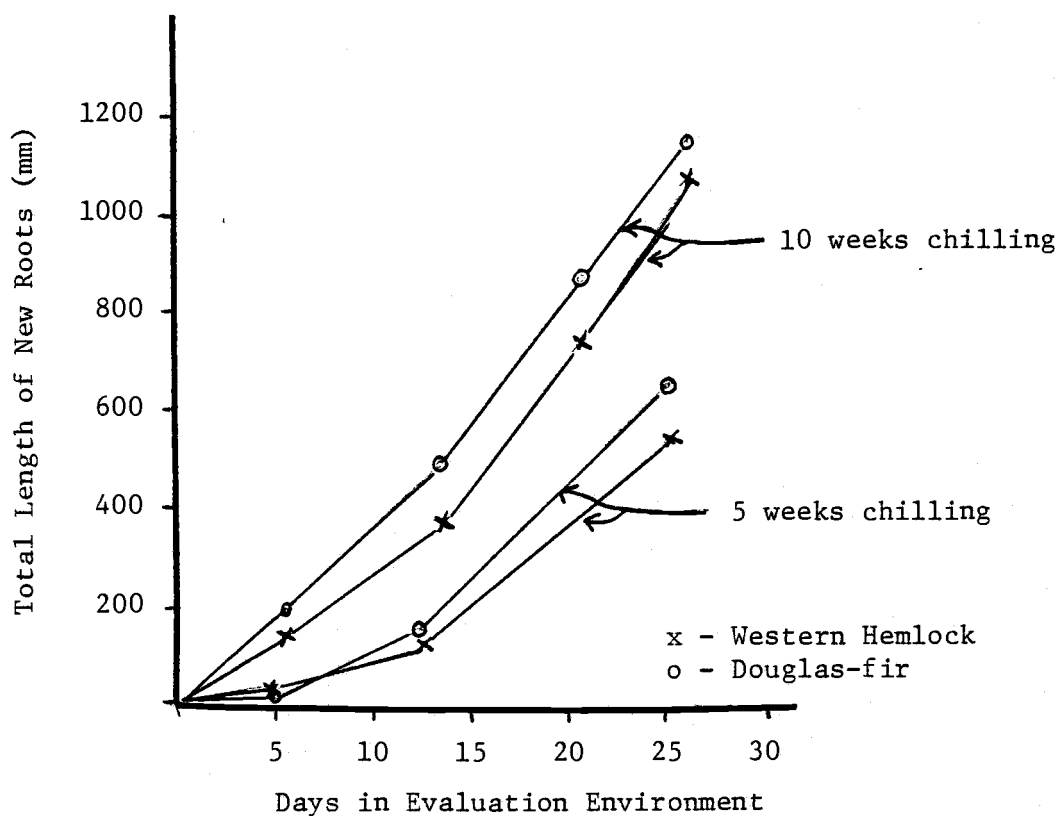
Figure 3. The change in cumulative root growth (length) during the 28-day evaluation period. Each point represents the mean of 90 seedlings. One standard error of the mean is approximately equal to 14% of the mean. After 5 days, the 5 weeks chilling curves for both species are significantly lower than the 10 weeks chilling curves for the rest of the 28-day period (p < .01).

The average number of roots produced in the 18 treatment combinations did not differ for the two chilling periods. Therefore, the difference in the total length (RRP) measured for the two sample dates was completely attributable to the differences in elongation rate.

The growth rate as measured through the transparent boxes was more than twice the growth rate as calculated from data collected at the final assessment after 28-days (Table 2).

The three root growth parameters--(total length, number, and growth rate of new roots) varied significantly ($p < .01$) with air temperature (Tables 3 and 4). The greatest number of new roots was obtained at 15°C or 20°C in both species while the most rapid growth rate was at 20°C. This effect resulted in a higher RRP (total length) at 20°C than at 15°C with 25°C exhibiting a marked root growth depression.

Photoperiod had a significant effect on the number of new roots recorded with each additional four hours producing an incremental increase in the number of new roots (Tables 3 and 4). The growth rate of the roots, however, remained unaffected by photoperiod. Consequently, the RRP as measured by the total length of new roots varied with photoperiod in the same manner as the number of new roots.

Soil type influenced both root growth rate and number of new roots produced by the Douglas-fir. But only the growth rate of the western hemlock roots varied with soil type. Both species, however, show significant ($p < .01$) differences in the RRP measured in the two soils (Tables 3 and 4) with the Douglas-fir showing the most growth in the sandy-loam while the western hemlock grew best in the perlite.

Table 2. The growth rate of the new roots of the western hemlock and
Douglas-fir seedlings in the perlite as measured by recurrent
visual observation and final assessment. Data is reported as
a grand mean of three temperature and three photo treatments
(3x3) ± S.E.

| Sampled After | Type of Assessment | Growth Rate (mm/day) | |
| | | Species | |
		Douglas-fir	Western hemlock
5 Weeks Chilling	Final	.82 ± .04	.60 ± .02
	Recurrent	1.95 ± .18	1.44 ± .10
10 Weeks Chilling	Final	1.31 ± .08	1.00 ± .07
	Recurrent	2.58 ± .25	2.15 ± .15

DOUGLAS-FIR

Table 3. Means for the three root growth parameters in Douglas-fir
as affected by the various environmental factors tested.
N.S. - indicates no statistically significant differences.
** - indicates significant differences at the p<.01 level.

	Number	Growth Rate (mm/day)	Length (cm)
DATE			
5 Weeks Chilling	53.87	0.957	149.87
10 Weeks Chilling	55.94 N.S.	1.315 **	208.79 **
TEMP (°C)			
15	58.35	1.089	180.31
20	60.56	1.261	214.74
25	45.82 **	1.058 **	142.94 **
PHOTOPERIOD (HRS)			
8	44.09	1.080	138.82
12	55.47	1.178	185.91
16	65.17 **	1.150 N.S.	213.26 **
SOIL			
Perlite	47.00	1.068	144.50
Sandy-Loam	62.82 **	1.205 **	214.16 **

WESTERN HEMLOCK

Table 4. Means for the three root growth parameters in western
hemlock as affected by the various environmental factors
tested. N.S. - indicates no statistically significant
difference. ** - indicates significant differences at
the p<.01 level.

	Number	Growth Rate (mm/day)	Length (cm)
DATE			
5 Weeks Chilling	70.88	0.621	124.45
10 Weeks Chilling	70.68 N.S.	0.902 **	182.96 **
TEMP (C)			
15	75.04	0.727	151.59
20	77.71	0.850	184.98
25	59.59 **	0.710 **	124.54 **
PHOTOPERIOD (HRS)			
8	52.55	0.722	108.38
12	67.88	0.763	144.21
16	91.92 **	0.801 N.S.	208.53 **
SOIL			
Perlite	72.88	0.804	167.05
Sandy-Loam	68.68 N.S.	0.720 **	140.36 **

Discussion

The root regeneration potential measured for both Douglas-fir
and western hemlock seedlings was significantly influenced by all the
environmental factors tested. This results leads to the practical
conclusion that in addition to soil temperature; the air temperature,
photoperiod,and the soil media used,should be carefully controlled
during the RRP evaluation period.

Air temperature affected RRP by influencing both the growth rate
and the number of new roots produced. The maximum RRP observed at 20°C
agrees well with the photosynthetic optima reported by Brix (1971)
for Douglas-fir and western hemlock, thus supporting the hypothesis of
Richardson (1953a) that root growth is dependent on current photo-
synthate. The slightly different temperatures at which the maximum
growth rate (20°C) and maximum number of new roots (15-20°C) occur may
indicate that these two root growth parameters are controlled by
different mechanisms within the plant. In the literature, the growth
rate of a root is most often correlated with available carbohydrate
(Richardson 1956, Winjum 1963, Little 1970, Etter and Carlson 1973)
while root initiation is seen as the result of hormonal stimuli (Zaerr
1967, Bhella and Roberts 1974, Farmer 1975, Carlson 1976, Webb and
Dumbroff 1978).

The change in RRP with photoperiod was due exclusively to a change
in the number of new roots measured (no change in growth rate). Heide
(1974) and Webb (1976a) studying norway spruce and sugar maple seed-
lings, respectively, also found no dependence of root growth rate

on photoperiod. Stahel (1972), on the other hand, found a marked effect of photoperiod on the growth rate of Sitka spruce roots. His seedlings, however, had actively growing shoots which are known to compete with root growth (Eliasson 1971) while the seedlings were dormant in the other investigations (Heide 1974, Webb 1976). In addition, Stahel found that root growth increased in the short day plants after shoot growth ceased. He concluded that root elongation under short days was inhibited by photosynthate being used in the shoot. The evidence then suggests that photoperiod does not influence the growth rate of roots when the shoots are dormant.

Richardson (1956) proposed a theory based on his observations of two year old sugar maple seedlings that might be invoked to explain the lack of response in growth rate to photoperiod. He concluded that in older seedlings the level of stored carbohydrate reserves determined the growth rate of the roots. More recent investigations, however, contradict this theory by finding no correlation of root growth with stored carbohydrates (van den Driessche 1978). In addition, Richardson's theory fails to explain the lack of dependence of root growth rate on photoperiod in first year seedlings (Heide 1974, Webb 1976) where stored carbohydrates are rarely present.

An alternative theory based on studies examining the effects of air temperature (Larson 1967) and light intensity (Barney 1951, Richardson 1953b) on the growth rate of roots, is that the rate of photosynthesis and not the total amount of carbon fixed influences the growth rate of the root. This would explain why light intensity has such a pronounced

effect in the studies cited while photoperiod does not. However, there are no direct comparisons between the effect of photosynthetic rate and total photosynthate on root growth to support or refute this theory.

The almost linear increase in the number of new roots with increasing photoperiod suggests a photosynthetically controlled response. This conjecture disagrees with the often proposed theory that root initiation is hormonally triggered phenomenon. Agreement with the hormonal theory can be achieved if the physiological status of the seedlings is again examined. After five or ten weeks of chilling, the seedling's buds would be in a post-dormant quiescent state. Campbell and Sugano (1975) have shown that the speed with which seedlings in this state break bud increases linearly with increasing photoperiod. Some evidence exists that hormonal increases may follow this increase in bud activity (Lavender and Hermann 1970). This evidence results in a hypothesis that the linear increase in root number with increasing photoperiod was due to a linear increase in hormones produced by the bud. This theory, however, is far from proven.

Of the environmental parameters tested, only the soil type induced a response that differed between the Douglas-fir and the western hemlock seedlings. In both species, the RRP differed significantly (p<.01) between the two soils with the Douglas-fir seedlings showing the highest RRP in sandy loam while the hemlock roots grew best in perlite. This interaction with soil medium has obvious applications to the nurseryman and as such deserves further study.

Increases in RRP with increased chilling has been noted by many observers (Webb 1977, Krugman and Stone 1966, Schubert and Baron 1965, Farmer 1975) and was recorded in this study. It was found to be due exclusively to an increase in growth rate of new roots (no increase in number of new roots). A possible explanation for this increase in growth rate may be that translocatable sugars shown to increase during chilling (Parker 1959, Krueger and Trappe 1967, Aronsson et al. 1976) and known to be advantageous for root growth (Winjum 1963, Little 1970, Etter and Carlson 1973) were more plentiful after ten weeks of chilling than after five weeks thereby increasing the growth rate of the roots.

The rapid increase at the beginning of the evaluation period in the growth rate of the roots observed after ten weeks of chilling may be due to rapid consumption of these available carbohydrates. Aronsson et al. (1976) have shown that sugar concentrations accumulated during chilling decrease to normal concentrations within two to four weeks after seedlings begin to deharden. When added to the observation of Krueger and Trappe (1967) that starch concentration in the roots of Douglas-fir seedlings remains constant during the spring root growth period but that a large loss of sugars in the shoot is observed, the two week spurt of root growth after ten weeks of chilling may be seen as the results of utilization of cold-induced translocatable sugars from the shoot.

The growth rate of roots as measured through the transparent containers (although consistently higher than those determined in the final evaluation) were accurate representations of the relative growth

rates after the two periods of chilling. The faster growth rate found with recurrent measurement reflects the fact that faster growing roots reach the transparent surface more quickly and, therefore, make up a higher percentage of the roots measured recurrently than they do of the growing root system as a whole. In addition, when individual roots ceased growth they were discarded from the sample. The use of transparent boxes to measure relative growth rates is acceptable but unrealistically high growth rates for the total root system are obtained.

In summary, environmental influences on RRP seem to be of two types 1) those that affect the growth rate of the roots and 2) those that affect the number of new roots. To better understand the physiological effects of environment both parameters should be examined.

III. EXPERIMENT II - THE LONG TERM EFFECTS OF AIR TEMPERATURE AND PHOTOPERIOD ON RRP

The prompt regeneration of new roots by transplanted tree seedlings has been recognized as an important factor in successful reforestation. While much of the variation in root growth observed in the field (Reed 1939, Morrow 1950, Stevens 1931, Hoffman 1972) can be explained by soil temperature and water availability (Lyr and Hoffman 1967, Bilan 1967), there are cases where roots do not regenerate even under favorable conditions (Morrow 1950). Such cases have been attributed to unknown endogenous factors within the plant constituting what Stone (1955) termed the root regeneration potential (RRP). Stone evaluated RRP by lifting the seedling, removing all white root tips, transplanting into an environment favoring root growth, and measuring the length and number of new roots after 28 days.

One would expect a high RRP to be especially important in reforestating dry or early-drying sites because 50 percent of the root volume may be lost during the nursery lifting operation (Hoffmann 1966 as cited in Lupke 1976), leaving predominantly nonfibrous, suberized roots (Burdett 1976) which are less able to take up water (Chung and Kramer 1975, Kramer and Bullock 1966). Lavender (1964) and Tranquillini (1972 as cited in Lüpke 1976) have confirmed that new root production is indeed essential under such conditions and RRP has been correlated directly with survival in Pseudotsuga menziesii (Stone et al. 1962, Lavender 1964), Pinus ponderosa (Stone and Schubert 1959), Pinus

taeda (Rhea 1977), Pinus contorta (Burdett 1976) and several broad-
leaved species (Webb 1977).

The present method of measuring RRP takes 28 days to complete by
which time the RRP of the seedlings remaining in the field has changed
substantially. This delay reduces the usefulness of this method for
determining the best time to lift seedlings in the nursery.

It has been reported in a large number of species that new root
production requires current photosynthate (Ursino et al. 1968, Eliasson
1971, Lavender and Hermann 1970, Carlson 1976, and Webb and Dumbroff
1978). This suggests a rapid method of predicting RRP may be developed
by measuring the amount of current photosynthate translocated to the
root.

