

AN ABSTRACT OF THE THESIS OF

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Title: Growth and Moisture Relations of Western Hemlock Seedlings  
as Affected by Root or Shoot Disturbance

Abstract approved Signature redacted for privacy.

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The need for increased efficiency in regeneration of western hemlock (Tsuga heterophylla (Raf.) Sarg.) requires greater understanding of seedling physiology. Experiments examined western hemlock seedling growth responses to in-bed root pruning, and pruning of shoots or roots prior to transplanting. Measurement of plant moisture stress (PMS) after physical root disturbance, and measurement of cytokinin activity in xylem sap were also taken.

Seedlings that were root pruned in the nursery bed initially had decreased root dry weights and increased shoot-to-root ratios. In time, both July and September pruned seedlings tended to match control seedling shoot-to-root ratios. The rate of recovery for the July pruned stock was faster than the September pruned seedlings.

In pruning experiments conducted in both the field and growth chamber, the shoot-to-root ratio was generally uniform among control, root pruned, and top pruned seedlings by the end of the test period.

Significant differences did exist between growth environments. Highest shoot-to-root ratios were recorded in the site where moisture stress was probably lowest.

Root pruning and root abrasion generally increased stress level in western hemlock and Douglas-fir seedlings compared to controls. However, abraded seedlings had significantly lower PMS levels than controls on the first morning following planting. The hemlock seedlings had generally higher PMS levels than the Douglas-fir.

Cytokinin activity in western hemlock xylem sap was negligible from August to early April. A sharp increase in zeatin-like and zeatin riboside-like substances was found in mid April.

These findings suggest that the balance of shoot and root growth is environmentally directed. Moisture stress may play a role in interfacing environmental stimuli and internal metabolism. Acknowledging the role of environmental factors in determining growth patterns, it is suggested that seedlings should be grown for specific cultural conditions.

Growth and Moisture Relations of  
Western Hemlock Seedlings as Affected by  
Root or Shoot Disturbance

by

John W. Morris

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Growth and Moisture Relations of  
Western Hemlock Seedlings as Affected by  
Root or Shoot Disturbance

INTRODUCTION

Although there are still spectacular stands of trees in the Pacific Northwest, the era has passed when timbermen simply climb the next ridge to find ready material for the saw. To meet the continuing demand for wood products with our fixed land area, foresters must now efficiently regenerate harvested lands. Efficient regeneration requires timely planning with a knowledge of site characters and seedling responses.

Site characters can be analyzed by their influences on the five factors that directly influence seedling growth; moisture, temperature, light, chemical, and physical influences (Greaves et. al. 1978). Differences in sites such as aspect or soil affect seedling growth by influencing one or a combination of the environmental factors. Aspect differences would probably moderate gradients of moisture, temperature, and light. Soil differences might affect the moisture, chemical, and physical influences.

However, it is the internal growth process of the seedling which integrates and manifests the factors of the growth environment. The effectiveness of an environmental stimulus to elicit a response depends on the receptivity of the seedling. Conifers which are adapted to the temperate climate of the Pacific Northwest go through periods of activity and quiescence. Douglas-fir (Pseudotsuga menziesii (Mirb.)

Franco) and a western hemlock (Tsuga heterophylla (Raf.) (Sarg.)) seedlings go through a period of dormancy where even under favorable conditions the shoots do not grow (Lavender and Cleary 1974, Nelson and Lavender 1978). It is apparent that the internal physiology of the seedling is not uniform throughout the year.

Precise measurement of internal physiology is difficult. Growth patterns can be measured as the final manifestation of internal processes. Alternatively, measures of chemical fluxes such as changes in hormone levels, which may direct the allocation and metabolism of substrates, may be useful in defining physiological stages of development.

Externally, morphological characters may control the degree to which some factors operate. Size of the root system may affect the volume of water removed from the soil. The relative size of the shoot and root may moderate the water balance within the seedling which in turn affects transpiration and photosynthesis (Brix 1962). Size of the root system may not be a fine enough measure when dealing with planted seedlings, as damage to the root tissue through pruning or improper handling may lower the efficiency of the root's absorptive capacity.

The studies reported here were aimed at understanding the growth dynamics of western hemlock seedlings. Interest was focused on root growth and hormonal relations. Specifically experiments attempted to reveal the effects of top or root disturbance on dry weight increase and moisture stress after planting, and the pattern of cytokinin content in the xylem sap as it related to root disturbance.

## LITERATURE REVIEW

## Seedling Growth Patterns

The growth of seedlings is a process which integrates the dynamics of the environment with the inherent potentials of the plant. However, growth of the various plant organs may be affected differently by changes in environmental conditions (Brouwer 1962). The physiological links between the shoot and root form a sensitive mechanism by which the plant adjusts shoot and root growth to meet environmental dynamisms (Luckwill 1960).

Seasonal changes in temperature, light, and moisture availability are common environmental changes to which the plant reacts. Keinholz (1934) studied the seasonal growth patterns of pine and found that roots were the first organs to break the quiescence of winter. Leader and cambial activity followed, increasing at approximately the same time. However, cambial activity continued for a longer period into the summer. The last tissues to show increase from winter were the needles. Summer and fall growth of the seedling finished with a second growth peak of the roots prior to winter's slow down. More recently Krueger and Trappe (1967) examined the seasonal growth of Douglas-fir seedlings. While the exact dates differ between the species, the same seasonal pattern emerged. Early spring root growth was followed by a shoot growth peak and declining rate of root increase. As shoot growth decreased in the fall, there was a second peak in root growth. Looking simply at the shoot, Owens and Molder (1973) examined the seasonal patterns of vegetative buds in western hemlock. The

apices became mitotically active during the last week of March. Swelling of the bud was caused by elongating leaf primordia which had initiated the year before. The apex however, remained small and produced bud scales. Axillary buds were initiated in mid April. Following bud burst in mid May, rapid shoot elongation occurred for seven weeks. The apex rapidly initiated leaf primordia until mid August and then more slowly until November. The usual growth pattern of the seedling that is seen, then, is early spring root growth prior to a burst of shoot growth as temperatures warm, and a final peak in root growth prior to the slowing of metabolic activity during the winter.

However, like the growth environments, the patterns of growth are subject to manipulation and change. McGee (1976) in studying northern red and scarlet oak seedlings, found seedlings burst bud earlier if they had been grown under shade rather than in the open. Root growth is not confined to the beginning and end of the growing season. For noble fir Wilcox (1955) found the regeneration of injured roots was most active during the months of June through September.

A significant point is the difference between the actual growth patterns of the seedling in the field, and the growth potentials which may be determined under laboratory conditions. The potential for new growth for Douglas-fir seedlings was found to vary during the year (Stone et. al. 1962). Stone and associates found the root regenerating potential (RRP) measured as growth of new roots on seedlings grown in the greenhouse, was low in the summer. However, RRP rose sharply in September and was high during the winter months. In April the RRP

dropped precipitously. Similarly, the RRP of ponderosa pine peaked in the winter. Brown (1977) followed the RRP of western hemlock seedlings and also found that potentials were highest during the winter months. Further, Stone and associates (1963) reported that differences existed between RRP of stock grown at different nurseries. Among the four California nurseries studied, the seedlings of the northern-most nursery began the autumn increase in RRP earlier, and the spring decline developed later.

