AN ABSTRACT OF THE THESIS OF

Pasi Kalevi Puttonen for the degree of Master of Science in Forest Science presented on November 18, 1983

Title: The Relationship of Abscisic Acid Concentration in Douglas-fir Needles to Seedling Vigor

Abstract approved: Denis P. Lavender

A quantitative and predictive measure of seedling vigor during the regeneration chain would be of utmost help for forest regeneration work. This was the impetus to study the possibility of using abscisic acid (ABA) concentration of Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) needles as an indicative measure of seedling vigor prior to outplanting.

The experiment included two lifting times (mid-November, mid-January), two pretreatments (normal dormancy, fall photoperiod), and three cold storage treatments (dark, light, and bareroot). Needle samples
were taken four times during the experiment:
1) after 48 hours in the cold room, 2) prior to taking
seedlings out of the cold room after 25 days of storage,
3) 48 hours in the growing environment, and 4) at the
time of bud break. Seedling vigor in relation to
treatments was assessed under two growing environments:
1) growth room and 2) cold frame. Seedlings were planted
in mid-December and mid-February. The concentration of
ABA in the needles was quantified by successive procedures
of extracting ABA through poly-N-vinylpyrrolidone (PVP),
diethylaminoethyl cellulose (DEAE), and octadecyl silica
(C_{18}) columns, preparative purification with high-
performance liquid chromatography (HPLC), and final
quantitation with gas-liquid chromatography (GLC)
using an electron capture detector.

Results indicate that time of lifting did not affect
survival greatly, but markedly influenced the time of
bud break. Storage treatments did not result in statisti-
cally significant differences in ABA concentrations,
survival or shoot growth. Generally, pretreatment with
supplementary light in the fall did not produce different
results from the normal dormancy pretreatment.

Considerable variability in ABA concentrations
amongst treatments was found, an occurrence which
probably precluded detection of its relationship to
seedling vigor. However, there was a tendency, though not significant, of increasing ABA concentrations from mid-November to mid-January. The failure to detect a relationship between ABA and seedling vigor may have been due to a transitory role of ABA in stress conditions or inability to quantitate ABA in its site of action, stomata.

Results of this study, along with those of previous studies, support continuance of research on ABA in physiology of plants and its potential use as an indicator of seedling vigor. However, a new synthesis of the role of ABA in seedling metabolism would be fruitful before undertaking new empirical studies as the whole concept of hormone action is undergoing a evolutionary realignment.

Keywords: Abscisic acid (ABA), Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco), seedling vigor, stress hormone.
The Relationship of Abscisic Acid Concentration in Douglas-fir Needles to Seedling Vigor

by

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The Relationship of Abscisic Acid Concentration in Douglas-fir Needles to Seedling Vigor

I. INTRODUCTION

Seedling quality

The need for planting stock of high quality has challenged nursery managers, silviculturists, and tree physiologists in developing meaningful methods for evaluating the quality of planting stock. The concept of seedling quality varies depending on the intensity of regeneration management in the field. It can describe whether seedlings are in a proper condition and ready for handling, whether seedlings are physiologically and morphologically adapted to a specific site, and in extreme cases, whether seedlings are alive or dead. Out of the stock quality concepts the term physiological condition has been widely used, and often indiscriminantly. It has served as an overall term to indicate the possibility of a seedling or a seedling lot to survive and withstand different types of stress.

The quality of planting stock, as defined at the New Zealand IUFRO workshop, "Techniques for Evaluating
Planting Stock Quality" (August 1979), is the degree to which it realizes the objectives of management. "Quality is fitness for purpose". As the purpose of a planting stock is to become established and grow successfully in a plantation, fitness is a function of survival and growth (Ritchie 1983).

Methods used for assessing seedling quality have been reviewed e.g. by Puttonen (1982) and Ritchie (1983). Seedling quality and its relationship to field performance have been traditionally evaluated indirectly by assessing morphological characteristics of seedlings, and more recently and directly, by physiological testing methods. The latter methods are often referred to as seedling vigor tests. One of the few large scale seedling vigor tests, based on drought resistance, is done by The Forest Research Laboratory (FRL) of Oregon State University, Corvallis (Hermann and Lavender 1979). Root regeneration potential as an operational measure for planting stock stress resistance was introduced by Stone et al. (1962). Seedling physiology has been evaluated by physical parameters, such as plant water status (Cleary 1971, Cleary and Zaerr 1980), electrical impedance (Glerum 1970, Askren and Hermann 1979), gas exchange (Troeng 1982), conductivity (Aronsson and Eliasson 1970), and by a thermal vision camera (Hagner 1969). Quantitative chemical parameters that have
been used are carbohydrate reserves (Krueger and Trappe 1967, Puttonen 1980), mineral nutrient content (van den Driessche 1971), and chlorophyll content of needles (Linder 1974). In fact, various living functions of plants, by evaluating metabolism are measured in these tests according to specific interests of the researcher. In a practical sense it is not possible to define planting stock quality using biological concepts alone, since the overall costs and benefits of regeneration must be concerned as well.

Physiological characteristics change rapidly with time and thus the period over which physiological data remains valid is short. Importantly, however, such data can be used to determine critical phases in producing and handling seedlings. These phases can be simulated and response of the seedlings examined (eg. Hallman et al. 1978). As summarized by Ritchie (1983), work on physiological grading of stock indicates that:

(1) "the seedlings physiological condition exerts a strong influence on seedling survival and growth potential,

(2) components of physiological condition are numerous, change rapidly over time, and can change independently of one another; and (3) physiological condition cannot be visually determined". He concluded that comparisons of seedling performance based upon morphological traits are valid only when seedlings are in the same physiological condition when tested. This requirement is very seldom
fulfilled or quantitatively satisfied - and that situation will persist.

Räsänen (1980) states "in the nursery and at the planting site, there is a complicated biological process at one level, technical problems at another and economic necessities at the third. ... No single phase in the production of seedlings can be examined without taking into account its effects on the treatments preceding and following it and on the final result", the established seedling. How an established seedling is defined depends on the management policy on a specific site. Forming a comprehensive picture of the growing process of a seedling lot, which could enable getting immediate output of the current state of the growth process and which could be used for directing the growing process in the nursery, is very demanding and has yet to be accomplished. Lately, some concepts derived from systems theory based on cybernetics have been proposed for studying such complex processes in regeneration research (Räsänen 1980).