Evidence indicates that the long-term environmental control of
root initiation and elongation is achieved through variation in the
levels of translocatable substances in the shoot. In seedlings of
deciduous species, the stimulus for vigorous root growth is localized
within the bud, and debudding greatly lowers this growth (Farmer 1975).
Furthermore, the bud must be fully formed and non-dormant in order to
elicit the greatest root growth response (Richardson 1958, Farmer
1975, Taylor and Dumbroff 1975, Webb 1976b, 1977).

In conifers, however, experiments involving either or both
debudding and defoliation (Gilmore 1965, Lavender and Hermann 1970,
Brown 1976) have established that both the buds and mature foliage
are sources of translocatable substances controlling root growth.
This result makes the identification of environmental and physiological

controlling factors more complex, and to date, neither these factors nor the chemical nature of the substance(s) they affect have been fully identified. However, Zaerr (1967) working with ponderosa pine seedlings found that debudding did not affect the RRP while addition of exogenous IAA increased the initiation of new roots. Only in the case of root initiation from cuttings has a correlation with bud dormancy similar to that in deciduous species been reported (Roberts and Fuchigami 1973). Much evidence, however, has shown that improved RRP in conifers is associated with the cumulative effects of chilling, and therefore, at least indirectly with bud dormancy which is released by chilling.

Krugman and Stone (1966) found that dormant ponderosa pine seedlings in controlled environments needed at least 90 consecutive cold (6°C) nights to increase significantly their RRP. The general relationship with chilling has been confirmed in nursery studies (Schubert and Baron 1965, Stone and Jenkinson 1971), but is not a precise one and varies greatly between provenances, and according to the timing of chilling. Stone and Norberg (1976) found that a one-month chilling of Abies concolor became progressively more effective in increasing RRP the later it was begun between October and January.

There is also evidence for an endogenous rhythm of RRP that is not controlled by chilling. Stone and Norberg's study showed that seedlings grown in a continuously warm environment had two RRP peaks, one of which corresponded to the peak found in the chilled seedlings. The RRP's were always higher for the chilled seedlings. Merritt (1968),

also,reported a significant periodicity of RRP in <u>Pinus</u> <u>resinosa</u> seedlings grown in a continuously warm environment. He concluded that "the gross pattern of root activity is endogenously controlled, although environmental variation may induce significant quantitative changes in activity and influence the timing of events."

The foregoing evidence suggests a number of possible ways the environment may affect the root regeneration potential expressed. The experiment described presently was designed to test two such hypotheses: that environmental treatments will bring about changes in the RRP in direct proportion to either, 1) the amount of chilling experienced, or 2) their effect on the level of dormancy.

In addition, the amount of ^{14}C- photosynthate translocated to the root was evaluated as a rapid method of predicting RRP.

Method

Douglas-fir seeds from 910-m elevation near Morton, Washington, and western hemlock seeds from 610-m elevation near Elbe, Washington, were sown in a 1:1 v/v, peat:vermiculite planting mix in 65-ml cylindrical plastic containers at a density of 1068 seeds m^{-2} in March 1977, and grown in a greenhouse at Rochester, Washington until June, 1977.

The trees were then moved to Corvallis, Oregon, placed in a greenhouse at 20°C with a natural photoperiod and a light intensity of ≃ 20 percent full sunlight (200-300 μ Einstein) and subsequently given one of the following treatments (Figure 4):

1. Natural temperature and natural photoperiod beginning the first week of June (NT/ND);

2. Warm temperature (20°C) and natural photoperiod beginning the first week of June (20°/ND);

3. Cold temperature (5°C) and short photoperiod (eight hours) beginning at ∿ one-month intervals (six subtreatments A-F throughout the experimental period)(5°/SD);

4. Warm temperature and short photoperiod beginning the first week of August (20°/SD);

5. Cold temperature and long photoperiod (16 hours) beginning the first week of August (5°/LD);

6. Warm temperature and long photoperiod beginning the first week of August (20°/LD).

Twenty-five seedlings of each species were sampled from the treatment environments at one- or two-month intervals (Figure 6): 20 seedlings for RRP measurements and five for dormancy measurement. The seedlings for RRP evaluation were removed from their plastic containers and planted into sandy loam in 7-liter pots, five seedlings per pot. The seedlings were watered thoroughly and placed in the strictly controlled evaluation environment selected on the basis of the optimum response in Experiment I (20°C soil, 20°C air temperature and 12-hour photoperiod). The pots were watered twice weekly to maintain the soil near field capacity. After 28 days in this environment, the seedlings were excavated and the number and length of new white roots over 0.5 cm in length recorded. The average growth rate per day of the root system of each seedling was calculated by dividing the total length of the new roots by the number measured and then dividing by 28 days.

The five seedlings for dormancy evaluation were kept at 20 ± 1°C under a 16-hr photoperiod. They were examined three times a week and the day of emergence of the first needles from a bud was recorded. Bud break data were transformed (Campbell 1974) into daily average rate of development (DARD)* values and averaged for the five seedlings of a treatment. Seedlings that did not flush in the total observation period (350 days) were arbitrarily assigned a DARD value of 0.28.

To test the feasibility of using the precent of ^{14}C-photosynthate translocated to the root as a rapid predictor of RRP, ten seedlings of each species were sampled at one or two month intervals from a sub-sample of treatments (Treatments: ND/NT, 5°/SD begun in September and December and 20°/LD were used). The seedlings were watered and placed into a chamber designed to allow uptake of $^{14}CO_2$ with a minimal amount of gas exchange with the ambient air (Figure 5). The light intensity in the chamber was $\approx 400\,\mu$ Ei and the temperature varied from 18-25°C.

Ten ml of distilled water and enough $Ba^{14}CO_3$ to generate 20 μ Ci of $^{14}CO_2$ per tree was placed in a beaker in the chamber. Three milliliters of 1 N HCL was placed in the test tube and the circulating pump and the light were turned on. The chamber was sealed and the acid dumped into the beaker, releasing the $^{14}CO_2$.

The chamber remained sealed for three hours. The vent was then opened and the exhaust from the pump was bubbled through 1 N NaOH

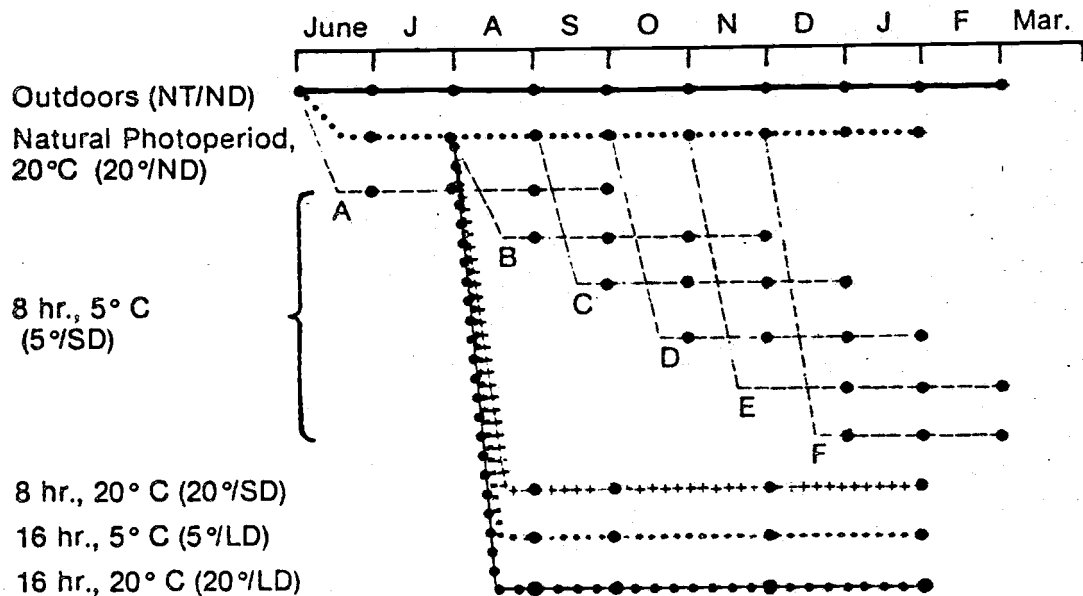*DARD = $\dfrac{100}{\text{number of days until bud-break}}$

Figure 4. Sequence of treatments measurements in Experiment 2. Horizontal lines represent treatments identified by abbreviations in parentheses. Large dots show times at which 20 seedlings were potted under standard conditions for the 28-day evaluation of root regeneration potential, and five transferred to a warm long-day environment for evaluation of dormancy.
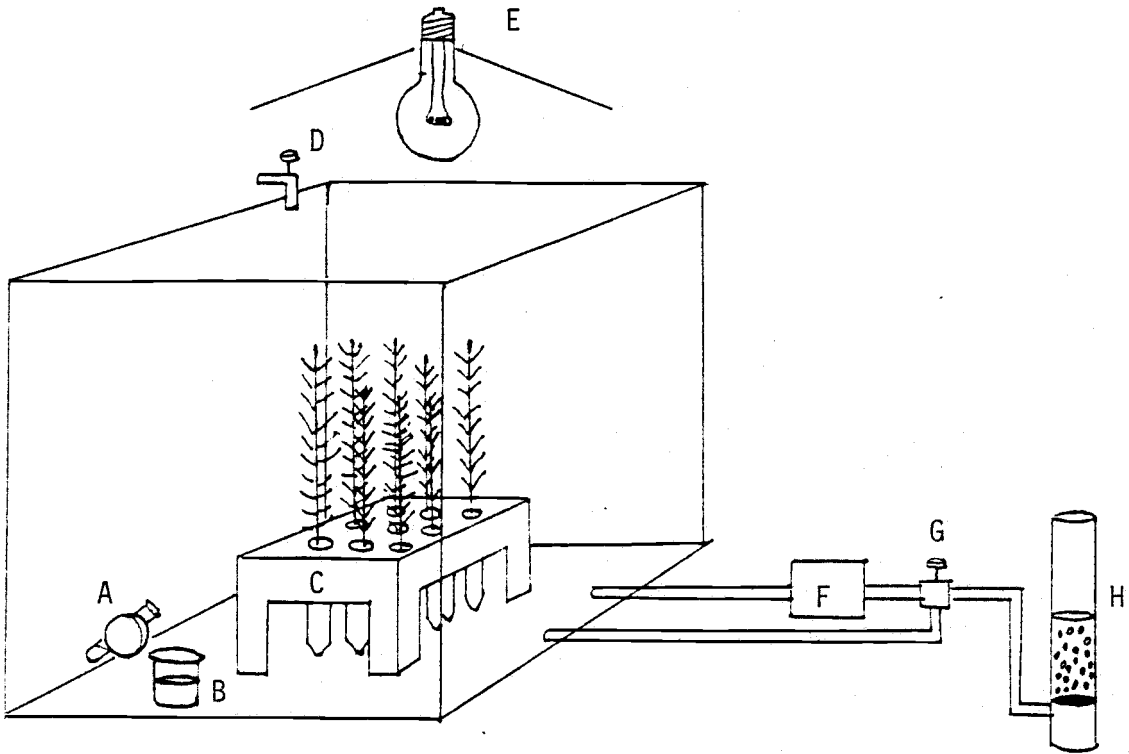
Figure 5. Apparatus for exposing the seedlings to radioactive $^{14}CO_2$. A. Test Tube containing 1 N HCl, B. Beaker containing $Ba^{14}CO_3$ dissolved in distilled water, C. Tray and trees, D. Air vent with valve, E. Light, F. Circulating pump G. Diverting valve and H. Scrubber filled with 1 N NaOH to trap $^{14}CO_2$. For further discription of technique see text.

to trap any radioactive CO_2 that may have not been fixed. The seed-lings remained in the chamber under these conditions an additional 21 hours.

Twenty-four hours after the initial exposure to the $^{14}CO_2$, the seedlings were removed from the chamber, the roots washed and the root and shoot placed into individual paper bags and dried at 70°C for twenty-four hours. After drying, the seedling roots and shoots were each weighed separately and recorded. The tissue was ground in a wiley mill to pass a size 20 mesh screen and a 5-10 mg sample was accurately weighed into a cumbusto-cone. These samples were oxidized on a Tri-carb Sample Oxidizer and the radioactivity determined on a Tri-carb Liquid Scintillation counter.

The relatively radioactivity in the total tissue was then calculated by multiplying the disintegrations per minute by the total tissue dry weight divided by the sample dry weight. No corrections for self absorption were applied.