These seasonal changes in the potentials of root growth may explain seasonal effects on survival and growth associated with the date seedlings are removed from the nursery and planted. Todd (1964) found that Douglas-fir survived better when lifted and planted after mid December. Seedlings that were lifted and stored at low temperature also survived best when lifted from the nursery after mid December. Hermann (1967) found that Douglas-fir seedlings lifted in January were less sensitive to root exposure than seedlings lifted in early November or late March. Also working with Douglas-fir, Lavender (1964) found winter lifted stock was less injured by cold storage than seedlings lifted in fall or spring.

In addition to seasonal effects, the effect of relative size of the shoot and root on seedling survival and growth has been investigated. Douglas-fir seedlings with well developed root systems had increased survival over seedlings with poorly developed root systems (Hermann 1964). In another study of seedlings classified as having large or small roots, Lopushinski and Bebee (1976) found that seedlings of three height classes with large roots survived at rates 22 to 26%

higher for Douglas-fir and 5 to 15% higher for ponderosa pine compared to seedlings with small roots. For both species shoot mass was 2.1 to 4.8 times greater for seedlings with larger roots than seedlings with small roots systems. Decreased top size can also affect growth. Seedlings that had from one fifth to three fifths of their tops removed suffered complete mortality during a study on Douglas-fir (Hermann 1964). Defoliated conifer seedlings were found to initiate fewer new roots per plant and fewer plants had new roots than intact control seedlings after a 30 day test period (Carlson 1977). While working with three month old Douglas-fir seedlings Krueger (1967) also found that defoliation decreased root initiation. Further, the study found that light intensity affected root growth. From 0 to 1050 fc (approximately one tenth full sun) more new roots grew the higher the light intensity. Brix (1970) looked specifically at the effect of light intensity on the growth of western hemlock seedlings. He found that when using green shade cloth to achieve light intensities of 10, 30, 50, 70, and 100 percent full sun, the greatest total dry matter production was under the 50 and 70 percent canopies. In addition, a nominal 50% sun cover was produced using spaced 1 inch slats. Dry matter production under this structure was significantly lower than total dry matter production under the cloth screen. It is also evident from Brix's data that the relative proportion of root, needle, and stem and branch weight change with light intensity. The greatest proportion of the shoot weight which was found as needles occurred at 50 and 70% full sun. Root weight had an increasing fraction of the total seedling

weight as light intensity increased. The increasing proportion of roots might be explained by an increasing transpiration potential which the roots attempted to meet.

#### Moisture Status and Seedling Growth

Maintenance of a favorable internal water status is important for seedling growth. Gross (1976) found that as soil water potential became more negative, rates of transpiration and photosynthesis decreased. Sudden increases in exposure also lead to damaging moisture stress levels in the seedling (Keller and Tregunna 1976). It was suggested that the photosynthetic apparatus was damaged as a consequence. The elongation rate of cultured radiata pine needles was found to be linearly related to the osmotic potential of the medium (Sand and Correll 1977). Stomatal closure of ponderosa pine and grand fir was a response associated with greater moisture stress levels in the leaf (Lopushinski 1969). Total closure of stomata was reached at 17.6 bars for ponderosa pine and 25.8 bars for grand fir. Leshem (1970) found the roots of Pinus halipensis reacted to water stress. Resting roots broke quiescence after watering, and quiescence was again induced when water stress levels approached 6 or 7 bars. Root growth and shoot development of germinating ponderosa pine and northern red oak seedlings also was found to decrease as the osmotic potential of the soil solution became more negative (Larson and Schubert 1969, Larson and Whitmore, 1970). Soil moisture is only one factor that affects the internal water status of the seedling (Cleary 1970). A direct measure of the internal water status can be made using a pressure chamber apparatus

(Waring and Cleary 1967). A detached twig is placed in the chamber with the cut surface extending outside. The pressure required to force the sap to the cut surface is termed the plant moisture stress (PMS) and is measured in bars of pressure. PMS is of equal magnitude to the tension of the water column in the twig and is a measure of water availability in the plant (Cleary and Greaves 1977).

Different levels of plant moisture stress may have profound effects on plant growth. Zaerr (1970) regulated the PMS levels of Douglas-fir trees by irrigation and found that trees with lower PMS had greater diameter growth than control trees. Cleary and Greaves (1977) reported that conifer seedlings have different tolerances to high PMS. Brix (personal communication) found that western hemlock seedlings could survive PMS levels of 60 bars while Douglas-fir seedlings could survive PMS levels of 110 bars. Further, when grown in the same pot and subjected to drying, western hemlock had greater PMS than Douglas-fir. Jones (1972) reported a size effect in Arizona mixed conifer seedlings. Seedlings less than about 15 cm tall had higher PMS levels than larger seedlings. Brix (1962) found that the relative rate of photosynthesis for loblolly pine seedling decreased markedly as PMS levels approached 10 bars. Photosynthesis and transpiration correlated well, both dropping as stress levels increased and rising following irrigation. In other studies of photosynthesis and its relation to PMS it was found that rates of photosynthesis declined for Douglas-fir, western hemlock, white spruce, and lodgepole pine when the PMS levels became 10.0, 10.7, 12.4 and 6.6 bars, respectively, and

became zero when PMS levels reached 53.9, 29.7, 28.6 and 22.4 bars (Brix personal communication).

#### Effects of Seedling Root Disturbance

Removal of part of the seedling root system can lead to substantial effects on shoot and root development. Humphries (1960) found that the more root removed from barley seedlings, the greater was the decline in shoot growth. However, removal of up to 50% of the root system had no effect on the rate of root increment. Dykstra (1974) found that the depth at which seedlings were undercut affected the amount of subsequent root growth. An undercutting depth of 7.5 cm produced greater subsequent growth than depths of 15 or 30 cm. Sutton (1967) found that complete pruning of lateral roots resulted in severe mortality of outplanted material. Less severe pruning did induce a greater proliferation of roots. First year height growth was not affected. In contrast, Larson (1975) found that shoot growth was directly correlated with the weight of the roots regardless of whether the relatively high or low root weights were the result of natural growth or pruning. Lateral pruning of conifer seedlings was found to be effective in producing dense and compact root systems without depressing height growth. However, timing of the operation during the year was critical (Eis 1968, Eis and Long 1972, 1973).

The technique of wrenching is a nursery practice providing a more severe root disturbance than undercutting alone. The process of wrenching, which follows undercutting, proceeds by passing an inclined blade under the seedling bed below the undercutting depth. Soil pore

space is increased and any new long roots are cut. (Cleary et. al 1978). Wrenching has the effect of redirecting growth, hardening seedlings, and generally reducing top growth (Edgren 1974). Koon and O De1 (1977) reported wrenching produced Douglas-fir seedlings with reduced shoot weight but not root weight, and that these seedlings had greater survival after planting. In contrast, Tanaka and associates (1976) reported no loss in top growth. This study also indicated that the beneficial effect of wrenching on seedling survival was greatest on droughty sites. Wrenched radiata pine seedlings were found to have greater height increment the first two years following outplanting than unwrenched controls (van Dorsser and Rook 1972). Further, these wrenched seedlings withstood exposure better. This was attributed not to the individual root, but to the denseness of the root system. The frequency of wrenching was found to affect levels of sugar and starch components in the seedling (Rook 1971). Seedlings wrenched monthly had twice the sugar and starch content of seedlings wrenched weekly or biweekly. Rook also reported that undercutting and wrenching resulted in a three-fold increase in the relative flow of  $^{14}C$  assimilates from foliage to roots. In an earlier study Rook (1969) found transpiration rates and turgidity of needles in seedlings wrenched biweekly to be higher than in controls.