Growing tree seedlings is a biotechnical process. Its progress can be measured and directed. Today, it is done to some extent through our knowledge of the growing processes in plants. We can either measure and direct the process itself, the growth and development of the seedling lot, or assess the product of the process, the seedlings.
Photosynthesis, transpiration, water potential, and nutrient concentration are states of the developmental process of seedlings which are transformed into a growing process by means of feedback regulation of the environment. The state of the growing process can be measured as shoot/root ratio, and growth rates of the different parts of the seedling, such as root growth (Räsänen 1980). Although, this approach is promising, it is far more conceptual than managerial.

Ritchie (1983) used somewhat analogous terms, i.e. "performance" and "material attributes" as concepts to reflect planting stock quality. Root growth potential, frost hardiness, and stress resistance are characteristics of performance attributes. Dormancy status, water relations, nutrition and seedling morphology are samples of material attributes. Since performance attributes are integrators of all or many seedling subsystems, they often predict seedling survival and growth potential well (Ritchie 1983).

When growing seedlings and regulating the growing process, it is important to measure or estimate characteristics which reflect the correct state of development and possibly predict future development. In different phases of the seedling production process the measurement is for different purposes and therefore, we have to
use diversified measurement or estimation methods. When seedlings are packed for shipment to planting areas, we have seedlings, which are the final product of the nursery process. The performance potential of which should be estimated for chosen economical risk levels of management. Thus, an indicator of seedling quality, both for bareroot and containerized seedlings, preferable a quantitative one, is needed. Since various plant growth regulators have been implicated with those growth processes vital to the establishment process, it is fitting that one of these substances, namely abscisic acid will be considered in more detail.

Abscisic acid

There is voluminous literature concerning plant growth substances, but very little is known about their role in seedlings during the part of the regeneration chain which starts at the nursery when seedlings are harvested and finishes when seedlings are established. According to Zaerr and Lavender (1980) there are few growth regulators which could be assayed for this purpose, but they conclude that one good candidate is abscisic acid (ABA). The relationship of ABA metabolism to stomatal conductance (reciprocal of resistance) in many plants, including Douglas-fir, has been noted widely both
in stressed and non-stressed conditions (e.g. Johnson and Ferrell 1982). Generally, during water stress the level of leaf ABA increases, causing stomata to close and override the other control factors (Walton 1980).

The increase of ABA concentration has been associated with threshold water potential (Newville and Ferrell 1980), and turgor potential (Pierce and Raschke 1978). However, in cotton seedlings the rise of ABA concentration was gradual with increasing water stress (Davenport et al. 1977). Water stress induces the synthesis and accumulation of ABA in chloroplasts where it migrates to guard cells. The turgor is maintained by imported K\(^+\) and Cl\(^-\) ions together with malate which is synthesized in guard cells. Turgor changes are regulated by movement of K\(^+\), H\(^+\), Cl\(^-\) ions and organic anions, particularly malate. ABA inhibits 1) uptake of K\(^+\) ions, 2) inhibits release of H\(^+\), and 3) promotes the leakage of malate from guard cells. These effects cause a drop in turgor potential of guard cells (Mansfield and Jones 1971, Raschke 1975). However, the exact manner of how ABA moves to guard cells is unclear. ABA may also be translocated from leaves to other regions such as roots where it may regulate root "gravireaction" (Pilet 1982). A recent study of the composite biosynthesis of ABA has been completed by Neill et al. (1982).
Webber et al. (1979) and Johnson and Ferrell (1982) have shown the seasonal changes in ABA concentrations of Douglar-fir needles and buds. The levels of ABA in needles were highest during dormancy induction in the late summer and in the autumn and decreased toward spring and the lowest concentrations were noted before bud burst in the spring. Diurnal changes of ABA in sorghum (*Sorghum bicolor* L. Moench) were reported by Kannangara et al. (1982). ABA exhibited a distinct diurnal variation and fluctuated over a range of 45 - 110 ng g\(^{-1}\) fresh weight with two distinct peaks at 8.00 and 18.00 hour. These peaks approximately corresponded to leaf water potential minima.

It thus seems that the ABA concentration is associated with the degree of stress in seedlings. Seedlings close to bud break might be more sensitive to stress, particularly to that which is caused by seedling handling. Stress is frequently reflected as higher ABA concentration in plant tissues, such as needles. If ABA is beneficial to plants during periods of water stress, increased ABA concentration may allow control of plant water losses after planting through its influence on stomatal action. Elevated levels of ABA are noted to occur in winter lifted seedlings which are stress resistant because of their state of deep dormancy. Seedlings that
are in the induction phase of fall dormancy have lower concentrations of ABA and are, therefore, sensitive to stress situations (personal communication, Dr. Richard Durley, OSU, Department of Forest Science). Furthermore, Blake and Ferrell (1977) noted that soil moisture potential was closely associated with levels of ABA in Douglas-fir seedlings and suggested that the roots may be drought sensing organs which mediate in translocation or production of ABA.

Based on the meager and often conflicting data that exists associating concentration of plant growth regulators to seedling physiology, but because of apparent associations of ABA to stress condition, it was hypothesized that the concentration of ABA in needles of seedlings prepared for outplanting could be used as an indicator of seedling vigor. The hypothesis thus formulated is that ABA concentrations in Douglas-fir seedlings differ because of different lifting times, status of dormancy and cold storage.

If the hypothesis is valid, ABA concentration, therefore, might be employed as a measure of seedling performance potential which is the cumulative result of all previous nursery cultural practices. Thus it could also be used as an indicator of seedling quality at any
point in the nursery production process.

Study objectives

The specific objectives of this research were to:

1. measure ABA concentration in needles of Douglas-fir seedlings subjected to various combinations of cultural practices. As a result of these treatments, the plants were subjected to a given level of stress.

2. relate ABA concentrations to seedling performance potential, as estimated by monitoring survival and first season height growth.