Results

When the new root growth in the evaluation environment was measured, the three parameters, average root growth rate, total number of new roots, and total length of new roots gave varying seasonal patterns. For example, the Douglas-fir seedlings preconditioned in the ND/NT environment had different patterns for each of the three parameters (Figure 6). The western hemlock seedlings showed similar seasonal growth patterns for both number and length of new roots, but these still differed greatly from the pattern of root growth rate
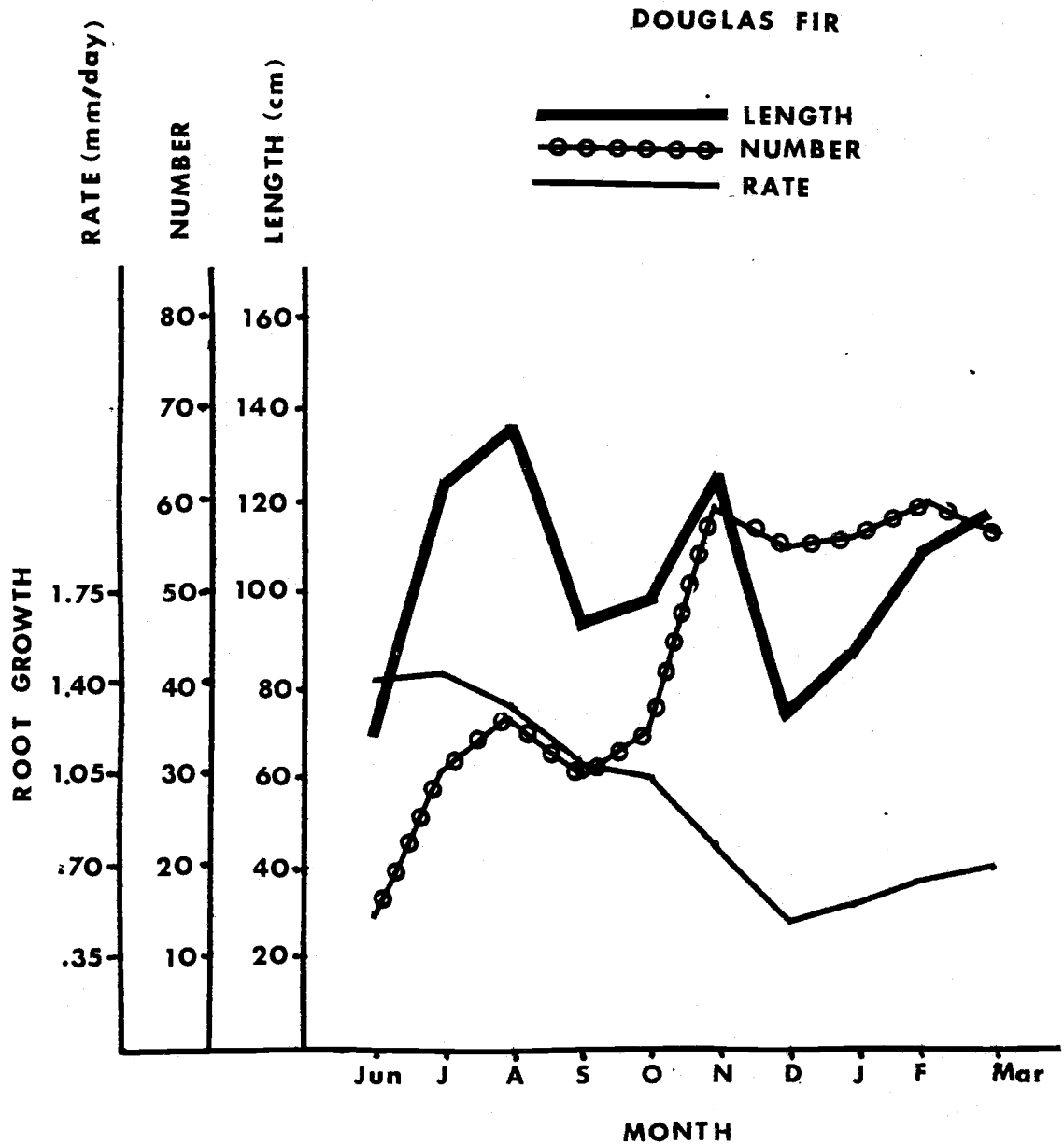
Figure 6. Seasonal pattern of RRP for Douglas-fir seedlings in the outdoor treatment (NT/ND); a comparison of the various growth parameters. Each point is an average of 20 individual measurements. The pooled standard errors are root length ± 10.7, new roots ± 4.5 and growth rate ± 0.06.
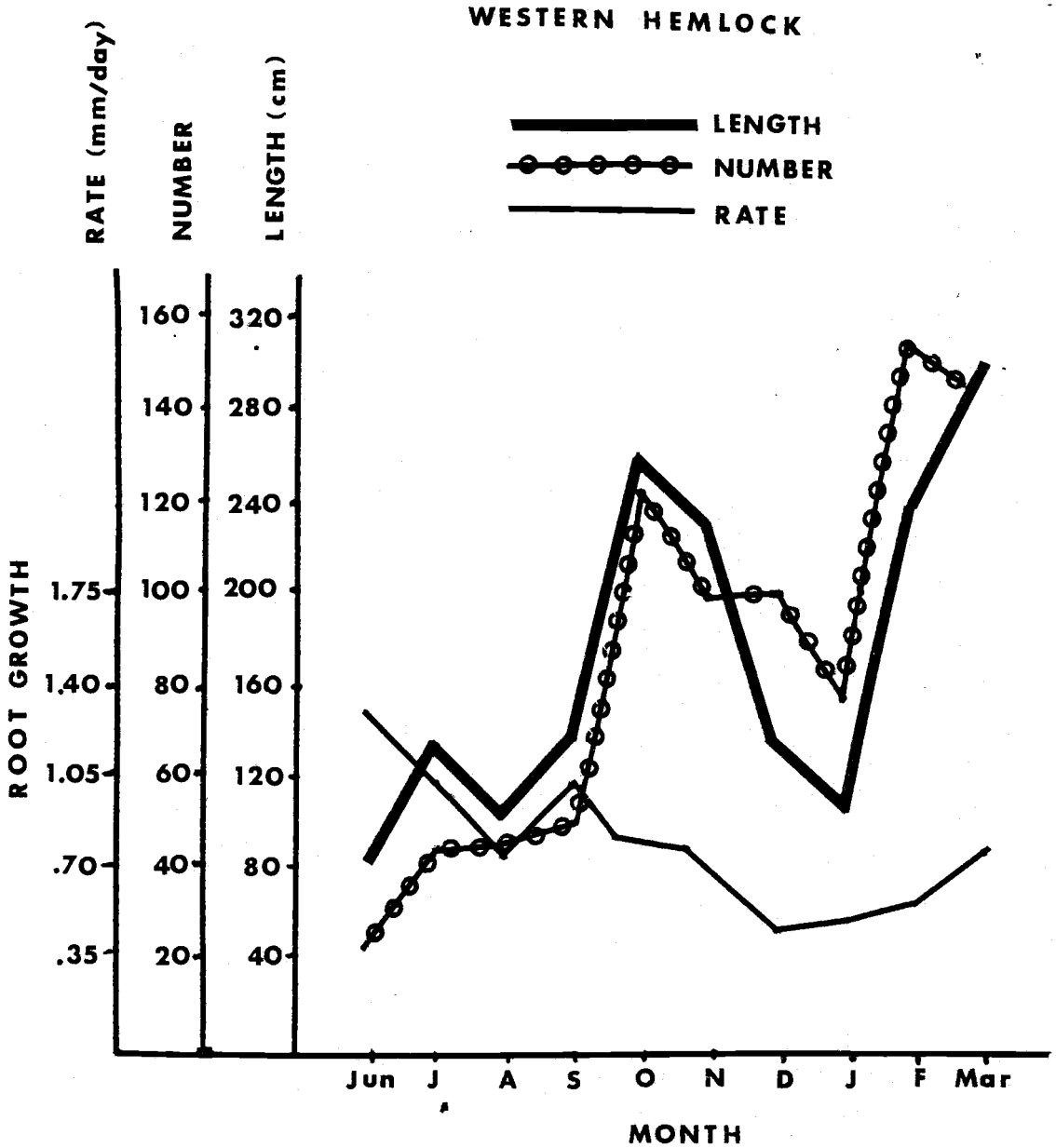
38

WESTERN HEMLOCK

Figure 7. Seasonal pattern of RRP for weastern hemlock seedlings in
the outdoor treatment (NT/ND); a comparison of the various
growth parameters. Each point is the average of 20
individual measurements. The pooled standard errors are
root length ± 18.9, new roots ± 8.5, and growth rate
± .12.

(Figure 7). The growth rate for the roots was similar for both spe-
cies; it declined slowly from June until December and then rose
slightly between December and March (Figures 6 and 7).

In general, a similar pattern of growth rate was observed for
all other treatments over time in both species. Figure 8 shows this
for Douglas-fir. At any given time, growth rates in the evaluation
environment were similar for all seedlings regardless of treatment with
two exceptions; seedlings of both species in the 5°/SD treatment begun
June and the 5°/LD treatment begun two months later showed signifi-
cantly higher growth rates.

In contrast, an examination of the number of roots regenerated
revealed significant differences associated with the various treatments
employed. For example, Douglas-fir seedlings from long- or natural
photoperiod 5°C environments sampled between September and December
produced significantly more new roots than the corresponding photo-
period treatments at 20°C (Figure 9). After December, however, the
unchilled seedlings began to produce an increased number of roots and
finally outproduced the chilled seedlings.

The effects (again on the number of new roots) of both the dura-
tion of chilling and the time of its initiation were examined by
comparing the 5°/SD treatments begun at various times of the year
(Figures 10 and 11). In Douglas-fir, the response of the number of
roots to chilling was quite regular, with chilling for four and eight
weeks resulting in progressive increases of 40-100 percent in RRP, with
12 and 16 weeks producing no additional response. The two earliest
5°/SD treatments begun in June and August are exceptions, however,
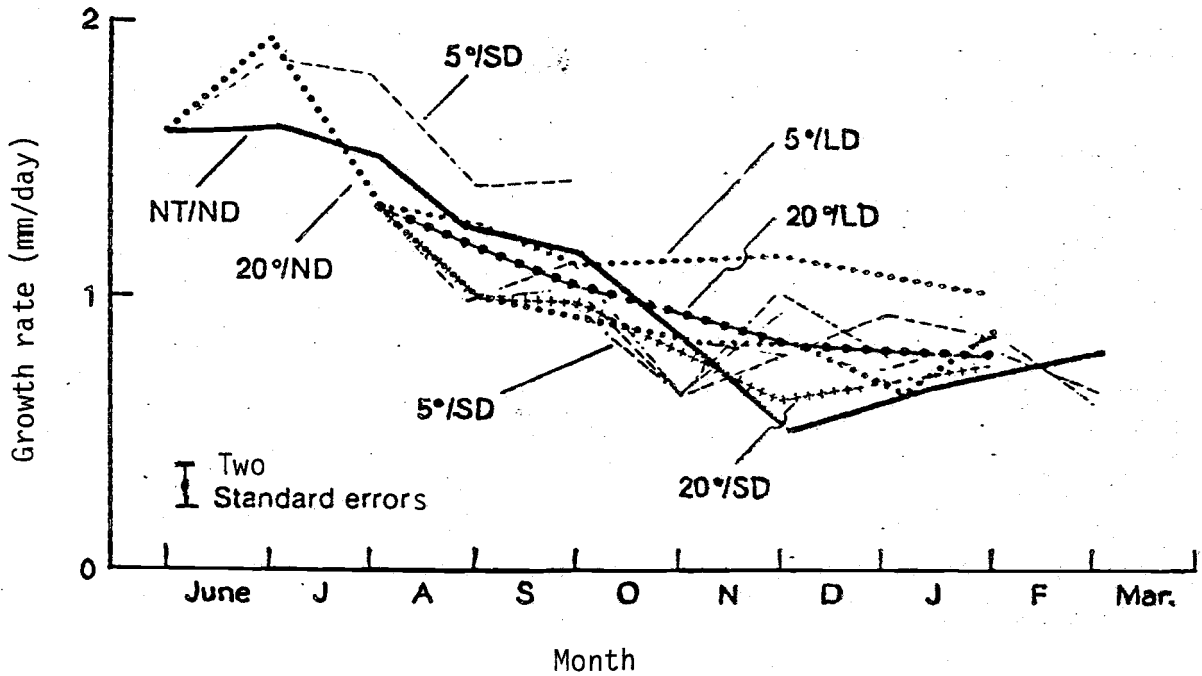
Figure 8. The effects over time of outdoor and controlled environment treatments on the average growth rate per root for the Douglas-fir seedlings. Each point represents the mean of the per root growth rate of 20 seedlings during the standard evaluation period.
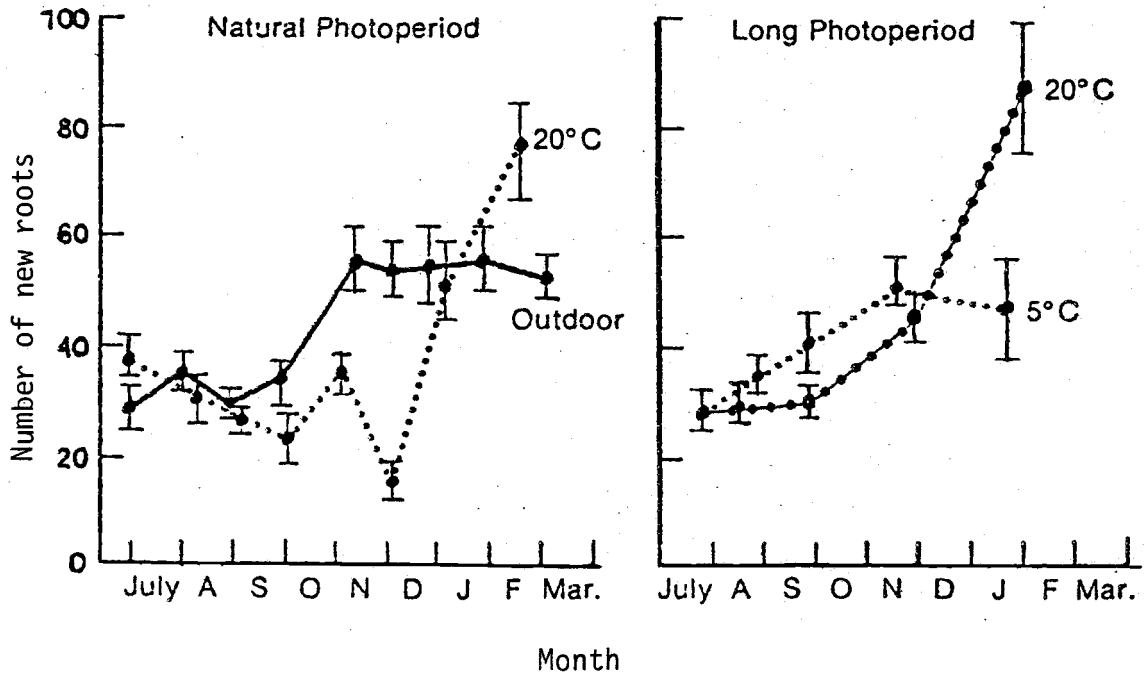
Figure 9. The long-term effect of constant warm or cold treatments on the number of new roots regenerated by the Douglas-fir seedlings. Each point represents the mean of 20 seedlings. The vertical bars represent two standard errors.