Root disturbance was coupled with cytokinin treatment in a study of Douglas-fir seedlings by Lavender and Wareing (1972). Two types of root damage were imposed. Treated seedlings were either abraded between the palms of the experimentors hands or their active roots were removed.

Control seedlings had the highest scores in survival, fresh weight increase, and shoot activity. Pruned seedlings were next in all three categories. Abraded seedlings were lowest in each growth parameter. Noteworthy too, was the result that the cytokinins kinetin and SD 8339 did not effect recovery from root damage.

## Cytokinins

Since the first characterization of auxin, plant hormones have been under investigation. Cytokinins are one class of hormones in plants generally defined by the ability to promote cell division in cultures of tobacco callus. However, a wide range of properties have been attributed to cytokinins, from enhancement of germination in lettuce seed (Miller 1956) to the retardation of chlorophyll breakdown in excised leaves (Richmond and Lang 1957). The first compound fully characterized as having cytokinin properties was kinetin. A purine derivative, it was found as an artifact in denatured herring sperm DNA. Letham and associates (1964) characterized the kinetin-like compound that Miller (1961) had found in Zea maize. Another N-6 substituted purine, it was named zeatin. Formally zeatin is 6-(4-hydroxy-3-methyl-trans-2 butenylamino)-purine. Since zeatin's characterization many natural and synthetic cytokinins have been identified. Similar to zeatin is another naturally occurring cytokinin, 6-(3-methyl-2-butenylamino) purine or 2iP. BAP, or 6-benzylamino purine, like kinetin, is another common synthetic cytokinin.

The site of synthesis for cytokinins is not clear. Reid and Burrows (1968) reported finding cytokinins in the spring sap of Acer

pseudoplatanus and Betula pubescens and suggested the source may be roots or xylem parenchyma of the trunk by de novo synthesis or release from storage. Evidence for manufacture in the root has been offered, however. Ringing of Salix babylonica stems increased the concentration of zeatin-like compounds below the bark ring (van Staden and Brown 1977) Weiss and Vaadia (1965) found kinetin-like activity in root apices of sunflower plants. Reduction in protein synthesis in rye seedlings and accelerated senescence of pea leaves which both follow root removal were reversed following application of a cytokinin (Feierabend 1969, McDavid, et. al. 1973). In studying the transport of cytokinins through the root, Mozes and Altman (1977) found that in Citrus aurantium BAP moved basipetally through intact portions at 2.6 to 5.1 mm/hr.

#### Cytokinins and Water Stress

Cytokinins have been implicated in plant water relations. Meidner (1967) found that kinetin treatment of barley leaves increased the rates of net assimilation of CO<sub>2</sub> and concluded that kinetin appeared to affect directly the stomatal mechanism. Endogenous levels of cytokinin were found to be affected by osmotic values of nutrient solution (Itai et. al. 1968). Sunflower roots suffered a reversible decrease in cytokinin translocation from the roots as osmotic values of the nutrient medium became more negative with the additions of salt (Na Cl) or Carbowax 6000. Itai and Basdia (1971) found that an air drying stress of only 30 minutes on intact Nicotiana rustica plants reduced the level of cytokinin in both the root exudate and leaves.

## Cytokinins in Trees

Cytokinins have been isolated from a variety of tree species. Hewett and Wareing (1973c) reported seven cytokinins in mature poplar (Populus x robusta) leaves. Horgan and associates (1975) isolated from the same poplar variety 6-(o-hydroxybenzylamino)-9-beta-D-ribofuranosyl purine. Willow (Salix babylonica) leaves were reported to contain a compound which, following hydrolysis with beta-glucosidase, chromatographed like zeatin (van Staden 1976). Using combined gas chromatography and mass spectrometry techniques Ribosyl-trans-zeatin, dihydro zeatin, and trans-zeatin were identified in sycamore maple spring sap (Purse, et. al. 1976). Less information is available on the cytokinins of gymnosperms, however. Van Staden (1978) reported several zeatin derivatives in leaves of representative of four divisions.

Compounds chromatographically similar to zeatin, zeatin glucoside, and zeatin riboside were found in all examined plants. In addition, a zeatin riboside glucoside-like compound was found in Ginkgo biloba and a 2iP like compound was found in Welwitschia mirabilis and Encephalaria natalensis. Lorenzi and associates (1975) reported finding zeatin riboside and zeatin-9-glucoside like compounds in Picea sitchensis needles and buds. Morris and associates (1976) reported Douglas-fir xylem sap contained zeatin-like and zeatin riboside-like compounds.

Studies of cytokinin periodicity may yield greater understanding of the role these compounds play in the physiology of the tree. Hewett and Wareing (1973b) found that from nondetectable levels in December and January, parallel increases occurred in the cytokinin levels in poplar sap and buds during February and March. The maximum level in

the sap occurred three weeks prior to the maximum in the buds. Similarly, Domanski and Kozlowski (1968) found that kinetin like activity was absent in dormant buds of Betula papyrifera and Populus balsamifera, but increased in both species until just prior to bud burst and decreased thereafter. Alvim and associates (1976) followed both cytokinin activity and abscisic acid (ABA) content in xylem sap of willow and found that spring growth was preceded by a decrease in ABA and an increase in cytokinin activity. The onset of dormancy was associated with low cytokinin activity and high ABA content. Roots of sugar maple (Acer saccharum) seedlings were shown to have increased cytokinin activity as root growth surged in the spring. Highest levels in lateral roots were found shortly before bud break and then declined (Brown and Dumbroff 1974, Taylor and Dumbroff 1975). Lorenzi and associates (1975) followed two zones of cytokinin activity in buds and needles of sitka spruce, a zeatin-9-glucoside like fraction (ZG) and zeatin riboside (ZR) fraction. Young needles and buds had low levels of ZG, and had ZR as the major cytokinin. After full expansion ZG increased and ZR decreased. The ZG fraction was suggested as a storage form.

Cytokinins, then, are widely distributed compounds and have been identified in some conifers. Their endogenous fluctuations associated with season and moisture stress, and their effects on cell division and protein metabolism permit speculation as to their role in the integration of shoot and root growth. The mechanisms of integration, however, await future research.

Root parameters, as outlined above, have been shown to affect the growth of the shoot. Considering the likelihood of root or shoot dis-

turbance for seedlings outplanted in a regeneration program, there are pertinent questions that remain for western hemlock. In nursery management, growing seedlings may have their roots undercut, laterally pruned or wrenched. How permanent are these root disturbances? Is the effect of root or shoot disturbance uniform on seedlings planted in different environmental conditions? Seedlings that are outplanted may suffer other root disturbances such as pruning to ease handling and planting. Additionally, rough or careless treatment in packing or shipping may disturb the root system of stock. Does root disturbance measurably affect seedling moisture stress immediately after planting? What are the long term effects of root disturbance? Are there hormonal changes in the young tree as a result of the disturbance?

Answers to these questions are needed to improve the success of western hemlock regeneration. The studies which follow are an effort to provide this information on the affect of root or shoot disturbance on the growth and moisture relations of western hemlock seedlings.