3. determine if the concentration of ABA in needles of planting stock is a useful operational indicator of seedling vigor.
II. MATERIALS AND METHODS

Seedling treatments

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) 2+0 seedlings of the 252 seed zone under 150 m (500 ft) elevation were lifted on April 15, 1981 at the D.L. Phipps State Forest Nursery near Elkton, Oregon. The seedlings were placed in plastic bags to prevent moisture loss and transported to The Forest Research Laboratory (FRL) of Oregon State University at Corvallis. On May 22 - 24, 1500 seedlings, 10 seedlings to a pot were planted in 11-liter pots containing fertile forest soil, then placed outside in a sunny place. The pots were watered once a week. In late July, pots were moved to a sheltered area (under oak trees) for better dormancy induction (Lavender and Wareing 1972). From this time on watering interval was 12 days, also for better dormancy induction (Lavender and Cleary 1974). The pots were randomly divided into two lots. One did not get any additional treatment during the fall to induce or break dormancy and hereafter is referred to as "the normal dormancy pretreatment". The other lot was exposed to a 16-hour daily photoperiod during September 2 October 15, 1982 by means of supplemental lighting from two 200 W incandescent bulbs. This treatment is referred to as "the fall photoperiod treatment".
On November 15, 1982, 24 pots from the normal dormancy lot were randomly selected for further treatments to assess stress that might develop in these conditions. Similarly, 14 pots from the extended fall photoperiod pretreatment were chosen. Pots of the normal dormancy lot were randomly allocated to three cold storage treatments: (1) dark storage in pots, (2) light storage in pots, and (3) bareroot storage in double plastic bags in the dark. The fall photoperiod lot was divided into dark storage in pots and light storage in pots treatments only. The light storage was chosen because of its favourable effect on cold stored potted seedlings (Lavender 1978). Bareroot dark storage is the most common nursery practise. Prior to cold storage seedlings were removed from their pots, repotted, and watered to simulate seedling harvesting and subsequent planting. Bareroot seedlings were stored in the dark in 2 mm plastic bags.

All cold storage treatments were in the same cold room (+5 C°), but the seedlings of the light treatments were exposed to 8-hour daily photoperiod in the cold storage. The photon flux density of the fluorescence light was 22.4 ± 2.2 µE m²s⁻¹ averaged over the pots (10 systematic measurements). The moisture of the pots was checked and the potted seedlings were watered once during the cold storage period. The storage period was 25 days.
The procedure was repeated for seedlings lifted on 12 January 1983. The storage treatments were as reported earlier, except that the daily photoperiod in the light storage was 16 hours. Thus, there were 2 pretreatments (normal dormancy, fall photoperiod), 2 lifting times (mid-November, mid-January), and 3 cold storage treatments (dark, light, and bareroot). As the bareroot storage was not applied to the fall photoperiod pretreatment the total number of treatments was 10. This produced a design which could not be analyzed as a complete factorial experiment, so the treatments were analyzed using planned comparisons among class means and multiple comparison tests (Snedecor and Cochran 1980).

Experimental design

After the 25 day cold storage period the pots and bags were randomized into their growing environments. The growing places were a growth room and cold frames located outside the FRL. At each of two planting times 200 seedlings were planted in the cold frame filled with sandy clay loam, and concurrently 190 seedlings (19 pots) were grown in the growth room. In the growth room the pots were randomly located on benches 1.2 m beneath the lights. The daily photoperiod was 16 hours, day tempe-
rature 21 °C and night temperature 16 °C. The growth room temperature was monitored with a recording thermo-hygrograph (Foxboro). The photon flux density of the fluorescence and incandescence light in the growth room was 101.0 ± 2.2 μE m² s⁻¹ averaged over the pots (21 systematic measurements). The pots were watered weekly and budbreak date was recorded daily. As some aphids appeared on some seedling pots they were sprayed bimonthly with Garden Malathion (active ingredient malathion 57 %). In spite of the spraying of bugs, pots of light and bareroot treatments of the mid-November lifting had to be removed from the study after 5 months due to fungi growth on the needles. This reduced the number of treatments by two in the growth room study (a completely randomized design).

The experimental design of both planting times in cold frames was a randomized complete block with four replicates. During the May - July period the nursery beds were irrigated once a month, and weeded twice during the study period. In total, 780 seedlings were utilized in this experiment: 390 at each lift. The number of seedlings allocated to treatments is summarized in Table 1.
Measures of growth responses

Throughout the assessment period the date of bud break for each seedling was recorded. Budbreak was defined as the time when the bud scales were opened and the new vegetative bud burst was 0.5 cm long. After leader extension was complete (early September 1983), seedling height, current leader growth for 1982 and 1983 were measured to the nearest 0.5 cm, and root collar diameter to the nearest 1 mm. The following

<table>
<thead>
<tr>
<th>Treatments</th>
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<tr>
<td>Dormancy status</td>
<td>Storage</td>
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<tr>
<td>Normal</td>
<td>Dark</td>
</tr>
<tr>
<td></td>
<td>Light</td>
</tr>
<tr>
<td></td>
<td>Bareroot</td>
</tr>
<tr>
<td></td>
<td><strong>240</strong></td>
</tr>
<tr>
<td>Fall photoperiod</td>
<td>Dark</td>
</tr>
<tr>
<td></td>
<td>Light</td>
</tr>
<tr>
<td></td>
<td><strong>150</strong></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
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index values were used to evaluate survival:

<table>
<thead>
<tr>
<th>Index value</th>
<th>Survival</th>
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<tr>
<td>0</td>
<td>Dead seedling</td>
</tr>
<tr>
<td>1</td>
<td>Unhealthy seedling</td>
</tr>
<tr>
<td>2</td>
<td>Healthy seedling</td>
</tr>
</tbody>
</table>

In the growth room the distance from each pot to the fan was measured as a possible covariate.

Sampling procedure

Samples for ABA quantitation in both lifting times were taken four times during the experimental procedure: (1) 48 hours after the seedlings were placed into cold storage, (2) prior to taking the seedlings to the growing environment, (3) 48 hours after seedlings were placed in their respective growing environments in the growth room and outside cold frames, and (4) during the period when budbreak occurred. The pots to be sampled, two pots out of the four in each treatment, were randomized. Five to ten needles from the lower part of 1982 leader were removed from each seedling in the pot, placed in a freezer bag and immediately quick frozen by submerging in liquid nitrogen. The samples were stored at -80 °C until further processed. Because diurnal fluctuations of ABA are known to occur (Kannangara et al.)
1982), only samples having the same time phase of the daily photoperiod in different treatments can be compared. Thus the sampling times in each phase were fixed at 7 hours after the start of the photoperiod.

Extraction and quantitation of abscisic acid

To examine plant growth regulators in a large number of samples accurately, as would be required for use in a seedling vigor testing program, a rapid and efficient technique to purify and quantify these compounds is required. The method to purify and quantitate ABA developed by Bell (1983) was therefore employed. Bell's (1983) method of purification was based upon an extraction process through columns of poly-N-vinylpyrrolidone (PVP, Polyclar), diethylaminoethyl cellulose (DEAE, DE52, Whatman) and octadecyl silica (C18). Thereafter, samples were further purified with high-performance liquid chromatography (HPLC) and finally quantified with gas chromatography (GLC). Among the advantages of the PVP - DEAE - C18 extraction method is that several samples (2 to 5) can be purified simultaneously. Buffers, solvents and column packing materials are readily available and inexpensive.