Figure 10. The effect of the duration and timing of chilling on the number of new roots regenerated by the Douglas-fir seedlings during the standard evaluation period. Each point represents the mean of 20 seedlings. Two pooled standard errors are indicated.
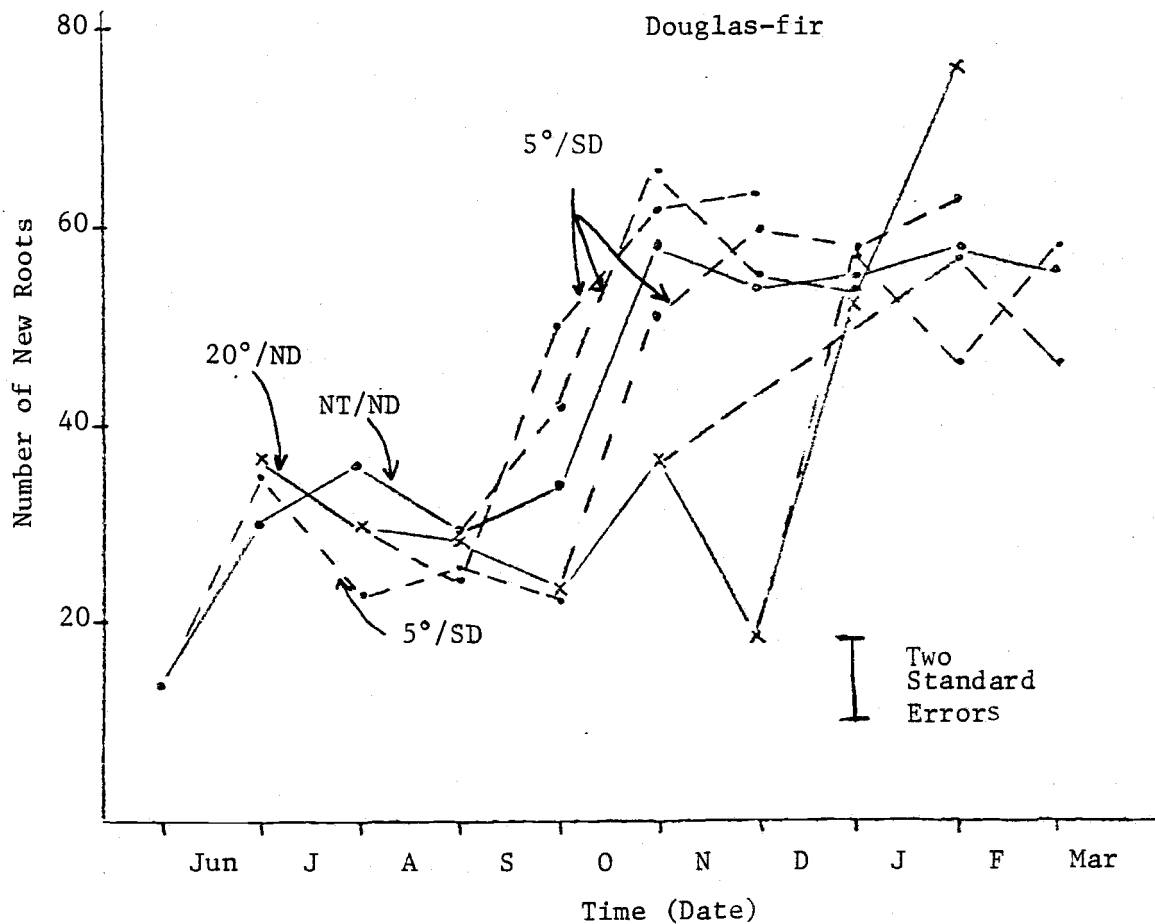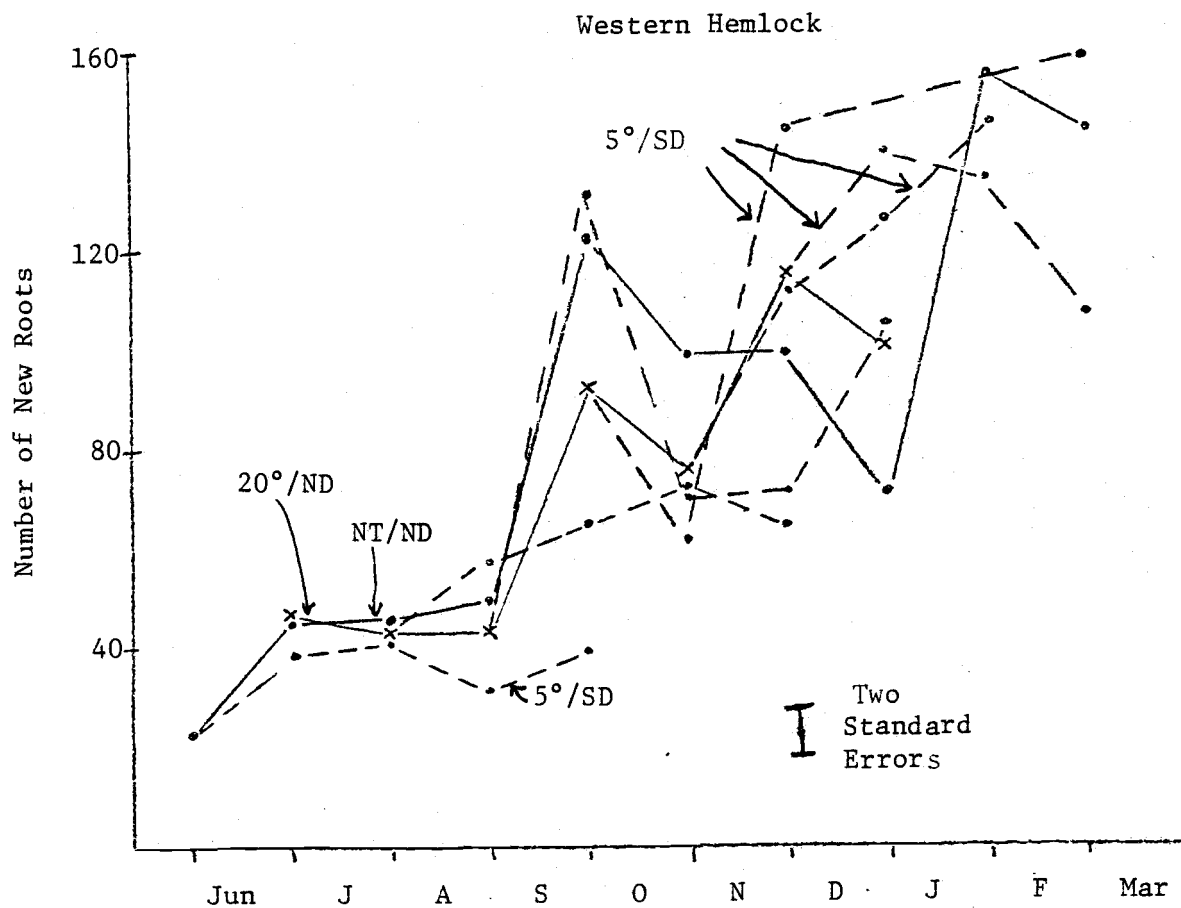
Figure 11. The effect of the duration and timing of chilling on the number of new roots regenerated by the western hemlock seedlings during the standard evaluation period. Each point represents the mean of 20 seedlings. Two pooled standard errors are indicated.

and did not show an initial increase in RRP (Figure 10). For western hemlock seedlings, however, chilling did not have a consistent effect on the number of new roots produced (Figure 11). The first month of chilling did, in general, increase the number of roots regenerated, but the response was variable. For example, the seedlings placed into the 5°/SD environment in October grew fewer roots during the first month than those that remained in the greenhouse.

The three parameters of root growth (length, number, and average growth rate) showed differing degrees of correlation with the dormancy as measured by the days until bud break. In both species, the length of new roots showed no relationship to bud dormancy. This was also true of the number of roots produced by the western hemlock seedlings (Figure 12). In contrast, the number of roots produced by the Douglas-fir was significantly correlated ($p < .05$) with the dormancy (Figure 13). The number of roots produced was greatest when the seedlings were not fully dormant and when bud break occurred within 50 days (> 2 DARDS) of placing them in the test environment. The growth rate of the roots in both species decreased as the buds became less dormant (DARD value increased)(Figures 14 and 15). As before, the 5°/SD treatment begun in June and the 5°/LD treatment begun two months later were both exceptions to the relationships described. In addition, samples of western hemlock seedlings that never broke dormancy during the 350 days of the experiment (DARD value of 0.28 arbitrarily assigned) did not conform to the relationship.

The percentage of the total ^{14}C photosynthate that was translocated to the root at different dates and for different treatments ranged from
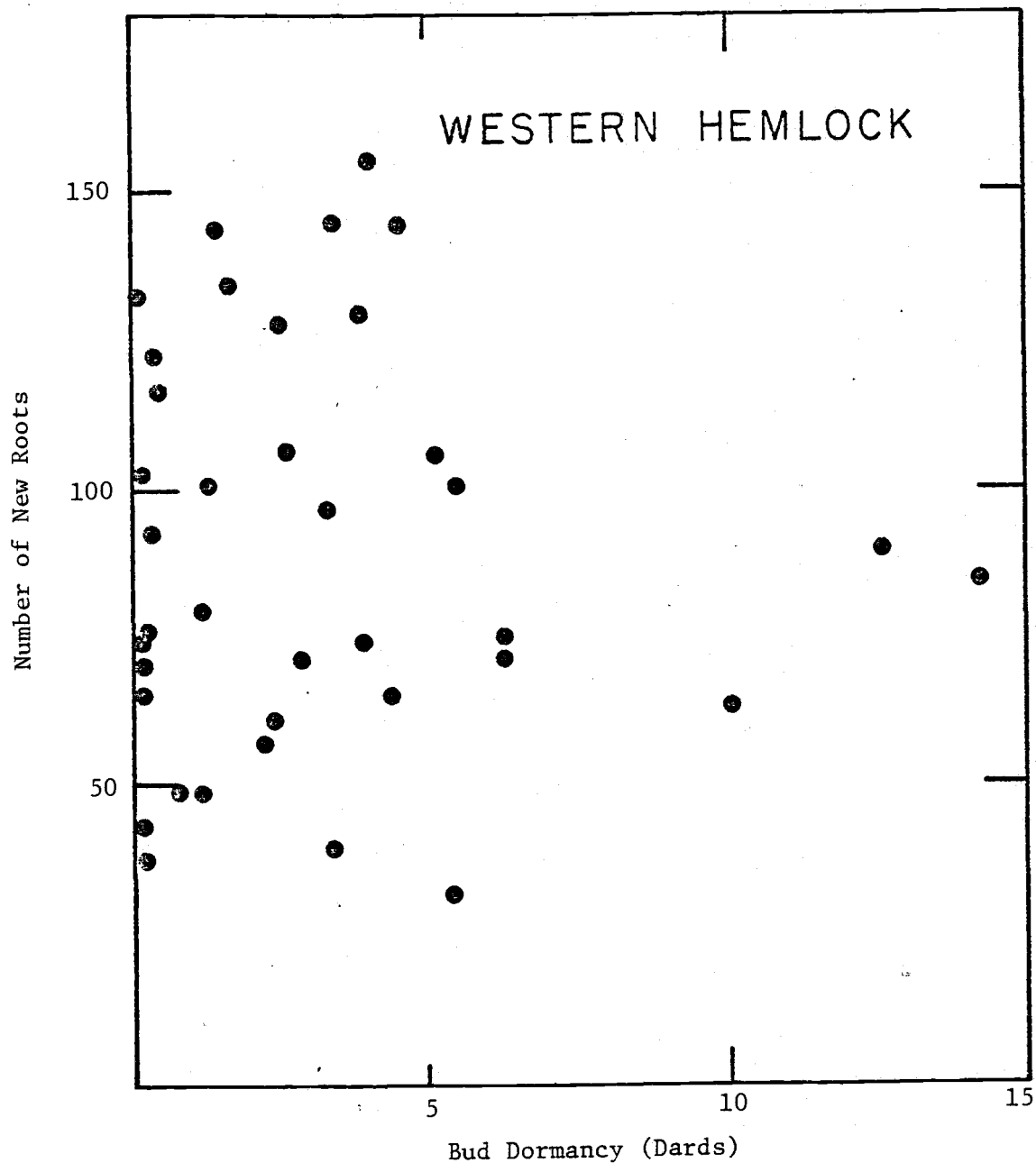
Figure 12. The relationship between the number of new root and the
dormancy status of the bud in the western hemlock seedlings.
Each point corresponds to a particular treatment and
sample date whose number of new roots was determined from
a sample of 20 seedlings and dormancy level from a sample
of five. No significant regression line was found through
the data.
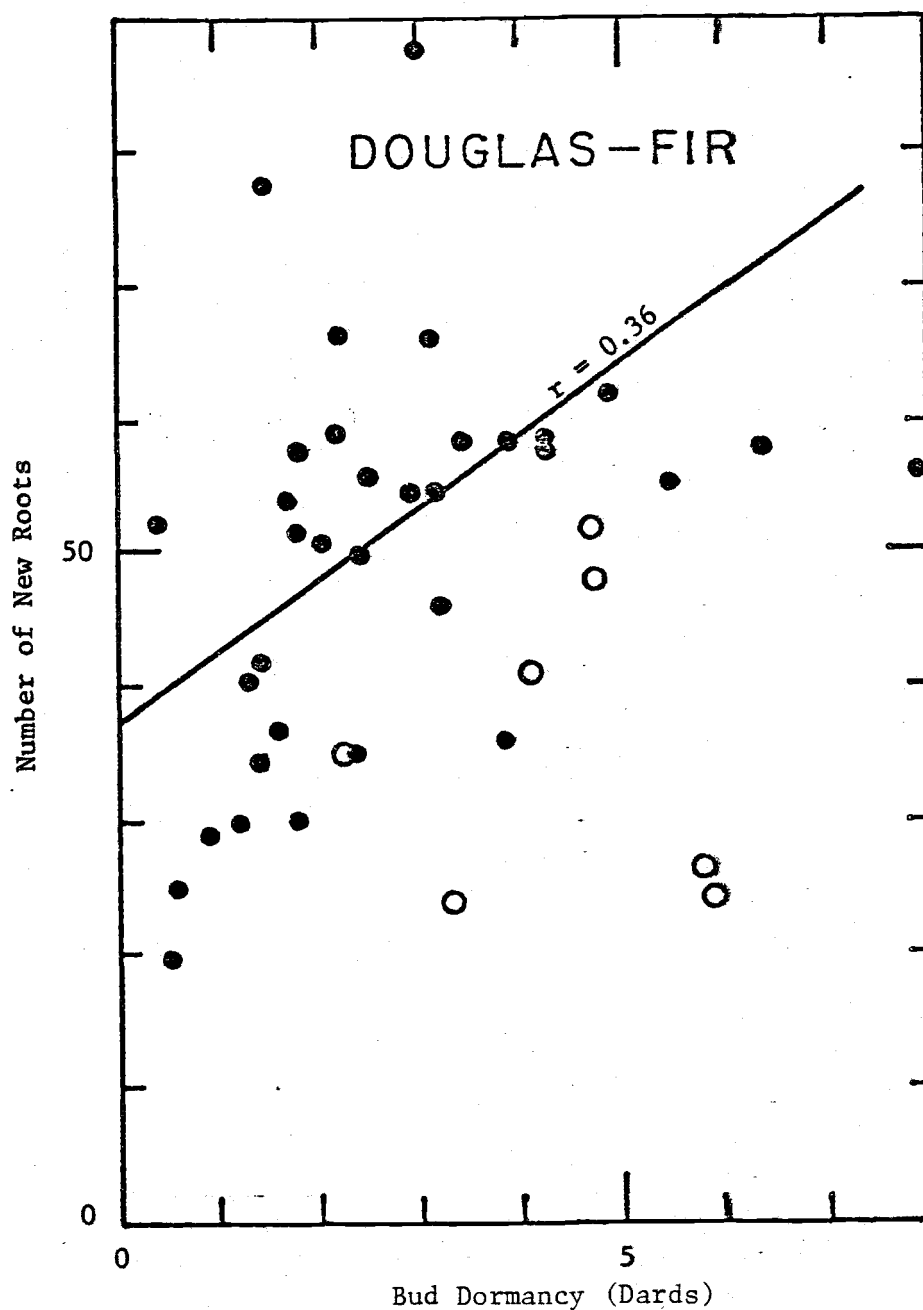
Figure 13. The relationship between the number of new roots and
the dormancy status of the bud in the Douglas-fir seed-
lings. Each point corresponds to a particular treatment
and sample date whose number of new roots was determined
from a sample of 20 seedlings and dormancy level from a
sample of five. The regression is determined by excluding
those samples from treatments 5º/SD and 5º/LD which
contained seedlings that lacked terminal buds (designated
as 0 on the graph). The r = 0.36 was significant at
p < .05.
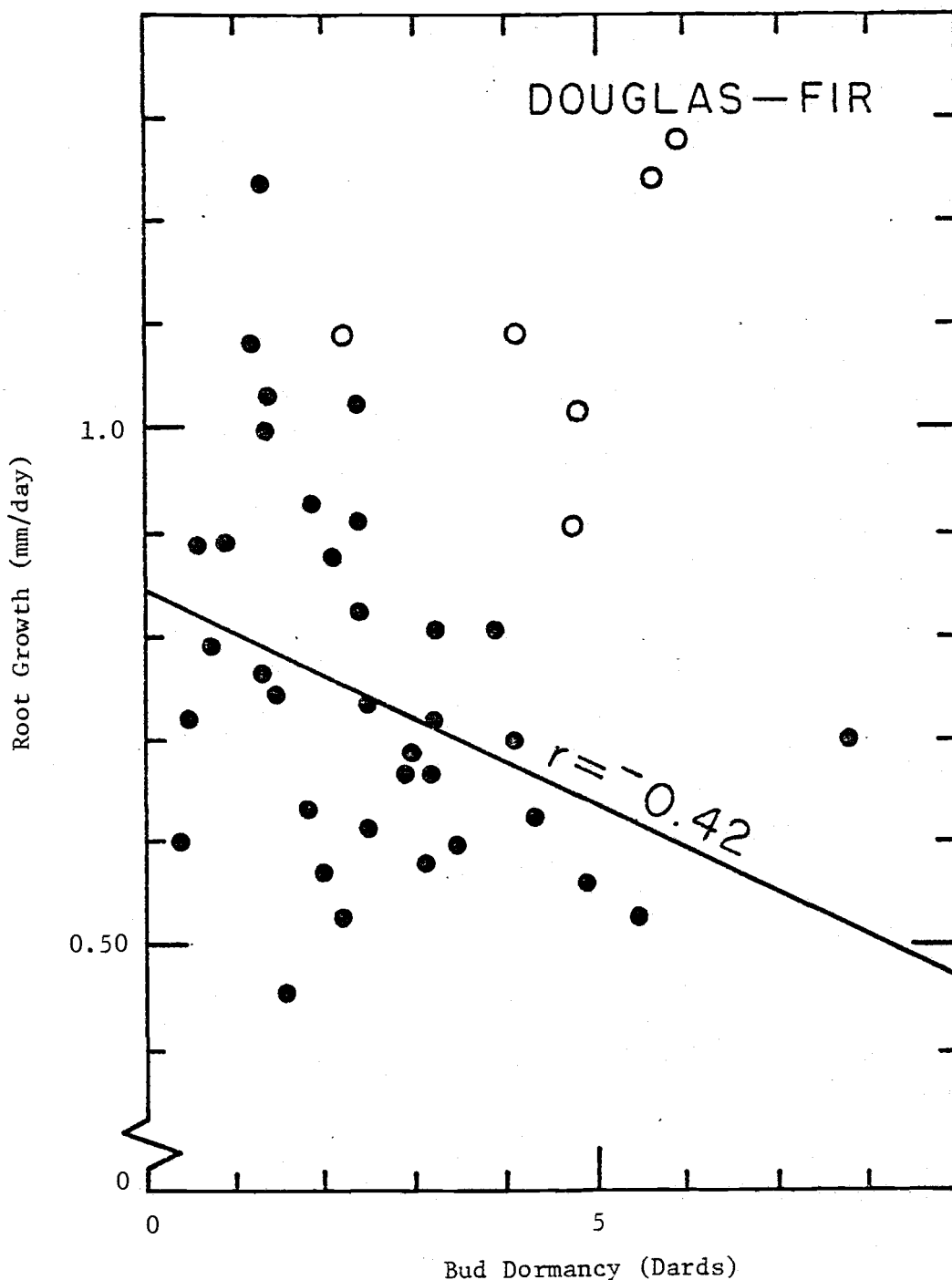
Figure 14. The relationship between the growth rate of new roots
of the Douglas-fir seedlings and the bud dormancy. Each
point corresponds to a particular treatment and sample
date whose root growth rate was determined from a sample
of 20 seedlings and dormancy level from a sample of five.
The calculated regression does not include six points
(designate 0) from seedlings lacking fully-formed terminal
buds (Treatments 5⁰/SD and 5⁰/LD). The r = -0.42 is
significant at p < .05.