## METHODS

## Seedling Growth and the Effect of In-bed Root Disturbance

For this experiment 1-0 western hemlock seedlings were lifted from the Industrial Forestry Association (IFA) nursery near Toledo, Washington. The seed source was low elevation coastal Washington (030-.5). The seedlings were lifted by shovel on February 18, 1977 and planted in a shaded cold frame at the Forest Research Laboratory in Corvallis, Oregon. The soil was a moderately heavy clay-loam of forest origin. The seedlings were planted at a spacing of 15 x 15 cm. and at the time averaged 3.4 cm in height.

Root disturbance was effected by pulling a pruning blade through the soil beneath a single row of seedlings. The blade was slanted downward at an angle of 15°. Thus the soil and seedlings were raised as it passed beneath. The blade was 15.5 cm across the cutting edge, 10 cm wide and 0.3 cm thick. The depth of the leading edge was 5.0 cm below ground level.

The seedlings were disturbed on one of two dates, July 1 or September 8. At intervals, seedlings were excavated from the cold frame using a trowel to loosen the soil. The soil was then washed gently from the roots in buckets of water.

## Dry Weight Measurement

Excavated seedlings were cut at the cotyledonary node and the shoot and roots of each seedling placed separately in paper bags for the dry weight determinations. The tissue was dried 48 hr at 70 C and allowed to cool to room temperature in a polyethylene bag before

weighing. The seedling parts were removed from the bags before weighing. Needle weights were determined after sorting out the stem segments.

The "t" test was used to detect differences between treatments or dates. The means and standard errors were calculated from the 15 or 20 individual seedlings that were excavated for each combination of date and treatment. A significance level of 5% was used.

### Pruning Effects Under Field Canopies

For examining pruning effects in the field environment, 2-0 western hemlock seedlings were lifted from the IFA nursery near Toledo, Washington on February 18, 1977. The seedlings were transplanted into pulp pots having a volume of 6800 cm<sup>3</sup>. The soil used was a sandy loam from a local forested site. Thirty-eight pots, each containing ten seedlings, were planted and then placed beneath the partial shade of an oak grove at the Forest Research Laboratory.

The seedlings were treated April 2, 1977 and at the time had an average height of 23.5 cm. The shoot pruning treatment consisted of clipping off that portion of the seedling shoot which stood more than 10 cm above the ground level. Roots were pruned by sawing the pot in two, 10 cm below the ground level. The upper disk of soil and roots was then transferred to a new pot containing the soil from the lower portion of the old pot. Control seedlings were left in the pots undisturbed. For each treatment, 120 trees or twelve pots were processed.

Four pots from each of the three treatments were buried under one of three canopy covers at the Northwest Forest Genetics Nursery near

Corvallis, Oregon. The closed canopy was beneath a young Douglas-fir plantation. The edge canopy was beneath the boughs of the trees on the east side of the plantation. The open canopy was in a grassy field near the plantation edge. Midday light intensities were approximately 13% of full sun under the edge canopy and 2% under the closed canopy. The seedlings were watered approximately biweekly to field capacity from June through September.

Height measurements to the nearest mm were taken during the growing season. Heights were defined as the distance from ground level to the base of the terminal needle or to the top of the terminal bud depending on the meristem stage. Dry weights were determined by removing the seedlings from the pots and then proceeding as previously described.

The elongation rates were computed from the changes in height of the individual seedlings. For a given growth period, treatment, and canopy, the elongation rate was computed as the mean increase in cm for living seedlings in each pot during the growth period divided by the number of weeks in the growth period.

The elongation rates and the tissue dry weights were compared by analysis of variance. The "F" test with a significance level of 5% was used to determine the significance of treatment differences. Differences among treatments were tested for significance with a "t" test at the same 5% error level.

#### Pruning Effects in Growth Chamber Environments

Two year old western hemlock seedlings, classed as 1-1 were used to investigate shoot and root pruning effects in three growth chamber

environments. The 1-0 seedlings were from the same seed source and nursery as previously noted. They were also lifted on February 18, 1977 and planted in pots as described above. The potted seedlings were grown through the summer under an oak grove at the Forest Research Laboratory.

On November 13, 1977 the seedlings were given one of three treatments; control, root pruned or top pruned, as described earlier, and placed into the growth chambers. Twelve pots were processed for each treatment. The three growth chambers each received four pots from each of the three pruning treatments. The growth chambers were set at different temperatures, either 15 C, 20 C, or 25 C. No diurnal changes in temperature were programmed. The photo-period was set for 16 hr and was maintained by "cool white" fluorescent lights supplemented with incandescent lights. Light intensity in all chambers was approximately  $26 \text{ w/m}^2$ . Humidity in the growth chambers was not controlled. The seedlings were grown in the chambers of 96 days, from November 13, 1977 to February 17, 1978 and watered weekly.

Measures of bud activity and shoot elongation were taken during the experimental period. Five scores, as defined in Table 1, were used to scale phenological development of the shoot. Shoot elongation was measured as the length from the fall bud scale scars to the base of the terminal needle or the apex of the bud depending on the stage of the meristem. Total heights were measured from ground level. All distances were measured to the nearest mm. Dry weights of the living seedlings were obtained using the procedure given above. However, for two pots in each treatment in each chamber, the roots and shoots were placed collectively in paper bags rather than individually.

Table 1

## Shoot Development Scores

SCORE	DESCRIPTION
0	no outward change in the autumn bud
1	swollen buds apparent, often characterized by a silvery sheen
2	tissue covering needles ruptured, needles still in a compact cluster
3	needles expanded, shoot apparently growing
4	new bud formed at apex

Analysis methods for elongation rate, tissue dry weight and weight ratios proceeded as outlined for the field grown seedlings. Analysis of variance procedures were used to detect significant differences between treatments. Again, a 5% error level was used.

#### Root Disturbance and Storage Effects on Seedling PMS

The effect of root disturbance and cold storage on the plant moisture stress levels of seedlings after planting was investigated using western hemlock and Douglas-fir. The 2-0 western hemlock was shovel-lifted from the IFA nursery at Toledo, Washington. As with the other experiments, the 030-0.5 seed source was used. The 2-0 Douglas-fir was machine lifted from the IFA nursery at Canby, Oregon. The seedlings were grown from the Wendling, Oregon seed source (471: mixed elevation). The mean heights for the hemlock and Douglas-fir were 12.5 and 25.0 cm, respectively. Both species were lifted on three dates; November 4, January 10 or March 9, 1978. A sample of the seedlings were planted one or two days after lifting while other samples were stored for one, two, or three months. The western hemlock seedlings were stored in polyethylene bags. The Douglas-fir seedlings were stored in polyethylene lined kraft paper bags. The storage facility used was a cold room set at 2 C.

The seedlings were given one of three root treatments; control, abraded or pruned. Control seedlings were simply removed from storage and planted. Abraded seedlings were treated by rubbing the root system between the palms of the hands for five seconds. Rubber gloves were worn during the abrading procedure. The entire root system was abraded

and rarely was any bark removed. Pruning was done using a paper cutter. For western hemlock, all roots five centimeters below the cotyledonary node were removed. The Douglas-fir seedlings were pruned 8 cm below the cotyledonary node. Both the abrasion and pruning were carried out in the cold storage room.

Seedlings of both species were planted in two locations. The first area was in shaded cold frames with an average spacing of 12.7 x 8.7 cm. The second location was in a growth chamber or greenhouse in pulp pots as described earlier. Again, ten seedlings were planted in each pot. For each combination of lift, storage, root treatment and location, forty trees were planted.