A large pool of phenolics and pigments are present in most coniferous plant tissues which interferes with
the isolation of plant growth regulators. Insoluble PVP has proven to be very effective at removing phenolics and pigments from various plant tissues for various analytical purposes (Loomis et al. 1979, Durley et al. 1982). Durley et al. (1982) used ammonium salts and PVP for ABA in sorghum. As phenolics do not form salts with ammonia so they adhere to PVP while ammonium salts (e.g. ABA) pass through PVP. For optimal purification the pH should be close to neutral, 6.5 - 6.7, and the extract have low methanol content (Bell 1983).

DEAE (DE52) is a crosslinked cellulose which is an anion exchanger. DEAE has worked well in many procedures to extract growth substances; auxins and cytokinins (personal communication, Dr. Roy Morris, OSU, Department of Agricultural Chemistry), and ABA (Bell 1983). As ABA has an acid-dissociation constant of 4 - 4.5, it adheres to DE52 at pH around 6.5 (Bell 1983). ABA was released from DE52 with 0.5 M acetic acid to a short C18 column. Further purification of ABA was accomplished by using the preparative HPLC by reversed-phase C18 column (Durley et al. 1982). GLC using an electron capture detector (ECD) is both sensitive and selective for ABA because the ring structure of ABA accepts electrons.
Quick frozen and cold stored (-80 °C) needle samples were ground with liquid nitrogen using a mortar and pestle. A needle sample of 250 mg was weighed and suspended with 5 ml of HPLC grade methanol (MeOH). About 40 mg of sodium diethyldithiocarbamic acid (DIECA, Sigma) was added as an antioxidant. The sample in methanol was further ground with a Polytron. Thereafter, $3 \times 10^4$ dpm of DL-cis trans($^{3}$H)ABA, (specific activity 1.85 MBq mmol$^{-1}$, Amersham Co.) was added as an internal standard to the sample. The sample was then centrifuged at $8 \times 10^3$ rpm for 10 minutes and placed in an ice-bath for stabilization while the columns for the extraction were prepared.

After testing the various amounts of PVP, DE$_{52}$, and C$_{18}$ in the column beds, the following volumes were chosen; 10 ml PVP, 10 ml DE$_{52}$, and 1 ml C$_{18}$. PVP and DE$_{52}$ columns were washed with 40 ml of 20 mM buffer and C$_{18}$ was equilibrated with 50 mM NH$_4$OAc. The sample was diluted with 20 ml of 20 mM ammoniumacetate buffer (NH$_4$OAc) pH 6.5. Buffer diluted methanolic extract was passed through PVP and DEAE columns using gravity flow only. Columns were washed with 35 ml of buffer. Needle pigments were removed by PVP while ABA passed through PVP and was concentrated on DEAE. After removing the PVP column the DEAE column was attached to the C$_{18}$ column and the system was
eluted with 20 ml of 0.5 mM acetic acid solution. The ABA passed through the DEAE column and was concentrated on the C18 column. The DEAE column was removed and the C18 column was washed with 2 ml of 50 mM NH₄OAc pH 3.5. Finally, the ABA was eluted from the C18 column with 4 ml of methanol.

After taking a 50 μl fraction for the recovery monitoring, the extract was evaporated to dryness in a "Speed-Vac", rotary vacuum evaporator (Savant). For methylation, the samples were redissolved in 100 μl of methanol and 1 ml of ethereal-diazomethane was added. The mixture was kept at room temperature for 10 min and then evaporated carefully to dryness in a Speed-Vac. To prevent evaporation of ABA the evaporation was stopped when a drop of solution remained in the vial. The methylated sample (ABA-Me) was redissolved in 25 μl of MeOH and 25 μl of HPLC buffer, and 50 mM triethylammonium acetate (TeAAc) pH 3.5. The buffer was prepared by filtering through Fluoropore (Millipore Ltd.), pore size 0.5 μm and then degasified. For ABA fractioning in the HPLC a mixture of 60 % MeOH and 40 % TeAAc was used. The HPLC chromatograph (Varian 5000) was operated isocratically with a reversed-phase C18 column (250 x 4.1 mm ID). The flow rate was 1 ml min⁻¹ and a sample of 15 - 20 μl was injected. ABA-Me was detected with an absorbance monitor at 254 nm. The
retention time for ABA-Me was about 8.2 minutes and the fraction eluted from this time to 10.7 minutes was collected. Thereafter, the sample was carefully evaporated to dryness in a Speed-Vac.

The ABA-Me was quantified with an Varian 3700 gas-liquid chromatograph equipped with a $^{63}$Ni electron capture detector. The dried sample was dissolved in 50 - 100 $\mu$l of n-hexane - pyridine solvent (10:1 v/v). After taking a 50 $\mu$l fraction for recovery monitoring, a sample of 1 - 2 $\mu$l was injected into a glass column (180 cm x 2 mm ID) packed with 7 % OV-101. As the performance of GLC decreases with increasing mass of the sample chromatographed (Littlewood 1970), sample size was kept small by diluting the original sample. The flow rate of nitrogen carrier gas was 35 ml min$^{-1}$, the injector temperature was 210 °C, the column temperature 145 °C and the ECD temperature 300 °C. After each injection the column was heated to 250 °C for 5 min. Each sample was quantified with 2 - 4 subsequent injections, and a blank was injected after each sample. The lower limit for ABA quantification by the GLC was about 5 pg, which reported by Brenner (1981), is the lower limit of detection with packed column GLC with ECD. The ABA-Me peaks were quantified using the standard curve formed by injection of known concentrations of ABA-Me. The ABA was calculated as pg ABA per g fresh
weight of needles. In total, 56 needle samples were measured. These samples were from the normal dormancy pretreatment only.

The time to extract one needle sample with PVP - DE$_{52}$ - C$_{18}$ columns required about four hours for four samples purified simultaneously. The drying of one to twenty samples in a Speed-Vac required another four hours. Methylation with ethereal-diazomethane and subsequent drying took about 50 minutes. Preparative fractioning of ABA-Me with HPLC required 40 minutes for each sample including stabilization of the baseline by removing the impurities. The fraction collected required about three hours to dry, and finally, three injections per hour into GLC could be made. Thus four samples could be quantified in 16 hours, or one sample in 13 hours.