48
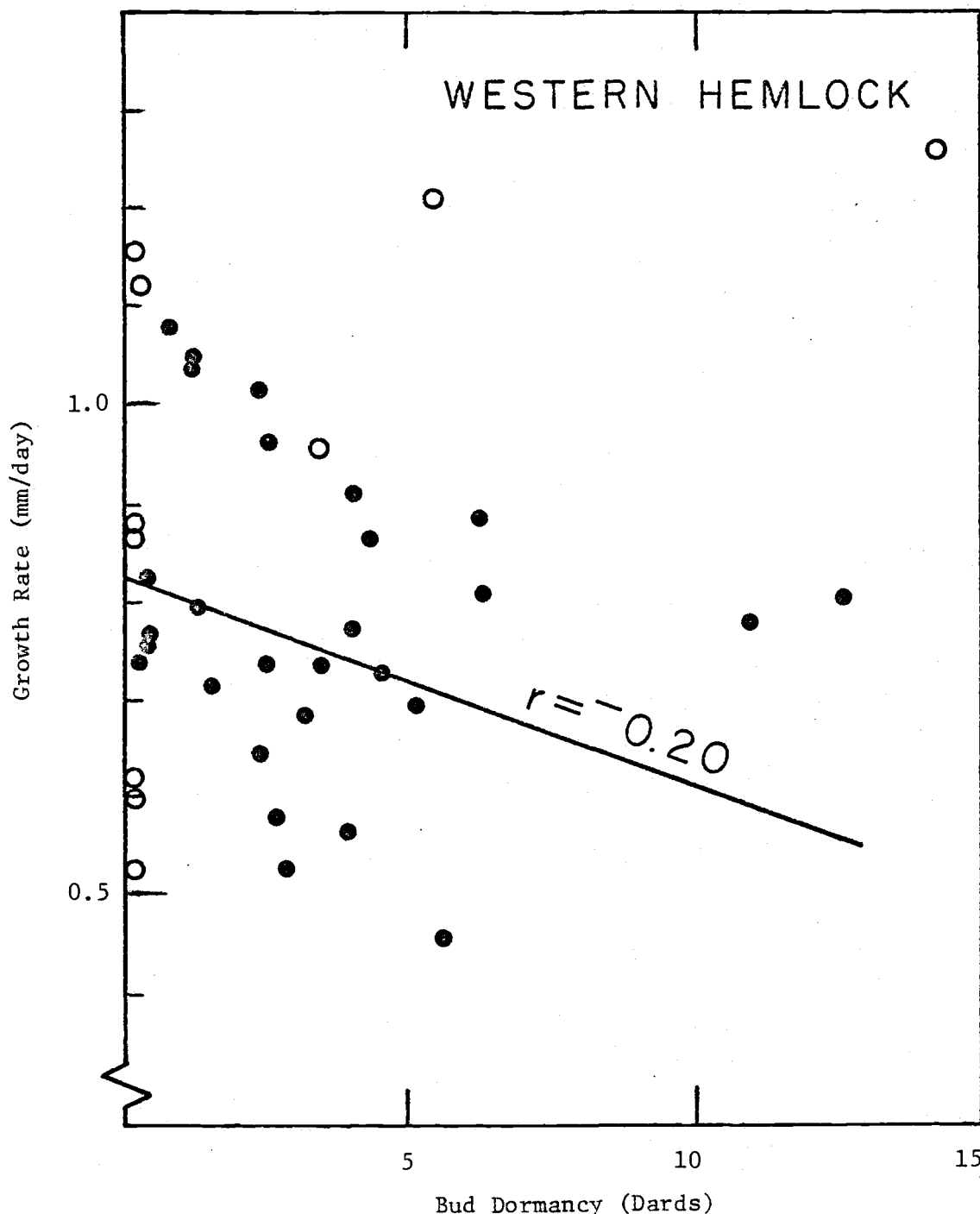
Figure 15. The relationship between the growth rate of new roots of the western hemlock seedlings and the bud dormancy. Each point corresponds to a particular treatment and sample date whose growth rate was determined from a sample of 20 seedlings and dormancy level from a sample of five. The calculated regression does not include six points from seedlings lacking fully formed buds (Treatments 5°/SD and 5°/LD) and those samples where the seedlings never broke bud (all designated 0). The regression line is not significant.

0.54-19.4 percent in the western hemlock and from 1.0-26.9 percent for the Douglas-fir. In all the treatment for both species with the exception of the 20°/LD treatment in the western hemlock a consistent seasonal pattern was evident. The percent ^{14}C translocated to the root was highest in the fall and decreased rapidly to a minimum in December or January (Figures 16 and 17).

The percent of the total ^{14}C fixed that was translocated to the roots within 24 hours was unrelated to the total length of new roots produced during the 28-day evaluation period (Figure 18). The percent ^{14}C in the root was weakly negatively correlated with the number of new white roots with correlation coefficients of r=-0.24 for the hemlock and r=-0.51 for the Douglas-fir (r=-0.51 is significant at p<.05).

A curvilinear relationship of the form ax^2+bx+c resulted in highly significant simple correlation coefficients (p<.01) when both the growth rate of the roots (Figure 19) and the dormancy status of the bud (Figure 20) were related to the percent of ^{14}C translocated to the root.