Winter rain generally maintained the moisture level in the soil of the cold frames. The seedlings in the greenhouse usually were watered once a week. From April through June the pots were watered twice a week. The greenhouse photoperiod was maintained at 16 hr. during the winter months with supplemental fluorescent lights. The temperature was set at a constant 20 C. However, warm sunny days often caused increases in the greenhouse temperature.

Measurements on the seedlings included PMS and bud burst at both locations, and new root growth at the greenhouse. Predawn moisture stress measurements were taken after planting using a pressure chamber apparatus and a branch 4 to 6 cm long cut from the seedling. Usually a minimum of eight seedlings were measured for each treatment on a given day. Normally no more than two readings were taken on any one seedling. Bud burst was recorded as the number of seedlings in each treatment that had broken bud after the growth period. An ocular

estimate of the percentage of the entire root mass that was made up of new white roots was also taken at the end of the growth period.

Plant moisture stress data were analyzed using analysis of variance. The "F" test with a 5% significance level was used to assess differences among months of storage, root treatment, and number of mornings since planting. For greenhouse grown seedlings data on new root percentage, flushing percentage, and survival percentage were taken once, at the end of the four week growth period. No variation associated with the number of days since planting could be assessed. The percentages computed for new roots and flushing were based on surviving seedlings only and not on the total number of trees planted. For seedlings grown in the cold frames PMS data were only analyzed for morning 15. Therefore, no analysis for the effects of different mornings was possible.

#### Seasonal and Root Disturbance Effects on Cytokinin Level

Naturally regenerated western hemlock trees were harvested from the 750 m level on Green Mountain in western Oregon's coast range for use in analysis of xylem sap for cytokinin activity. The trees were approximately seven years old, 2.5 meters tall and average 2.7 cm in basal diameter. Trees were selected July 23 and 24, 1977 and tagged in pairs. One of each pair had its roots disturbed at this time. Disturbance was provided by breaking up the root system and soil approximately 20 to 30 cm from the trunk all around the tree with a pickaxe.

The ten-tree samples were harvested just before dawn at monthly intervals for the control trees and bimonthly for the root disturbed

trees. Collected trees were first sawn off at the base and then the entire tree placed in a polyethylene bag. The trees were then transported to the Forest Research Laboratory forty miles away. Trees were doused with water prior to cold storage at 2 C for up to two days.

Extrusion of the xylem sap was possible by use of large pressure chamber. After being misted with distilled water, bark from the lower 15 cm of the trunk was removed and allowed to protrude from the pressure chamber. Pressures to 28 bars were used to extrude the sap from the tree. Trees were under pressure for approximately 20 minutes. The sap from individual trees were frozen using liquid nitrogen. The bulk, 250 ml, samples of generally five trees were stored frozen at -14 C until fractionation.

#### Sap Fractionation and Cytokinin Trace Enrichment

The frozen xylem sap was first thawed and filtered through a Whatman #1 filter paper. Two microliters of kinetin or in some cases radioactive kinetin was then added to the xylem sap. Next, the sample was acidified to pH 3.50 with glacial acetic acid. Trace enrichment proceeded by pushing the sample through a 1.0 by 5.6 cm porasil column. The column was eluted with 50 ml 95% ethanol and evaporated to dryness under vacuum. The residue was partitioned three times in a 2:1:1 v:v:v ethylacetate: t-butyl alcohol; tris buffer (0.1 M pH 8.0) solvent system. The three combined ethyl acetate- t-butyl alcohol washes were then dried with anhydrous sodium sulfate. The final solution was evaporated to dryness under nitrogen and placed in cold storage at -70 C.

### High Performance Liquid Chromatography

Following trace enrichment, xylem sap samples were further fractionated using High Performance Liquid Chromatography (HPLC). The column used was a Waters Associates 3.9 mm by 30 cm micro C18 column. Two solvents were used, 95% ethanol and 0.02M ammonium acetate buffer pH 3.50 in a 20 min gradient. The gradient ran from 5 to 60% of the 95% ethanol increasing linearly from the time of injection. The flow rate through the column was 2.0 ml/min. Standard solutions for zeatin, zeatin riboside, and kinetin were used to determine the retention times for these compounds in this system. Collections were then made from the enriched xylem sap samples that corresponded with each of the three cytokinins. Samples were freeze-dried and stored at -20 C until bioassayed.

### Cucumber-cytokinin Bioassay

Cytokinin activity was assessed by measuring chlorophyll increase in etiolated cucumber cotyledons. The bioassay was adapted as follows from Fletcher and McCullagh (1971). Cucumber seeds were first planted in vermiculite and incubated in the dark four days at 28 C. While working under a green safe light, cotyledons were removed from the seedlings and placed in 100 ul of test solution for a 16 hr dark imbibition period. Following a two hour exposure to fluorescent lights (1400 fc), the cotyledons were then frozen and later the same day ground in a solution of tetrahydrofuran and ethyleneglycol monoethyl ether 1:1 v:v with a teflon/glass motor driven homogenizer. After centrifugation, the solution was read on a spectrophotometer at 665 and 698 nm.

The difference in absorbance indicated the relative chlorophyll content. The test solutions which the cotyledons imbibed were made by adding 1.0 ml of buffer (10mM  $\text{PO}_4^{--}$  <sup>3mM</sup>  $\text{Ca}^{++}$  (CaCl<sub>2</sub>) pH 5.0) to the freeze-dried chromatograph sample and agitating it. Bulk samples from the same date and treatment were combined prior to the bioassay. The one ml sample was then divided into ten 0.1 ml aliquots for ten cucumber cotyledon pairs. Two determinations of chlorophyll content for five cotyledon pairs were then made.

#### Computation of Zeatin Equivalents

To compute the zeatin equivalent for each freeze-dried chromatograph sample, a standard curve was computed using solutions of known molarity; 0.5, 5.0, and 25.0 micro molar zeatin. These three points were then fitted to a regression line. The regression line related two variables; LZ, the logarithm of the zeatin concentration, and OD, the optical density of the chlorophyll solution. OD was computed as the difference in light absorbance at 665 and 698 nm. The regression equation for all days combined was,  $\text{OD} = 325.2 + 207.7(\text{LZ})$ . This equation had an  $R^2$  value of 0.587. The individual days, however, had an average  $R^2$  value of .980. From the several regressions the zeatin equivalents were computed for the chromatograph samples and for a test solution of buffer. The zeatin equivalent was computed as the difference between the sample and the buffer.

Analysis of the cytokinin data rests on an intuitive approach. While two xylem sap samples were taken for each treatment, these were combined after chromatography in an effort to observe some apparent

activity. Therefore, the lack of replication prevents the effects associated with the month of collection from being statistically tested.

## RESULTS

### Seedling Growth and the Effect of In-bed Root Disturbance

Shoot and root dry weights both increased during the study period. Shoot weight increased moderately until winter, and then more rapidly in early spring (Figure 1). Root dry weight accumulated faster in late autumn and early spring (Figure 2).

The seedling shoot-to-root ratio did not show the same pattern of change as the dry weights. The control seedlings showed the highest ratios in the spring and lowest in the fall (Figure 3). Figure 3 shows also that root disturbed seedlings initially had significant increases in the shoot-to-root ratio resulting from a loss of root mass. The difference from the control seedlings decreased with time. Seedlings treated in July matched the control shoot-to-root ratio in 6 wk. September treated seedlings also decreased their shoot-to-root ratio after treatment, but were slower to match control seedlings.