The recoveries of ABA from the extraction through PVP - DE$_{52}$ - C$_{18}$ columns averaged 86 ± 3.4 percent. The average of the recoveries after evaporation in the Speed-Vac was 75 ± 3.0 percent, and after methylation and HPLC the recoveries averaged 43 ± 2.5 percent. The total recovery was 27 ± 2.1 percent.
III. RESULTS

Abscisic acid

Mid-November lifting - The results from the ABA analysis will be presented in two parts, first, for seedlings lifted in mid-November, and second, those lifted in winter. All statistical tests were made using the 5 percent level of significance but also P-values are reported in comparisons. Thus in a small-sized experiment like this, a large real difference must exist if the null hypothesis is to be rejected. Typically, field tests of seedling quality are based on small sample size because of practical limitations. As the consequences of being wrong in seedling quality testing are often economically serious, even 1 percent level of significance may be desirable in decision making.

The concentrations of ABA in needles of seedlings placed into cold storage in mid-November were 8 - 105 ng g⁻¹ fresh weight (Figure 1). Within the second sampling time (prior to outplanting) ABA concentration was highest in light stored (48 ng/g) and lowest in bareroot stored seedlings (8 ng/g). After 48 hours in the cold frame (sample 3) there was little difference in ABA levels of dark and light stored seedlings, but the ABA level in bareroot stored seedlings was more than 13 times higher.
Figure 1. Concentration of ABA in needles of Douglas-fir seedlings lifted in mid-November. The bars represent standard errors of means. Needles were sampled just prior to outplanting (sample 2) and after two days in the growing environment (sample 3).
than in dark stored. Variation within the bareroot stock was, however, considerable. Thus, for seedlings lifted in mid-November there were no significant differences in ABA concentrations between seedlings treated dark, light, or bareroot storage in the samples within the same collection time (Figure 1). Based also on t-tests, there were no differences in ABA levels amongst samples collected prior to the planting (sample 2) and samples collected after two days in the growing environment (sample 3). The lack of significance at 5 percent level is due to great variation in ABA within a treatment. P-values were typically from 0.13 to 0.65, with a median of 0.22. Also, the small number of samples analyzed per treatment (two to six) prevented detection of statistical significance.

Mid-January lifting - The ABA levels in the mid-January experiment varied even more than in the fall. ABA concentration varied between 2 - 295 ng g$^{-1}$ fresh weight depending on sampling time (Figure 2). Highest ABA concentrations were in seedlings stored 48 hours in the cold room (sample 1) with bareroot seedlings having the highest concentrations. By the end of the fourth week of cold storage (sample 2) the ABA concentrations were more uniform within and amongst samples. Dark stored and bareroot seedlings lifted in mid-January exhibited a tendency toward significance in
ABA concentrations, when samples were collected prior to removing seedlings from cold storage (Figure 2, (68 ng/g vs 23 ng/g respectively, P-value 0.067). Samples from the first collection time (48 hours after lifting) and sampling time 2 showed no significant differences between storage treatments in ABA levels. P-values ranged from 0.23 to 0.64. Even the seemingly great difference in bareroot stored seedlings between the samples 1 and 2 was not statistically significant because of extreme variation (P-value 0.64). As for the fall lifted seedlings, no significant differences of ABA levels in mid-January lifted seedlings were detected for any combination of treatments between sampling time 1 and time 2. Comparisons between other collection times are not relevant due to seasonal changes in ABA levels (Webber et al. 1979, Murphy and Ferrell 1982). There were no significant differences between the two lifting times when samples of the same collection time (sampling time 2) were compared. However, there was a tendency of increasing ABA concentration from mid-November to mid-January (P-value 0.12). As only few samples were analysed from the sampling at about time of bud break these figures are only suggestive. The alternative hypothesis of this study was that the ABA concentrations differ due to different lifting times, pretreatment, and cold storage. However, this alternative was rejected.
Figure 2. Concentration of ABA in needles of Douglas-fir seedlings lifted in mid-January. Needles sampled 48 hours after the start of cold storage (sample 1), prior to outplanting (sample 2), and at time of bud break in the cold frames (sample 4).
Growth room study

Survival - Differences in survival amongst treatments were tested for significance using the z-test, which is comparable to chi-square test ($X^2$) of independence in a 2 x 2 table (Snedecor and Cochran 1980). The arc sine transformation for proportions was applied as the range of the dead percentage was from 0 to 30% (Snedecor and Cochran 1980). However, no differences in proportions of dead seedlings were found when any combination of treatments was compared (Figure 3). Calculated P-values for comparisons of mortality (%) ranged from 0.2 to 1.0. Size of treatments was usually 40 seedlings, although for three treatments only 20 seedlings were available because of fungal attack.

Days to budbreak - Only one of the mid-November lifted seedlings broke bud during the 9 month growth room period. It took 199 days to bud flush for that seedling from the fall photoperiod and dark storage treatment. Not even the 25 day cold storage treatment had an effect on budbreak. Thus, results of budbreak are reported only for the mid-January experiment. Distance of pot to the fan in the growth room was applied as a covariate, but showed no significant difference.
Figure 3. Mean survival (%) of the seedlings in the growth room after one growing season. Each mean is based on a sample of 40 seedlings. A. Seedlings with normal dormancy. B. Seedlings with fall photoperiod.
In planned comparisons with \( n \) treatments, \( n-1 \) comparisons can be made. This is equal to the number of degrees of freedom in the analysis of variance. Days required for budbreak varied from 40 to 52 days (Figure 4). The dormancy status at the time of lifting appeared to effect the time to budbreak. Seedlings with normal dormancy broke bud earlier than those of the fall photoperiod treatments. The comparison of treatments by contrasting means are presented in Table 2. Fall photoperiod and subsequent dark storage resulted in significantly more days to budbreak (contrasts 2, 3). Storages of the normal dormancy pretreatment did not differ as a difference of 7.5 days is required for significance (critical F-value 4.6 at 5 percent level).

Table 2. Comparison of days to budbreak of trees grown in growth room by contrasting treatment means.