The percentage of ^{14}C-photosynthate translocated to the root on any date was not related to the total amount of carbon fixed or the carbon fixed per gram of shoot. Length, number and growth rate of new roots also showed no correspondence to these measurements of the amount of carbon fixed.
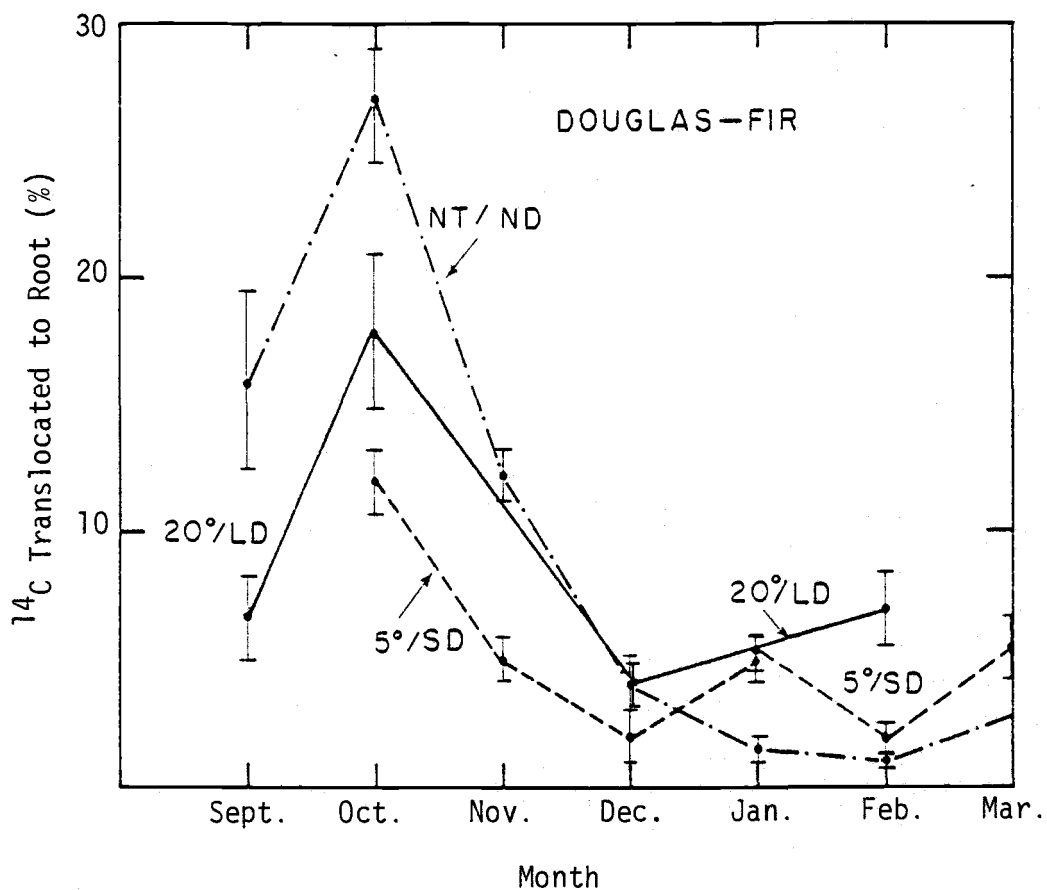
Figure 16. Seasonal pattern of percent ^{14}C-photosynthate translocated to the roots of Douglas-fir seedlings in the first 24 hours after removal from the treatment environment. Each point is the mean of ten seedlings. Vertical bars represent two standard errors.
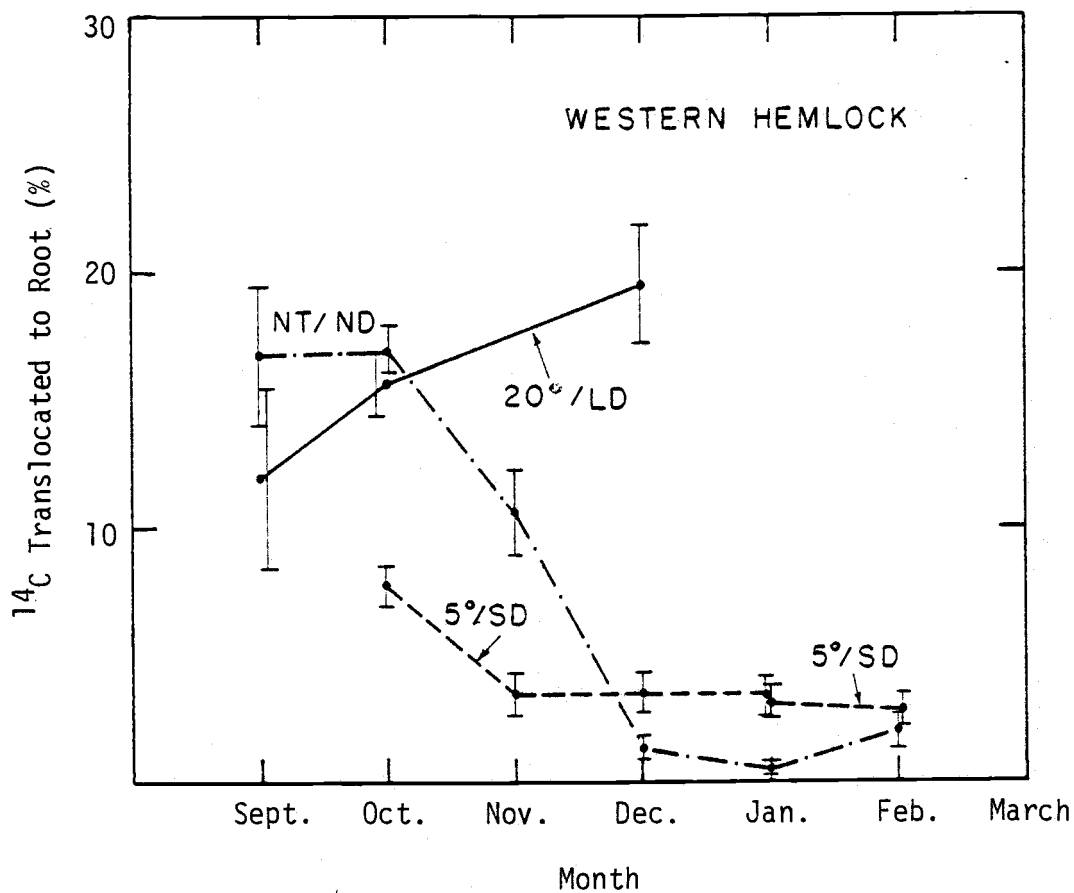
Figure 17.   Seasonal pattern of percent ^{14}C-photosynthate translocated
to the roots of western hemlock seedlings in the first
24 hours after removal from the treatment environment.
Each point is the mean of ten seedlings. Vertical bars
represent two standard errors.
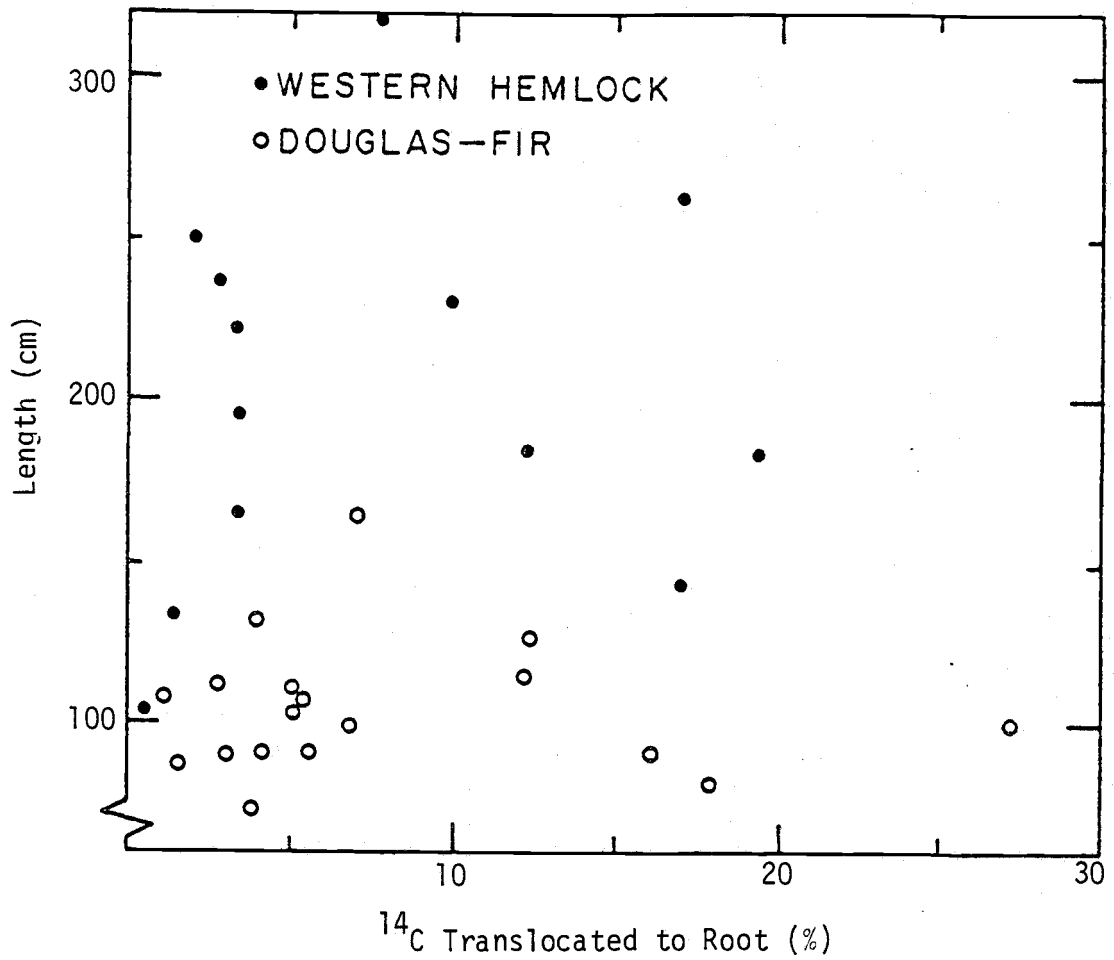
Figure 18. The relationship between length of new roots and the
percent ^{14}C-photosynthate translocated to the root.
Each point corresponds to a particular treatment and
sample date whose length of new roots was determined
from a sample of 20 seedlings and the %-^{14}C translocated
to the root from a sample of ten.
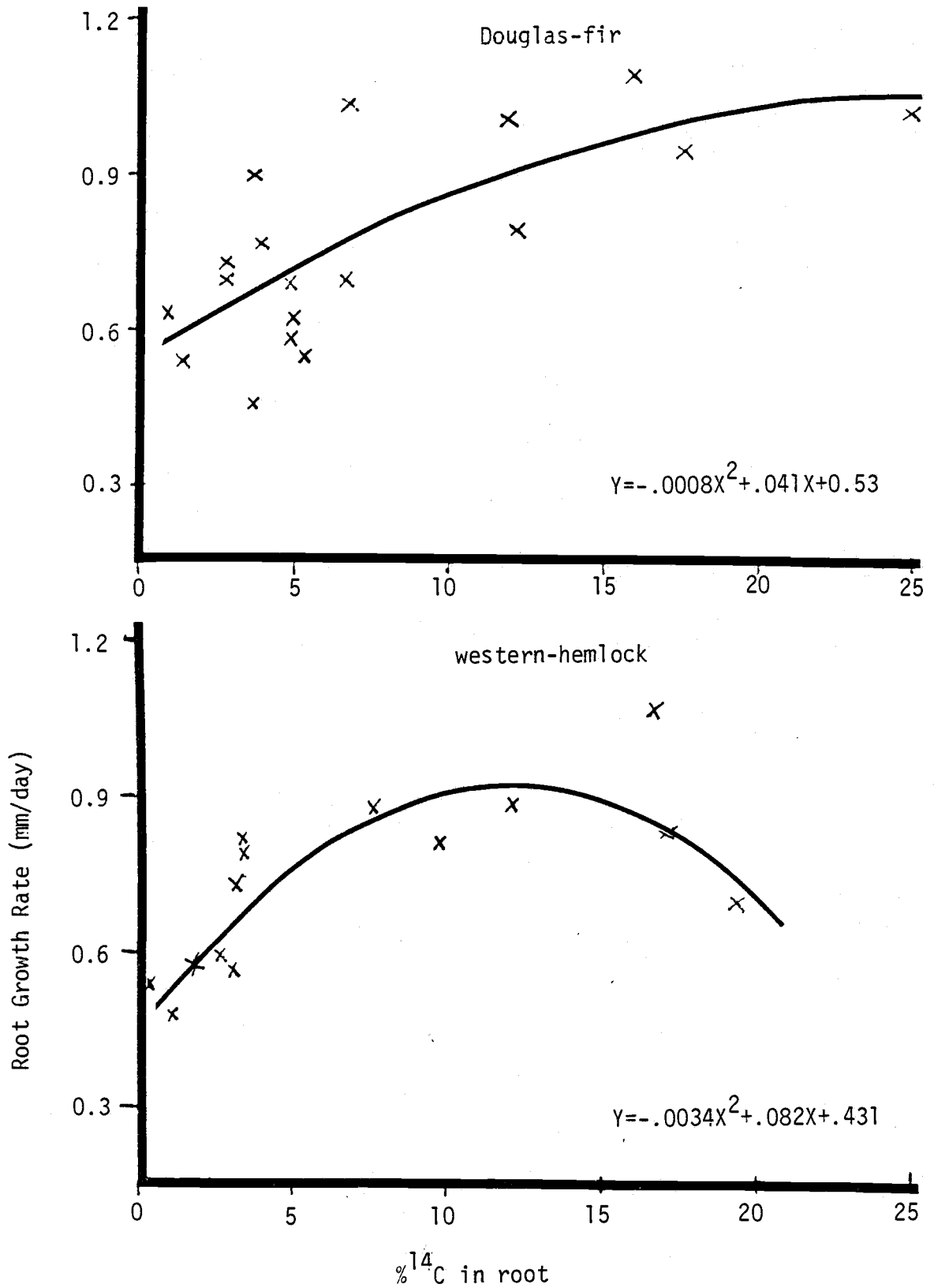
Figure 19.

Figure 19. The relationship between the average growth rate of the root and the percent ^{14}C photosynthate translocated to the root for both Douglas-fir and western hemlock seedlings. Each point represents the mean root growth rate of twenty seedlings and the average percent ^{14}C translocated to the root for ten seedlings. The line shown in each graph is the multiple regression line given by the equation shown.

Douglas-fir
$Y = .012X^2 - .463X + 5.424$

$r = .65$

western-hemlock
$Y = .0292X^2 - .755X + 5.743$

$r = .76$

Bud Dormancy (DARD)

% ^{14}C translocated to the root

Figure 20.

Figure 20. Relationship between the bud dormancy and the percent ^{14}C
            translocated to the root for both Douglas-fir and
            western hemlock seedlings. Each point represents the
            mean dormancy measurement of five seedlings and the
            average percent ^{14}C translocated to the roots of ten
            seedlings. The line shown in each graph is the
            multiple regression line given by the equation shown.

Discussion

Survival in the field has been correlated to the length of new roots (Brown 1976) grown in the evaluation environment. Root regeneration potential, however, has been reported as the number of new roots (Krugman, et al. 1965, Winjum 1966, and Lüpke 1976) the length of new roots (Krugman and Stone 1966, and Stone and Norberg 1976) or both the length and number (Schubert and Baron 1965, and Stone and Schubert 1959). The results from this experiment indicate that in some species such as western hemlock both parameters produce RRP peaks that occur at approximately the same time (Figure 8) and therefore should give similar predictions of survival in the field. In Douglas-fir, however, measurements of the root number and length produce quite divergent seasonal patterns (Figure 7) which result in differing conclusion about the RRP during a la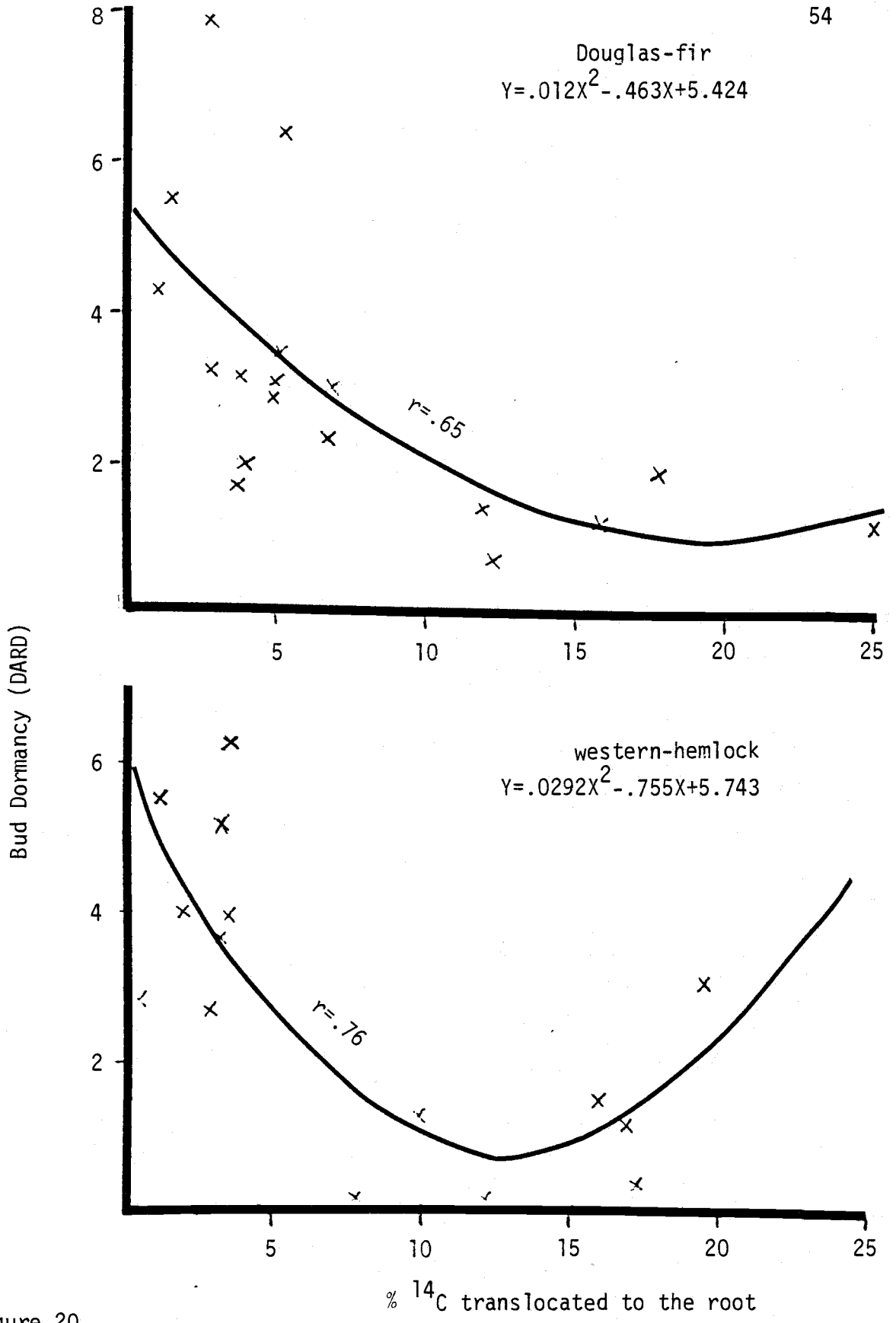rge part of the season. It seems desirable to standardize the parameter reported as RRP; length is the logical choice if correlation with survival is desired.