### Pruning Effects Under Field Canopies

#### Elongation Rate

The rate of seedling leader elongation was affected by both the pruning treatment and the canopy it was grown under. As shown in Figure 4, the highest rates of elongation for all seedlings were in spring and early summer. The greatest rate measured for the unpruned control seedlings was during the period April 7 to June 23. The average rate fell to .46 cm/wk in July and approached 0.0 for the period of August to December. The peak rate of .68 cm/wk measured

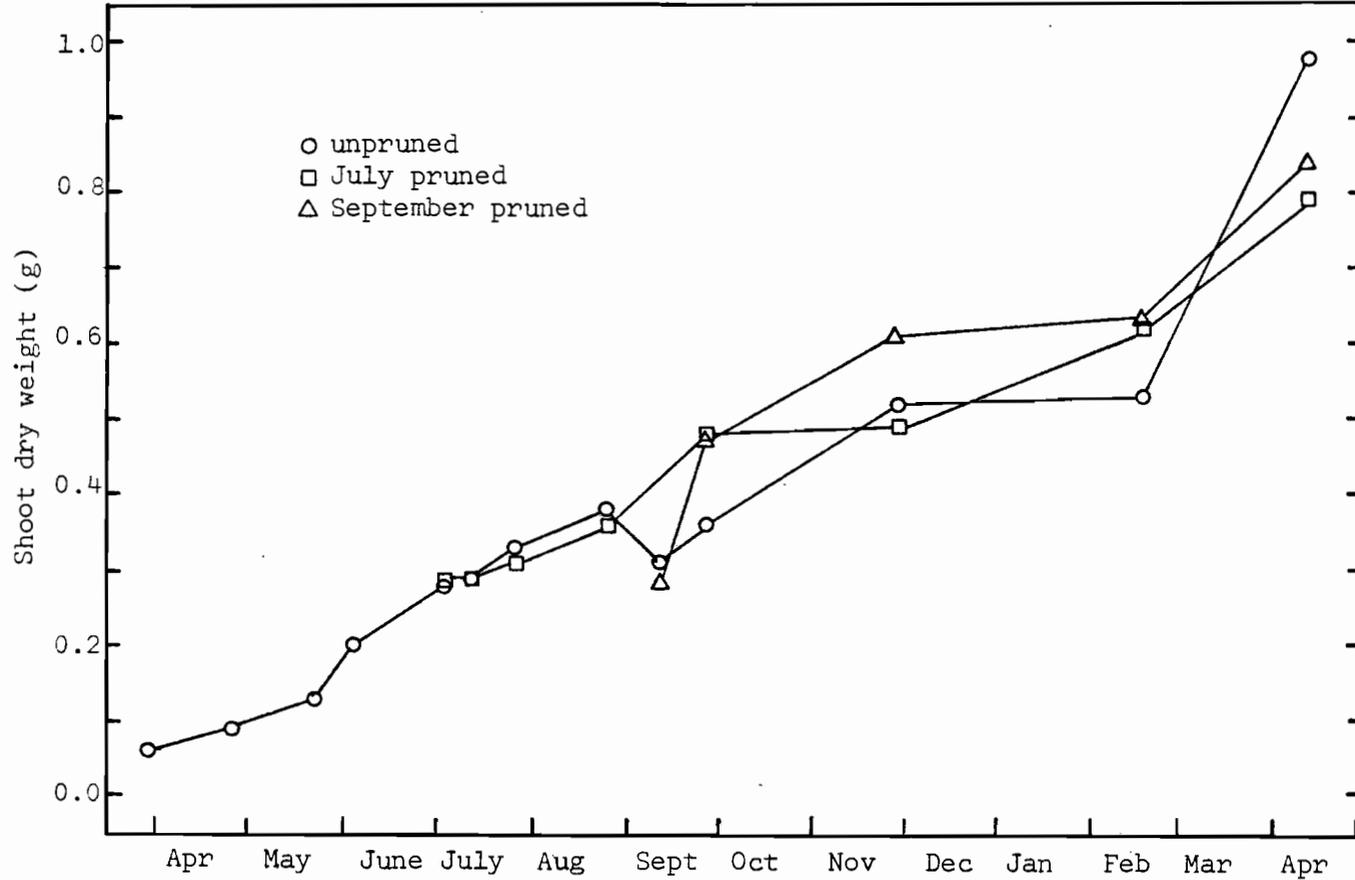


Figure 1. Seasonal shoot weight of western hemlock seedlings grown in a shaded coldframe. Immediately after treatment shoot dry weights of root pruned seedlings did not differ significantly from the undisturbed seedlings. The following April shoot weights of controls were significantly higher than July and September pruned seedlings as indicated by "t" tests at the 95% confidence level.

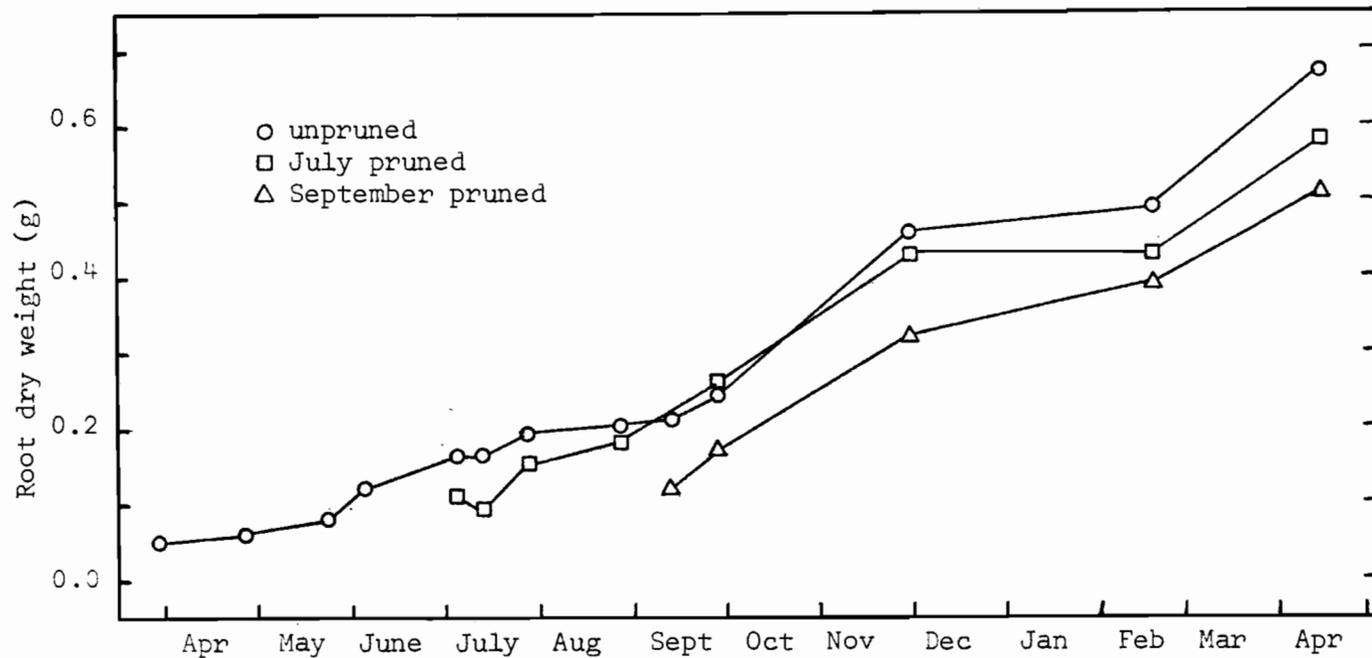


Figure 2. Seasonal root weight of western hemlock seedlings grown in a shaded cold frame. Root dry weights of root pruned seedlings were significantly different from control immediately after treatment. The following April no significant differences were found between treatments.