<table>
<thead>
<tr>
<th>Treatment comparisons</th>
<th>Mean square</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Dormancy:normal vs fall</td>
<td>134.1</td>
<td>5.5</td>
<td>0.025 ***</td>
</tr>
<tr>
<td>2. Fall photo: dark vs light</td>
<td>307.5</td>
<td>12.5</td>
<td>&lt;0.005 **</td>
</tr>
<tr>
<td>3. Dark storage:normal vs fall</td>
<td>243.3</td>
<td>9.9</td>
<td>0.008</td>
</tr>
<tr>
<td>4. Light storage:normal vs fall</td>
<td>1.9</td>
<td>ns</td>
<td>&gt;0.500</td>
</tr>
</tbody>
</table>

\[ \text{MSE} = 24.5 \quad \text{df} = 14 \quad 6 \text{ treatments} \]

(MSE = Mean square error, df = degrees of freedom)
Figure 4. Average number of days to budbreak in seedlings grown in the growth room. The bars represent standard errors of means.
Shoot growth - The seedlings of the mid-November lifted stock did not break bud. Apparently the chilling requirement was not fulfilled before lifting the seedlings or during the cold storage period. Thus, only the shoot growth of the mid-January lifting is presented. The average shoot growth of 1983 varied from 1.8 cm to 2.7 cm. Although leader growth was relatively small it was uniform in all treatments (Figure 5). Seedlings having fall photoperiod and dark storage had significantly shorter leader than light stored seedlings of the same treatment (Table 3, contrast 5). Other comparisons did not result in significance as a difference of 0.8 cm was needed to exceed the critical F-value of 4.4. The greater the heatsum was prior to budbreak the shorter was the leader. This suggests that leader growth ceased when a threshold temperature sum was reached, at least under constant photoperiod and temperature. The treatment with fall photoperiod, and dark storage had significantly shorter leader growth, but it also had significantly more days to budbreak than the other treatments (Figures 4, 5). Leader growth in 1982 was not significant as a covariate.
Figure 5. Mean shoot growth of the seedlings grown in the growth room, mid-January lifted seedlings. The bars represent standard errors of means.
Table 3. Comparisons of current year's leader growth of trees grown in the growth room.

<table>
<thead>
<tr>
<th>Treatment comparisons</th>
<th>Mean square</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Dormancy: normal vs. fall</td>
<td>82.8</td>
<td>ns</td>
<td>0.15</td>
</tr>
<tr>
<td>2. Storage: dark vs. light</td>
<td>90.3</td>
<td>ns</td>
<td>0.12</td>
</tr>
<tr>
<td>3. Storage: dark vs. bareroot</td>
<td>0.5</td>
<td>ns</td>
<td>&gt;0.50</td>
</tr>
<tr>
<td>4. Storage: light vs. bareroot</td>
<td>5.8</td>
<td>ns</td>
<td>&gt;0.50</td>
</tr>
<tr>
<td>5. Fall photoperiod:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dark vs. light storage</td>
<td>137.8</td>
<td>4.5</td>
<td>0.049</td>
</tr>
</tbody>
</table>

MSE = 30.6  df = 18  6 treatments

Stem diameter - The average root collar diameter by treatments varied from 7 to 7.5 mm. Statistically a difference of 0.5 mm can be shown to be significant at a probability level of 5%. However, this figure is not practically pertinent as stem diameter is more subjected to measurement errors than due to treatment differences.
Nursery study

Survival - As in the growth room there were no significant differences in survival between treatments except for one pair of comparisons (Figure 6). Seedlings lifted in mid-November, and without supplementary photoperiod in the fall, and dark cold storage had significantly more dead seedlings than the seedlings having the same treatments but lifted in mid-January (Figure 6). P-value for this difference was 0.007. For other comparisons the P-values were typically greater than 0.20. The only significant difference in survival between the growth room and the nursery experiments was in the seedling stock having fall photoperiod, lifted in mid-November and cold stored in light (P-value 0.01).
Figure 6. Mean survival (%) of the seedlings in the cold frames after one growing season. Each mean is based on a sample of 40 seedlings.
A. Seedlings with normal dormancy. B. Seedlings with fall photoperiod.
Days to budbreak - All surviving seedlings broke their terminal buds in the nursery. Lifting time had a significant effect on bud break (Table 4, contrast 1, 6 and 7) as fall lifted seedlings required nearly twice as many days to flush their buds than winter lifted (Figure 7). The state of dormancy at the time of lifting did not effect bud break (contrast 2). Storage treatments did not have an effect (contrasts 3 - 5). A difference of at least 9.3 days was required for significance between two means (critical F-value = 4.21). Blocking did not contribute to precision in detecting budbreak differences (P-value 0.34).

Table 4. Comparison of days to budbreak of trees grown in the cold frames.

<table>
<thead>
<tr>
<th>Treatment comparisons</th>
<th>Mean square</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Experiment: 1 vs. 2</td>
<td>54 316.9</td>
<td>1 315.5</td>
<td>0</td>
</tr>
<tr>
<td>2. Dormancy: normal vs. fall</td>
<td>108.0</td>
<td>ns</td>
<td>0.13</td>
</tr>
<tr>
<td>3. Storage: dark vs. light</td>
<td>83.2</td>
<td>ns</td>
<td>&gt;0.50</td>
</tr>
<tr>
<td>4. Storage: dark vs. bareroot</td>
<td>17.6</td>
<td>ns</td>
<td>&gt;0.50</td>
</tr>
<tr>
<td>5. Storage: light vs. bareroot</td>
<td>4.8</td>
<td>ns</td>
<td>&gt;0.50</td>
</tr>
<tr>
<td>6. Normal dormancy:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 1 vs. 2</td>
<td>31 915.6</td>
<td>772.9</td>
<td>0</td>
</tr>
<tr>
<td>7. Fall photoperiod:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 1 vs. 2</td>
<td>22 410.1</td>
<td>542.7</td>
<td>0</td>
</tr>
</tbody>
</table>

MSE = 41.3  df = 27  10 treatments
Figure 7. Average number of days to budbreak in seedlings grown in the cold frames. The bars represent standard errors of means. A. Seedlings with normal dormancy. B. Seedlings with fall photoperiod.
Shoot growth - Current leader growths of the treatments were compared with Tukey's HSD (Honestly significant difference) multiple comparison procedure. With n treatments there are n(n-1)/2 possible pairwise comparisons. Error rate of the test is experimentwise. In general, Tukey's method is rather conservative. The results are presented in Table 5. The difference between a specific pair of means is significant at the 5 percent level if it exceeds 1.6. Means underscored by the same line are not significantly different. The state of dormancy did not have any effect on shoot growth (P-value > 0.50, Figure 8). Interestingly, in both the normal dormancy and fall photoperiod pretreatments there was a significant difference in shoot growth of dark stored seedlings between the two lifting times (Table 5). Thus, lifting in the fall and subsequent dark storage is more stressful than dark storage of winter lifted seedlings. One would expect a maximum difference between fall lifted, dark storage treatment and winter lifted, light storage treatment, but these storage treatments did not have any effect on shoot growth. The use of leader growth of 1982 as a covariate did not change the conclusions.