Although there are no other results of RRP measurements analyzed by the method used in this paper available in the literature, data obtained from Brown (1976) on western hemlock containerized seedlings, when reevaluated, produced seasonal patterns for the three parameters of root growth strikingly similar to those obtained in this study (Figure 21). His data adds credence to the endogenous pattern in the growth rate of the roots found in this study. His results also substantiate the conclusion that in western hemlock the seasonal pattern of length and number of new roots is similar.
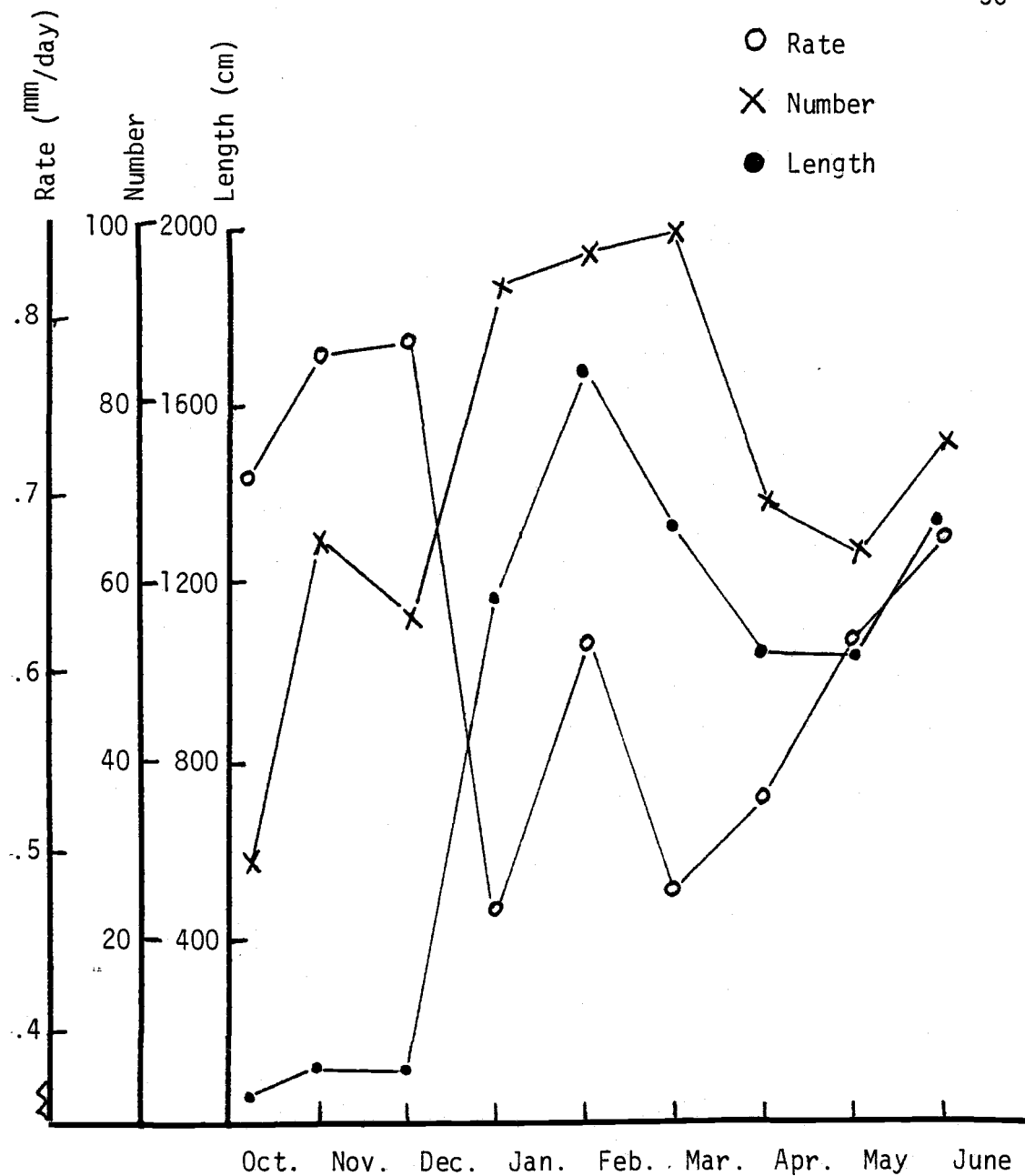
Figure 21. Seasonal pattern of RRP for western hemlock seedlings grown in containers in an outdoor environment; a comparison of the various growth parameters. Each point is the average of 20 measurements. (Data adapted from Brown, 1976).

One possible explanation of the agreement between length and number in western hemlock and disagreement in Douglas-fir is the difference of root growth habit in the two species. Western hemlock produces a much larger number of roots, 0.5 cm or larger after one month than the Douglas-fir and the variation from month to month is also larger. This large variation in number masks the small variation in growth rate in the composite parameter of length. In the Douglas-fir, the variation in the number is moderate from month to month and, therefore, the variations in growth rate play a larger role in determining the variation reflected in total length of new roots.

In the previous experiment, the data indicated that the growth rate of the roots may be related to the short term photosynthetic rate of the seedlings. Decreasing photosynthetic rates are known to occur during the winter months following bud set (Fry and Phillips 1977) and may be the cause of the seasonal decline in root growth rate observed in this experiment.

Results from this experiment indicate that contrary to the conclusions of Stone and his co-workers (1959, 1962), it is important to consider both the number of new roots and their growth rate in order to understand the basic environmental control of root growth. The first experiment in this thesis has shown that different environmental factors affect either the growth rate or the number of new roots which in turn affects the total length of the new roots. In this experiment, the growth rate of the roots seemed unrelated to the treatment imposed, while the number of new roots responded to almost every environmental change. The growth rate of the roots was only affected by the date of

bud set. If buds were set, the growth rate declined until December at an almost constant rate that was unrelated to the treatment imposed (Figures 9 and 10). However, maintaining seedlings in an environment that prevented bud set (5°/SD begun June, 5°/LD) allowed the seedlings to maintain a near constant root growth rate.

The enhancement of RRP by chilling treatments, hypothesized at the outset of this study, was observed with respect to the number of new roots produced but not with respect to their average growth rate. These results, at first, seem to be a direct contradiction of the results reported in the first experiment in this thesis that chilling affects the growth rate of the new roots and not the number. This difference can be reconciled by considering the age and physiological state of the seedlings in both studies. In my earlier study, the seedlings were two years old and probably contained substantial stored carbohydrate that could be hydrolized by chilling and thus increase the growth rate of the roots. The first year seedlings used in this study would have fewer reserves. In addition on both evaluation dates in my earlier study the seedlings were in a post-dormant state. Results from this study indicate that after the chilling requirement is fulfilled the number of roots regenerated tends to level off. This would explain the lack of effect chilling had on the number of roots produced in the previous experiment.

The number of new roots increased with increasing chilling as the dormancy of the Douglas-fir deepened from June (non-dormant, no

enhancement after 16 weeks of chilling) to October (fully dormant, 100 percent enhancement after four weeks of chilling) strongly suggesting a direct relationship with bud dormancy. Dormancy was shown by Campbell and Sugano (1975) to be quantitatively related to both the time of initiation and duration of chilling in Douglas-fir.

Although a general increase of root number with increased chilling is observed in the hemlock, the relationship is not as consistent as seen in the Douglas-fir and anomalies in the data do exist. This may be related to the shorter chilling requirement of western hemlock, four weeks (Nelson 1978) as compared to eight to ten weeks for Douglas-fir (Campbell and Sugano 1975). After the chilling requirement has been fulfilled, additional chilling would have less predictable results. A rapid increase in the number of new roots produced by both species was observed in the outdoor treatment after the accumulation of sufficient natural chilling in October.

The presence of a terminal bud plays an important role in determining the effect of chilling on the number of roots produced. For example, chilling begun in June had no effect on root initiation and these seedlings never developed a terminal bud. Seedlings whose chilling treatment began in August did not have terminal buds at the outset of the treatment period and for the first four weeks chilling had no effect. During these four weeks, however, buds developed, after which chilling increased the number of roots produced.

The results not only support many others cited earlier that chilling enhances RRP, but they also lead to the more specific conclusion that a

dormant bud must be present for chilling to be effective. It can not, however, be concluded from this data that there is invariably a direct correlation between bud dormancy and RRP. A more direct correlation between the measured bud dormancy (DARD) and the root parameters failed to give concrete results but hinted at some possible relationships.

In Douglas-fir, the bud dormancy was weakly correlated with both the number of new roots and their growth rate. In western hemlock, only growth rate was correlated with the bud dormancy. These poor correlations can be partly explained by the small sample used for dormancy determination (five trees per treatment) and the large variation within each sample.

The correspondence of bud dormancy with the number of new roots is probably the most physiologically significant result. The relationship in Douglas-fir is similar to that demonstrated in deciduous trees (Webb 1977, and Richardson 1958) that fully dormant buds-{one that will not grow when placed in a favorable environment(Dard value low)inhibit root growth while fully formed non-dormant buds (Dard value high) stimulate it.

The correlation of bud dormancy to root growth rate probably reflects both the period since bud set (as shown in the relationship of growth rate to sample date) and a true dependency on bud dormancy. The first relationship increases the variance of the second.

Although chilling had a pronounced short term effect on root growth, seedlings that remained in the continuously warm treatments (20 °/ND, 20 °/LD), showed a two or three-fold increase in the number of new roots regenerated (Figure 11). This suggests, as Merritt (1968)

did, the eventual dominance of some endogenous trigger for root growth whose timing is influenced by chilling. Worral (1971) reported a similar situation with regard to initiation of cambial activity in unchilled Douglas-fir seedlings.

The percentage of ^{14}C translocated to the root within twenty-four hours is not a good indicator of the subsequent RRP as determined by the length of new root produced during the 28-day evaluation period. It, therefore, is not a practical method for rapid prediction of RRP.

The use of ^{14}C, however, may give some insight into the basic physiological processes controlling root growth. The results of this experiment indicate that the number of roots produced during the subsequent evaluation period is lower when more ^{14}C is translocated to the root during the first twenty-four hours after the seedlings are removed from the treatment environment. Although this negative correlation is admittedly weak, its negative nature clearly indicates a lack of dependence of root number on current photosynthate. The number of new roots produced in other species has been suggested to be under hormonal control (Carlson 1976).

The growth rate of new roots, in contrast, has been suggested by some investigators to be controlled by the level of current photosynthate (Carlson 1976, Richardson 1953). Results of Etter and Carlson (1973) using dormant lodgepole pine indicate that the amount of sucrose translocated from the shoot to the root was greater for seedlings with new root growth than for those seedlings with no root growth. Their results agree with the present results obtained for the growth rate of

the roots of Douglas-fir and western hemlock seedlings. An increase in growth rate was closely correlated to an increase in the percent ^{14}C-photosynthate translocated to the roots.

The relationship between bud dormancy and the percent ^{14}C-photosynthate translocated to the roots is best interpreted in conjunction with the similar relationship of root growth and the percent ^{14}C-photosynthate translocated to the roots. These two relationships produced similar but opposite curves with similar correlation coefficients. This difference is best explained by example. When the buds are dormant (low DARD value) and the growth rate of the root system is high, a larger percentage of the ^{14}C-photosynthate is translocated to the root. And conversely, when the buds are non-dormant (high DARD value) and preparing for shoot growth, and the root growth rate is low, a smaller percentage of the ^{14}C-photosynthate is translocated to the root. This interpretation agrees well with the general hypothesis that shoot growth and root growth compete for the available carbohydrate (Eliasson 1971). Furthermore, it supports the more specific hypothesis that it is the growth rate of the roots and not the number of roots initiated that competes with the shoots for current carbohydrate.

The seedlings ability, within the first twenty-four hours outside the treatment environment, to distribute current photosynthate in a manner that reflects both the one month growth rate of the roots and the long term dormancy status of the bud seems quite astounding. However, others have found that ^{14}C-photosynthate translocated to the root after an even shorter period of time (less than twenty-four hours)

was related to the total amount that remained in the root after one month (Schier 1970, Ziemer 1971, Shiroya, et al. 1966). All these results indicate that twenty-four hours is long enough to establish the general allocation pattern for the following month.

The seasonal pattern and the magnitude of percent ^{14}C-photosynthate translocated to the root found in this study agrees well with the one found by Shiroya et al. (1966) for white pine. The fact that this seasonal pattern of allocation does not depend on the total amount of ^{14}C-fixed indicates a true variation in the source-sink relationships and not merely a spill-over of excess photosynthate to the root when photosynthesis is high. The one divergent point in the seasonal pattern of percent ^{14}C-photosynthate translocated to the root of the western hemlock seedlings was for the 20°/LD treatment sampled in December. The erratic behavior of this result may be related to the seedlings continued growth in the 20°/LD environment.