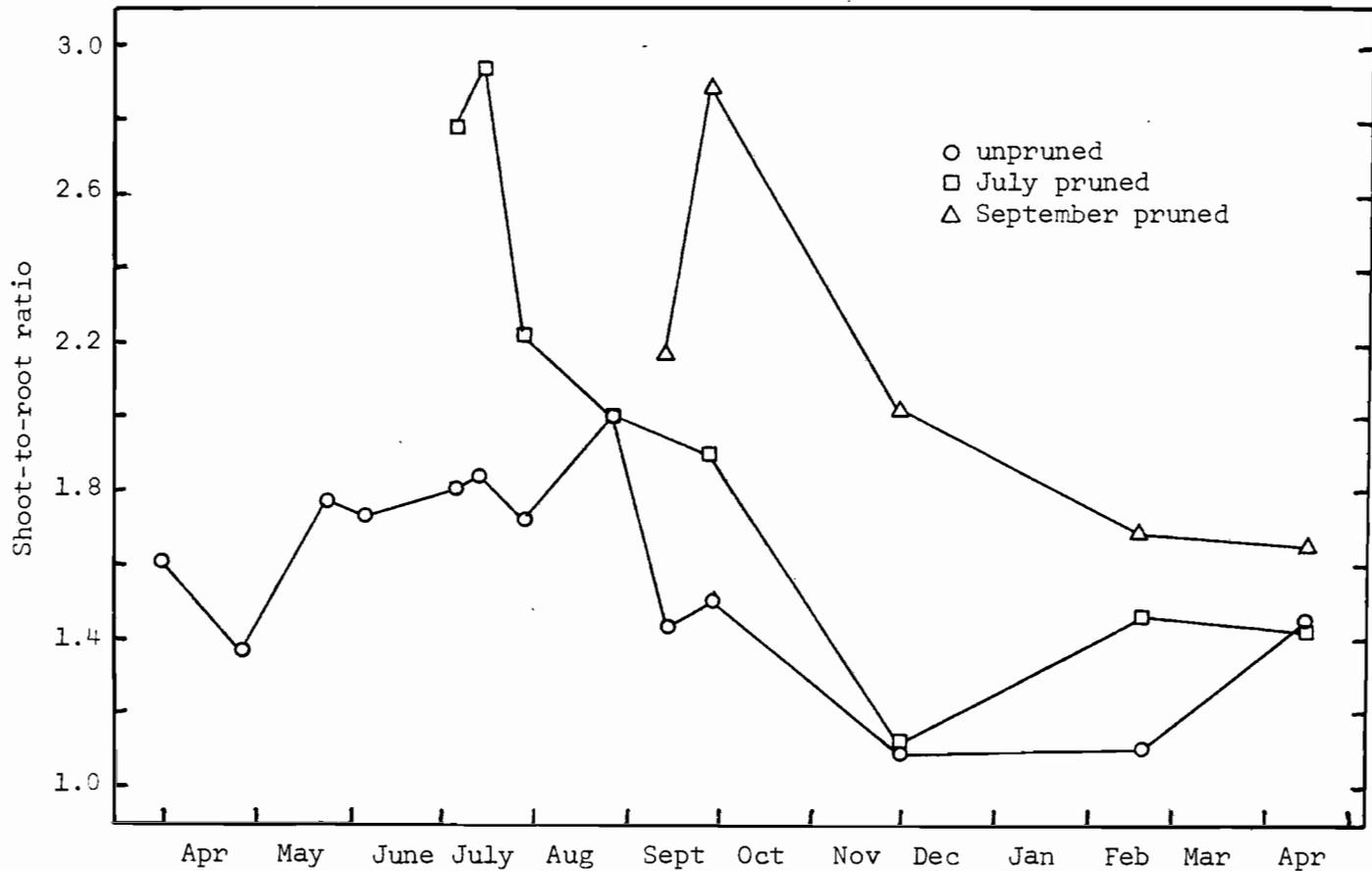


Figure 3. Shoot-to-root ratios of western hemlock seedlings grown in a shaded cold frame. Shoot-to-root ratios of control seedlings were significantly higher in early summer than late fall and winter. Ratios of both July and September pruned seedlings were significantly higher than unpruned seedlings immediately following treatment. The following April "t" tests indicated no significant differences between treatments.

for the root pruned seedlings was also during the period of April 7 to June 23. This rate fell to .62 in July and was close to 0.0 cm/wk from August 23 to December 20. The peak rate for the top pruned seedlings occurred in July and was computed as .92 cm/wk. The elongation rate for the top pruned seedlings rose May to June to July and then declined through the fall. Top pruned seedlings had the greatest growth rates of the three seedling treatments from July until October when all seedlings had generally set bud.

The different seedling canopies were associated with differences in rate of terminal elongation as diagramed in Figure 5. The greatest average rate measured was under the edge canopy during the period of July 11 to July 25. Seedlings grown under the edge canopy grew faster than seedlings under the other canopies until August. Seedlings grown under the closed canopy roughly paralleled those under the edge canopy but at a lower level. Seedlings grown in full sun initially grew at the lowest rates. For the period June 23 to July 11 these seedlings had a growth rate of .35 cm/wk compared to 0.71 and 0.86 cm/wk for edge and closed canopy grown seedlings. However, seedlings grown under the open canopy maintained growth for a longer period than those under the closed canopy and at a greater rate than the edge grown seedlings from August to October.

#### Shoot-to-Root Ratios

Seedling shoot-to-root ratios changed significantly during the summer. In April there were significant differences in shoot-to-root ratio between treatments, but not between canopies (Table 2). In December the differences between treatments were no longer signifi-