Leader growth exhibited a tendency to decline as the number of days to bud break increased, i.e. with
Figure 8. Mean shoot growth of the seedlings grown in the cold frames. A. Seedlings with normal dormancy. B. Seedlings with fall photoperiod. The bars represent standard errors of means.
Table 5. Comparison of current year's leader growth of trees grown in the cold frame with Tukey's multiple comparison test. Means underscored by the same line are not significantly different at the 5 percent level.

<table>
<thead>
<tr>
<th>Lifting time</th>
<th>Mid-November</th>
<th>Mid-November</th>
<th>Mid-November</th>
<th>Mid-November</th>
<th>Mid-January</th>
<th>Mid-January</th>
<th>Mid-January</th>
<th>Mid-January</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dormancy status</td>
<td>Fall photo-period</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Fall photo-period</td>
<td>Normal</td>
<td>Normal</td>
<td>Fall photo-period</td>
</tr>
<tr>
<td>Storage</td>
<td>dark</td>
<td>dark</td>
<td>light</td>
<td>bareroot</td>
<td>light</td>
<td>light</td>
<td>bareroot</td>
<td>light</td>
</tr>
<tr>
<td>Mean</td>
<td>2.7</td>
<td>2.9</td>
<td>3.1</td>
<td>3.2</td>
<td>3.3</td>
<td>3.6</td>
<td>3.7</td>
<td>3.8</td>
</tr>
</tbody>
</table>

MSE = 35.1  df = 27
10 treatments, Tukey's test value 1.4
increase of temperature sum. However, there was great variation within each treatment and pretreatment. This relationship indicates the dependency of leader growth on the progress of annual physiological cycle which was somewhat better demonstrated in the growth room study. Blocking was efficient in reducing variations in seedbed (P-value 0.022).

Stem diameter - In the growth room experiment, mean stem caliper between some treatments were shown to differ significantly, as only a difference of 0.4 mm was required for significant difference. Sometimes a morphological characteristic, sturdiness, is used to describe seedling stock. As this index is calculated by dividing height by diameter, and diameter being nearly constant, height might be a simplier index.
IV. DISCUSSION

The quantitation of four ABA samples required about 16 hours and thus is relatively slow. Based on an approximation of sample size when variance, desired power of the test, and size of the meaningful difference between treatments are known (Snedcor and Cochran 1980), a substantial sample size is needed for each seedling lot to estimate confidently levels of ABA. This method of ABA quantitation is not very efficient in this respect. Moreover, a method for screening seedling quality should give results in a shorter time. Furthermore, the ABA levels in needles did not seem to follow any hypothesized pattern or differ significantly in response to different treatments.

Rigorously taken, the quantitation of ABA does not meet the requirements set by Scott (1982), namely, that the identity of a growth substance in a plant extract can only be conclusively established using techniques which provide information on its chemical structure. This would require use of mass spectrometry. Scott's principle (1982) is thus far more severe than that of Reeve and Crozier (1980) who used information theory for evaluation of the accuracy of a plant growth substance analysis. This theory involves the comparison of the information needed to solve an analytical problem and the information
yielded by the chosen procedure. A crucial problem in making such an estimate is that plant extracts contain an immense number of uncharacterized compounds (Scott 1982). In this study, ABA was verified comparing chromatographs to standard ABA.

After 25 days of cold storage no significant differences were found in ABA concentrations of seedlings lifted in mid-November or mid-January (Figures 1 and 2, sample 2). Variability of ABA concentrations was typical. Murphy and Ferrell (1982) also reported large variability of ABA concentration in needles of field grown 5 - 6 m high Douglas-fir trees. Older needles had more variation than young ones. Similarly, Johnson and Ferrell (1982) found large inherent variability between branches and among three year-old Douglas-fir seedlings. Generally, the ABA concentrations of this study are comparable to studies of dormant Douglas-fir needles (Webber et al. 1979, Murphy and Ferrell 1982, Bell 1983). Dark stored seedlings of the mid-January lifting had seemingly higher ABA concentrations than light or bareroot stored seedlings. The low levels of ABA in bareroot stored seedlings is an anomaly. The definite trends found by Bell (1983) in ABA levels of needles of Douglas-fir seedlings were not totally confirmed by this study. In Bell's study, ABA levels increased the later the seedlings were lifted.
In mid-November lifted seedlings the relation of ABA levels in dark and light stored seedlings was similar in both Bell's and the present study. In mid-January lifted seedlings, however, the ABA concentrations of seedlings stored in the light were higher than those stored in the dark (Bell 1983); for the present study the reverse was true.

In the winter lifted seedlings, the levels of ABA were highest in dark stored and lowest in bareroot stored seedlings. Johnson (1981) suggested ABA to be photosensitive, photoisomerizing to a biologically inactive isomer, and photo-oxidizing into inactive metabolites. Thus it could be assumed that photoperiod during cold storage should decrease the levels of ABA in seedlings if not stressed. If the photoperiodic treatment in the cold room had accelerated the decrease of ABA concentration in leaves or buds, the light stored seedlings should have been sooner and more metabolically active after transferring them to growing environments. Probably, the light intensity in the cold room was not high enough to promote this process. Since ABA is an inhibitor of growth (Milborrow 1978), it is often supposed that rapidly elongating shoots contain little if any ABA in the growing region. The evidence presented by Powell (1982) does not support the possible role of ABA in regulating shoot growth. Furthermore, Powell
(1982) reports studies where ABA concentration of dormant bud was perhaps not controlled by chilling temperature. These do not imply that ABA has no important role to play, e.g. changes in tissue sensitivity to ABA could be one system which might not rely on changes in ABA concentration.

Sembdner et al. (1980) concluded that ABA in spring sap of birch trees has no physiological significance with respect to the transition from dormancy to budbreak. Bell (1983) also suggested that ABA in needles of Douglas-fir was not a dormancy hormone. Although ABA was discovered in investigations on bud dormancy and abscission, the consensus of data to date does not support its function as a dormancy hormone.

ABA concentrations prior to outplanting were determined after constant storage conditions which could have equilibrated levels of this plant growth substance. However, if the treatments have long term effects on ABA levels significant treatment differences should have been apparent. As this did not occur it is possible that ABA had only a transitory effect in stress situations. It can serve more as a short term stress resistance substance. Davies et al. (1980) suggested that a rapid release of a small quantity of ABA just sufficient to cause maintenance of plant turgor would be a more
desirable character than an accumulation of sufficient ABA to close stomata for a prolonged period of time.