In summary, RRP measurements need to be consistantly expressed as length if correlations with survival are desired. But the examination of its two component parameters, growth rate and the number of new roots produced, may lead to a better understanding of the basic physiological phenomenon of root growth. This study demonstrates that chilling can be used in predictive equations of RRP in nurseries for both Douglas-fir and western hemlock. However, under less natural conditions, factors other than chilling may trigger root growth. More experimentation is needed to understand the seasonal pattern of

growth rate and the relationships between bud dormancy and both the number of new roots and their growth rate. Finally, although the percent of ^{14}C translocated to the root cannot be used to predict RRP, measurements of ^{14}C-photosynthate translocation may help to understand how environmental stimuli affect root growth.

BIBLIOGRAPHY

Aronsson, A., T. Ingestad and L. Loof. 1976. Carbohydrate metabolism and frost hardiness in pine and spruce seedlings grown at different photoperiods and thermoperiods. Physiol. Plant. 36:127-132.

Barney, C. W. 1951. Effects of soil temperature and light intensity on the root growth of loblolly pine seedlings. Plant Physiol. 26:146-163.

Bhella, H. S. and A. N. Roberts. 1974. The influence of photoperiod and rooting temperature on rooting of Douglas-fir. J. Amer. Soc. Hort. Sci. 99:551-555.

Bilan, M. V. 1967. Effects of low temperature on root elongation in loblolly pine seedlings. IUFRO Proc. 14th (1967), Munich, Part 4, Sec. 23.

Brix, H. 1970. Effect of light intensity on growth of western hemlock and Douglas-fir seedlings. Bi-monthly Res. Notes. Can. For. Serv., 26:551-555.

Brix, H. 1971. Growth response of western hemlock and Douglas-fir seedlings to temperature regimes during day and night. Can. J. Bot. 49:289-294.

Brown, C. 1976. Patterns of growth and seasonal changes in the concentration of abscisic acid and indoleacetic acid in the roots of western hemlock. M.S. Thesis, Oregon State University, Corvallis, Oregon. 97 p.

Burdett, A. N. 1976. The relationship between root fibrosity and root growth potential in bare-root lodgepole pine. B.C. For. Serv. Int. Rpt. on E.P. 746.03. 5 p.

Campbell, R. K. 1974. Use of phenology for examining provenance transfers in reforestation of Douglas-fir. J. Appl. Ecol. 11:1609-1080.

Campbell, R. K. and A. I. Sugano. 1975. Phenology of bud burst in Douglas-fir related to provenance, photoperiod, chilling and flushing temperature. Bot. Gaz. 136:290-298.

Carlson, L. W. 1976. Root initiation of lodgepole pine and white spruce seedlings grown under varying light conditions. Bi-monthly Res. Notes. Can. For. Serv. 32:21-22.

Carlson, L. W. 1977. The effect of defoliation on conifer seedling root initiation. Bi-monthly Research Notes. 33:1.

Chung, H. H. and P. J. Kramer. 1975. Absorption of water and 32P through suberized and unsuberized roots of loblolly pine Can. J. For. Res. 5:229-235.

Cooper, A. J. 1973. Root temperature and plant growth: A review. Commonwealth Bureau of Horticulture and Plantation Crops, East Malling, Maidstone, Kent. 73 p.

Day, R. J. and G. R. MacGillivray. 1975. Root regeneration of fall lifted white spruce nursery stock in relation to soil moisture content. For. Chron. 51:196-199.

Driessche,R., van den. 1978. Seasonal changes in root growth capacity and carbohydrate in red pine and white spruce nursery seedlings. Paper presented at IUFRO meeting, September 11-15, 1978. Nancy France. 12 pp.

Eliasson, L. 1971. Adverse effect of shoot growth on root growth in rooted cuttings of Aspen. Physio Plant. 25:268-272.

Etter, H. M. and L. W. Carlson. 1973. Sugars, relative water content and growth after planting of dormant lodgepole pine seedlings. Can. J. Plant. Sci. 53:395-399.

Fairbairn, W. A. and S. A. Neustein. 1970. Study of response of certain coniferous species to light intensity. Forestry 43:57-71.

Fry, D. J. and I. D. J. Phillips. 1977. Photosynthesis of conifers in relation to annual growth cycles and dry matter production II Seasonal photosynthetic capacity and mesophyll ultrastructure in Abies grandis, Picea sitchersis, Tsuga heterophylla and Larix leptolepis growing in S.W. England. Phys. Plant. 40:300-306.

Gilmore, A. R. 1965. The apparent source of a root growth stimulus in loblolly pine seedlings. Univ. of Illinois, Agr. Exp. Sta. Forestry Note 112: 4 p.

Good, R. E. and N. F. Good. 1976. Growth analysis of pitch pine seedlings under three temperature regimes. For. Sci. 22:445-448.

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

Hellmers, H. 1963a. Effect of soil and air temperatue on the growth of redwood seedlings. Bot. Gaz. 124:172-177.

Hellmers, H. 1963 b. Some temperature and light effects in the growth of Jeffrey pine seedlings. For. Sci. 9:189-201.

Hellmers, H. and D. A. Rook. 1973. Air temperature and growth of radiata pine seedlings. New Zealand J. of For. Sci. 3:271-285.

Heninger, R. L. and D. P. White. 1974. Tree seedlings growth at different soil temperatures. For. Sci. 20:363-367.

Hoffman, G. 1972. Wachstumsrhythmik der Wurzeln und Sprossachsen von Forstgehoelzen. Flora (Jena) 161:303-319. En. sum.

Jenkinson, J. L. 1975. Seasonal patterns of root growth capacity in western yellow pines. Proc. Soc. Amer. Foresters: p. 445-453.

Kaufman, M. R. 1977. Soil temperature and drought effects on growth of Monterey pine. For. Sci. 23:317-325.

Kramer, P. J. and H. C. Bullock. 1966. Seasonal variations in the proportion of suberized and unsuberized roots of trees in relation to the absorption of water. Amer. J. Bot. 53:200-204.

Krueger, K. W. and J. M. Trappe. 1967. Food reserves and seasonal growth of Douglas-fir seedlings. For. Sci. 13:192-202.

Krugman, S. L., E. C. Stone, and R. V. Bega. 1965. The effect of soil fumigation and lifting date on root regeneration potential of Monterey pine planting stock. J. For. 63:114-119.

Krugman, S. L. and E. C. Stone. 1966. The effect of cold nights on the root-regenerating potential of ponderosa pine seedlings. For. Sci. 12:451-459.

Larson, M. M. 1967. Effect of temperature on initial development of ponderosa pine seedlings from three sources. For. Sci. 13:286-294.

Larson, M. M. 1970. Root regeneration and early growth of red oak seedlings: Influence of soil temperature. For. Sci. 16:442-446.

Larson, M. M. and F. W. Whitmore. 1970. Moisture stress affects root regeneration and early growth of red oak seedlings. For. Sci. 16:495-498.

Lavender, D. 1964. Date of lifting for survival of Douglas-fir seedlings. Res. Note 49. Forest Research Laboratory, Oregon State University, Corvallis, Oregon. 20 p.

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

Lavender, D. P. and R. K. Hermann. 1970. Regulation of the growth potential of Douglas-fir seedlings during dormancy. New Phytol. 69:675-694.

Lavender, D. P. and P. F. Wareing. 1972. Effects of daylength and chilling on the response of Douglas-fir seedlings to root damage and storage. New Phytol. 171:1955-1067.

Little, C. H. A. 1970. Derivation of springtime starch increase in balsam fir (Abies balsamea). Can. J. Bot. 48:1995-1999.

Lüpke, B. 1976. Wurzelregeneration von jungen Forstpflanzen nach dem Verpflanzen. Forstachiv. 47:245-251. From a translation by Scientific Translation Service, Ann Arbor, Michigan.

Lyr, H. and G. Hoffmann. 1967. Growth rates and growth periodicity of tree roots. Internat. Rev. For. Res. 2:181-236.

Merritt, C. 1968. Effect of environment and heredity on the root growth pattern of red pine. Ecology 49:34-40.

Minore, D., C. E. Smith and R. F. Woollard. 1969. Effects of high soil density on seedling root growth of seven northwestern tree species. Pac. Northwest For. and Range Experiment Station. USDA For. Serv. Res. Note PNW-112.

Morrow, R. R. 1950. Periodicity and growth of sugar maple surface layer roots. J. For. 48:875-881.

Nelson, E. A. 1978. The physiology of dormancy of western hemlock (Tsuga heterophylla [Raf.] Sarg.) seedlings. PhD Thesis Oregon State University, Corvallis, Oregon 206 pp.

Neter, J. and W. Wasserman. 1974. Applied Linear Statistical Models. Richard D. Irwin, Inc. Homewood, Illinois. 842 p.

Parker, J. 1959. Seasonal variations in sugars in conifers with some observations on cold hardiness. For. Sci. 5:56-63.

Reed, J. F. 1939. Root and shoot growth of shortleaf and loblolly pines in relation to certain environmental conditions. Duke Univ. Bull. No. 4. 52. p.

Rhea, S. B. 1977. The effects of lifting time and cold storage on root regenerating potential and survival of sycamore, sweet gum, yellow poplar, and loblolly pine seedlings. M.S. Thesis, Clemson Univ., Arkansas.

Richardson, S. D. 1953a. Studies of root growth in Acer saccharinum
    L. I. The relation between root growth and photosynthesis. Proc.
    Kon. Ned. Akad. Wetenschap. C56:185-193.

Richardson, S. D. 1953b. Studies of root growth in Acer saccharinum L.
    II. Factors affecting root growth when photosynthesis is curtailed.
    Proc. Kon. Ned. Akad. Wetenschap. C56:346-353.

Richardson, S. D. 1956. Studies on root growth in Acer saccharinum L.
    III. The influence of seedling age on the short-term relation
    between photosynthesis and root growth. Proc. Kon. Ned. Akad.
    Wetenschap C59:416-427.

Richardson, S. D. 1958. Bud dormancy and root development in Acer
    saccharinum. In: Physiology of Forest Trees, ed. K. V. Thimann.
    Ronald Press, New York. 409-425.

Roberts, A. N. and L. H. Fuchigami. 1973. Seasonal changes in auxin
    effect on rooting of Douglas-fir stem cuttings as related to bud
    activity. Physiol. Plant. 28:215-221.

Schier, G. A. 1970. Seasonal pathways of ^{14}C-photosynthate in red pine
    labeled in May, July and October. For. Sci. 16:1-13.

Schubert, G. H. and F. J. Baron. 1965. Nursery temperature as a factor
    in root elongation of ponderosa pine seedlings. Pac. Southwest
    Forest and Range Experiment Station. U.S. For. Serv. Res. Note.
    PSW-66.

Shiroya, T., G. R. Lister, V. Slankis, G. Krotkov and C. D. Nelson.
    1966. Seasonal changes in respiration, photosynthesis and trans-
    location of ^{14}C labeled products of photosynthesis in young Pinus
    strobis. Ann. Bot. 30:81-91.

Stahel, J. B. 1972. The effect of daylength on the root growth of
    Sitka spruce. For. Sci. 18:27-31.

Stevens, C. L. 1931. Root growth of white pine (Pinus strobus L.)
    Yale Univ., School of Forestry Bull. #32. 61 p.

Stone, E. C. 1955. Poor survival and the physiological condition of
    planting stock. For. Sci. 1:90-94.

Stone, E. C. and J. L. Jenkinson. 1971. Physiological grading of
    ponderosa pine nursery stock. J. For. 69:31-40.

Stone, E. C., J. L. Jenkinson and S. L. Krugman. 1962. Root-
    regenerating potential of Douglas-fir seedlings lifted at different
    times of year. For. Sci. 8:288-297.

Stone, E. C. and E. A. Norberg. 1976. An evaluation of periodicity in the root growth capacity of white fir (<u>Abies</u> <u>concolor</u>). Unpub. progress report for: Members of the True Fir Management Coopera- tive, California.

Stone, E. C. and E. A. Norberg. 1979. Use of root growth capacity in developing propagation regimes, storage criteria, and nursery stock certification. Presented at a meeting of the True Fir Management Cooperative in Redding, California on May 31, 1979. 35 pp.

Stone, E. C. and G. H. Schubert. 1959. Root regeneration of ponderosa pine seedlings lifted at different times of year. <u>For</u>. <u>Sci</u>. 57:837-841.

Stone, E. C., G. H. Schubert, R. W. Benseler, F. J. Baron and S. L. Krugman. 1963. Variations in root regenerating potentials of ponderosa pine from four California nurseries. <u>For</u>. <u>Sci</u>. 9: 217-225.

Taylor, J. S. and E. B. Dumbroff. 1975. Bud, root and growth regulator activity in <u>Acer</u> <u>saccharum</u> during the dormant season. <u>Can</u>. <u>J</u>. <u>Bot</u>. 53(4) 321-331.

Ursino, D. J., C. D. Nelson and G. Krotkov. 1968. Seasonal changes in the distribution of photo-assimilated ^{14}C in young pine plants. <u>Plant</u> <u>Physiol</u>. 43:845-852.

Webb, D. P. 1976a. Root growth in <u>Acer</u> <u>saccharum</u> Marsh. seedlings: Effects of light intensity and photoperiod on root elongation rates. <u>Bot</u>. <u>Gaz</u>. 137:211-217.

Webb, D. P. 1976b. Effects of cold storage duration on bud dormancy and root regeneration of white ash (<u>Fraxinus</u> <u>americana</u> L.) seedlings. <u>Hort</u>. <u>Sci</u>. 11:155-157.

Webb, D. P. 1977. Root regeneration and bud dormancy of sugar maple, silver maple, and white ash seedlings: Effects of chilling. <u>For</u>. <u>Sci</u>. 23:474-483.

Webb, D. P. and E. B. Dumbroff. 1978. Root growth in seedlings of <u>Acer</u> <u>saccharum</u>. Presented at IUFRO meeting, Sept. 11-15, 1978. Nancy, France. 14 pp.

Winjum, J. K. 1963. Effects of lifting date and storage on 2+0 Douglas-fir and noble fir seedlings. <u>J</u>. <u>For</u>. 61:648-54.

Worrall, J. 1971. Absence of "rest" in the cambium of Douglas-fir <u>Can</u>. <u>J</u>. <u>For</u>. <u>Res</u>. 1:84-9.

Zaerr, J. B. 1967. Auxin and root-regenerating potential in ponderosa pine seedlings. _For_. _Sci_. 13:258-264.

Ziemer, R. R. 1971. Translocation of ^{14}C in ponderosa pine seedlings. _Can_. _J_. _Bot_. 48:167-171.