As the site of ABA action is in the guard cell, ABA should be measured only in those cells or in chloroplasts of the mesophyll cells where it is produced. To average ABA concentrations over the entire needle serves to dilute the concentration of ABA and obscures measured levels at which it is active in the plants. Moreover, fluxes of ABA or any other plant growth substance, are usually impossible to measure, although to describe dynamic nature of the compounds would require this measurement. However, as today's quantitation techniques require a labeled internal standard, quite large amounts of plant material are needed so that endogenous concentration of a plant hormone is higher than that of standard. Consequently, this constraint of the method precludes the precise localization of ABA biosynthesis and action.

The seedlings were watered once during the cold storage and thus they might not have experienced wilting and subsequent increase of ABA concentration. The discussion of the action of ABA both in dormancy and under conditions of water stress contains numerous discrepancies. Stomatal movements are poorly correlated with changes in ABA levels during the onset of water
stress as it was noted by Beardsell and Cohen (1975), that stomata closed before any increase in ABA could be detected in the leaves of maize and sorghum when subjected to water stress. Dörffling et al. (1980) suggested that only a very small amount of ABA, below the limit of detection, is transported from the mesophyll to the guard cells. This is partly supported by Raschke (1982) who claimed that the amount by which epidermal ABA increased when stomata close is of the order of fmol per mm² of stomata (1 fmol = 10⁻¹⁵ mol). Ackerson (1982) proposed that small amounts of apoplastic ABA (outside the cell plasmalemma), not dependent upon water stress, induces initial stomata response by ABA translocation within the transpirational stream to initiate stomata closure. Davies et al. (1980) concluded that attention has been misdirected to the amount of ABA produced under water stress, based upon the supposition that a greater quantity should be indicative of drought resistance.

The tenuous existence of a straight-forward relationship between ABA contents of leaves and stomata movements perhaps reflects the ideas of Trewavas (1981) who states that the most likely role of plant growth substances is an integration of plant development under imperatives of a changing environment. Thus the involvement of other regulators in modulating stomatal sensitivity to ABA needs a thorough exploration as e.g. suggested by
Snaith and Mansfield (1982) for auxin. Trewavas (1981) also emphasizes the different sensitivity of tissues to plant growth substances during plastic plant development. A change of the sensitivity of guard cells to ABA may occur under prolonged moisture stress (Kriedemann et al. 1972). A pretreatment with exogenous ABA decreased the sensitivity to the next dose of the substance in strawberry plants (Kubik and Antoszewski 1983). A similar decrease of sensitivity to ABA was obtained by shortening photoperiod. A shorter photoperiod, however, increased variability of ABA concentrations.

When seedlings were lifted and transferred to cold storage there was a drastic change in root temperature. While being outside, shoots were exposed to cool night temperatures during the fall, but roots in the pots were insulated from temperature fluctuations. Since roots are very sensitive to temperature changes plant growth substances produced by roots could have gained a new level which caused a new balance or imbalance of the substances in the whole plant (Lavender and Wareing 1972). According to Jaffe (1980), full growth potential of seedlings is never obtained since even slight mechanical perturbation retards growth of all plant species, a phenomenon called "thigmomorphogenesis".
Lavender (1978) reported that exposure to long photoperiod during the chilling period accelerated subsequent bud activity whereas no photoperiod effect was noted if long or short periods were applied after the chilling requirement is satisfied. Johnson (1983) described similar results for loblolly pine. In this study, however, there were no significant differences in bud breaking capacity between light and dark storage in either lifting time. Generally, light stored seedlings lifted in the fall fully broke their buds a few days earlier than those stored in the dark or bareroot seedlings. Lifting time had a drastic effect on time of bud burst, as is commonly observed. The winter lifted seedlings had a bud break time one half of that for fall lifted seedlings. Fall lifted seedlings grown in the growth room did not break their buds at all, a response which is in accordance with the results of Wommack (1964) and Hermann (1967). Douglas-fir needs 8-12 weeks of constant 4°C or longer periods of time under fluctuating temperature to be released from dormancy. After November 15 the seedlings were stored 4 weeks at 5°C. The longer the chilling period the more rapid will be the bud break in favourable conditions and buds will also break over a wider range of temperatures (Vegis 1964). Vegis (1964) suggested that if postdormant plants are exposed to temperatures which lie over the maximum temperature of the narrow temperature range for
bud break, the bud enter secondary dormancy. Further chilling is required to overcome this effect. Practical implications of the chilling requirement of coniferous seedlings have been presented by Lavender and Cleary (1974).

Some seedlings of the both lifting times were dug out after one growing season, and the condition of their root system was evaluated cursorily. The root system of mid-November lifted seedlings, planted in mid-December and grown in the cold frame, was stagnated. Seedlings of mid-January lifting had a more extended and branchy root system. The low capacity of Douglas-fir for root regeneration in fall has been reported e.g. by Hermann et al. (1972).

Long photoperiods tend to promote dormancy release when seedlings are in deep dormancy but the effect is negligible as chilling progresses (Campbell 1978). In this experiment this could not be verified as the dormancy cycle in the seedlings was still in the induction time of dormancy. Moreover, the bud breaking times were very similar in both pretreatments. Inability of the fall lifted seedlings to burst bud in the growth room is an anomaly. In the cold frames the rate of bud burst as bud activity stages could have been more distinct feature, but this was not recorded. In a
practical sense during regeneration, the final tally of bud burst is critical for survival.

This study did not support the hypothesis that the concentration of ABA could be a useful vigor index of seedlings to be outplanted. It might be that seedlings were not exposed to treatments harsh enough to promote changes in ABA. As discussed earlier, there were treatment effects in morphological characteristics of seedlings. Possibly, the study had perhaps too much a nature of a growth room study, whereas studies intended to evaluate methods for practical purposes call more for evaluations and techniques based on field studies. Since the exact role of ABA in plant metabolism is somewhat unresolved, more clear ideas of its role in plants is needed before undertaking new empirical experiments.

Present analytical methods for plant growth substances have the potential for detecting picogram quantities. Although with help of such precise and accurate methods definite relationships between seedling vigor and levels of plant hormones may be revealed, the time required for hormone analysis makes it impractical for large scale use. If this kind of relationship can be verified, it could be employed in an operational nursery. It would, however, be prudent to relate ABA concentrations to other physiological parameters that
are equally well correlated with seedling vigor, thus eliminating the tedious routine procedure of quantifying growth substances. Results of this study along with those of previous studies emphasize the need for additional research on the role of ABA in physiology of plants, especially in the light of both established theory and recent evolutions of the theory of plant hormones (Trewavas 1981, 1982). A synthesis of the role of ABA in plants derived from both schools of thought could be very fruitful for subsequent empirical studies.
V. BIBLIOGRAPHY