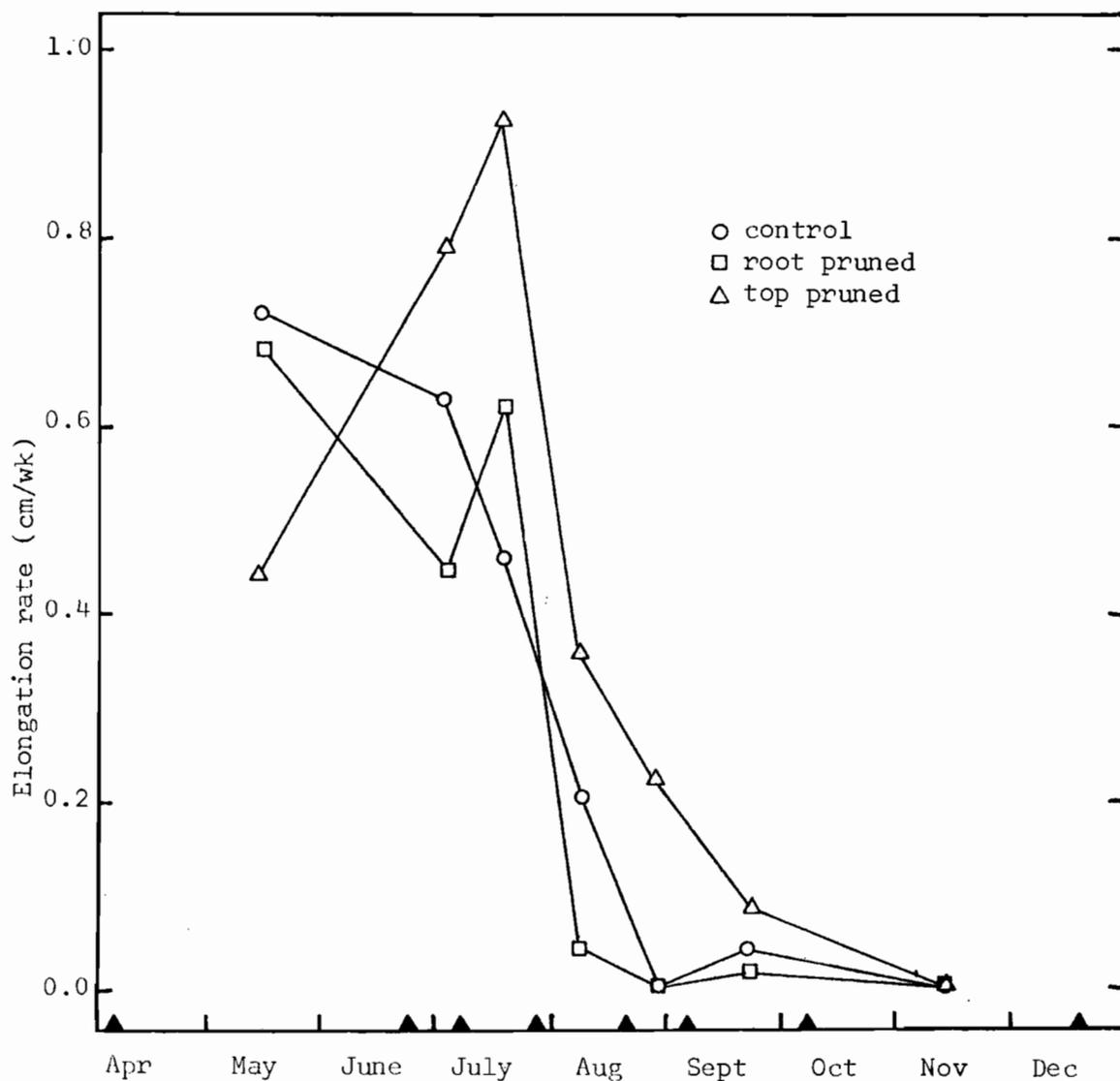


Figure 4. Treatment effects on shoot elongation rates of western hemlock seedlings grown in the field. Top pruned seedlings had a significantly greater mean shoot elongation rate than the control or root pruned seedlings as judged by "t" test at the 95% confidence level. The standard error for the difference of two treatment by date means was 0.124 cm. The solid triangles along the horizontal axis denote the dates of height measurement.

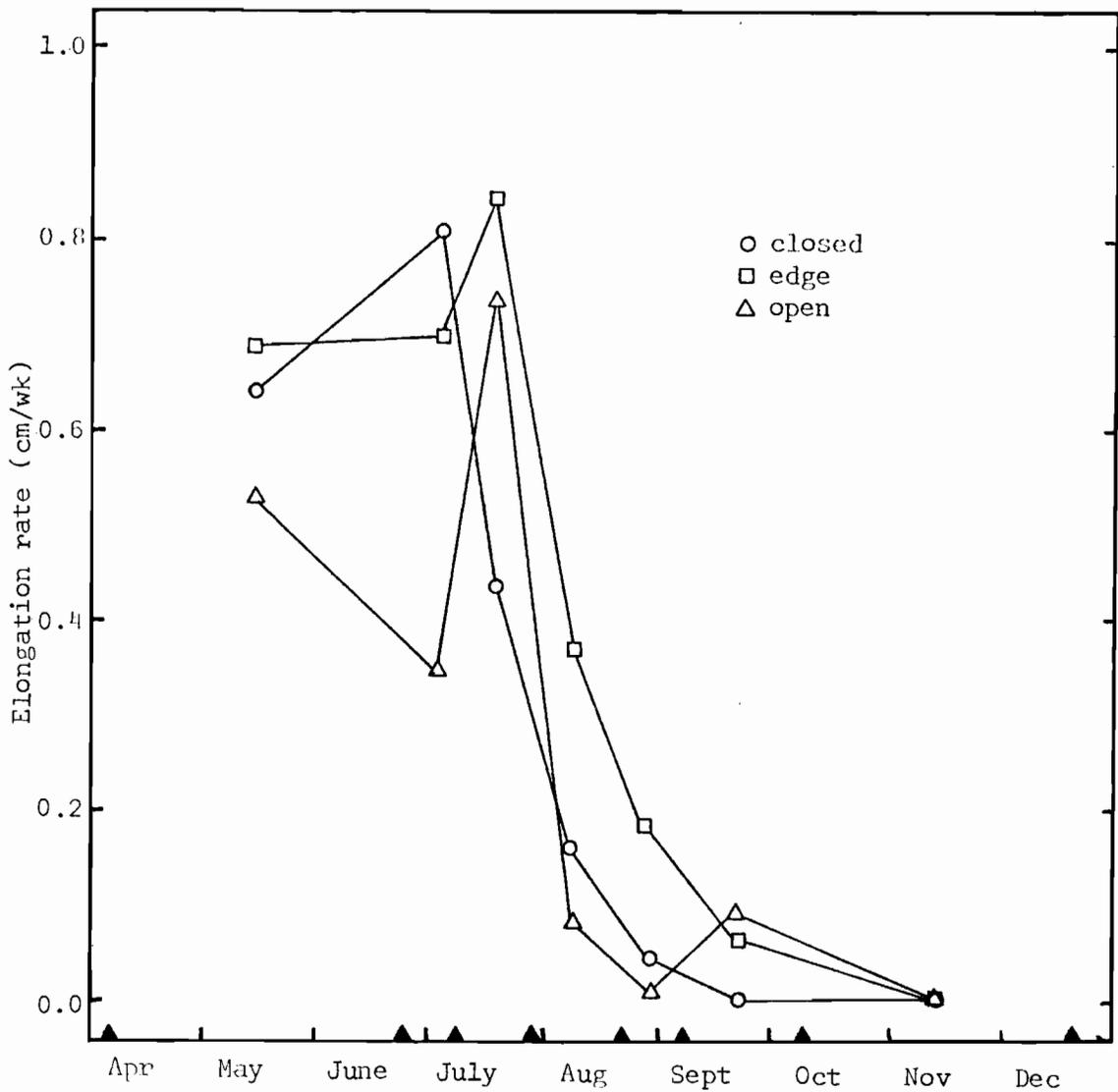


Figure 5. Canopy effects on shoot elongation rates of western hemlock seedlings grown in the field. Seedlings grown under the edge canopy had a significantly greater mean shoot elongation rate than seedlings in the open or closed canopy as determined by "t" tests at the 95% confidence level. The solid triangles along the horizontal axis denote the dates of height measurement.

Table 2

Shoot-to-Root Ratios of Western Hemlock  
Seedlings Prior to Summer Field Growth

Shoot-to-root ratio by pruning treatment	Control	Root Pruned	Top Pruned
	3.82 <sup>a</sup>	4.72 <sup>b</sup>	2.19 <sup>c</sup>
Shoot-to-root ratio by canopy type	Closed	Edge	Open
	3.45 <sup>a</sup>	3.82 <sup>a</sup>	3.46 <sup>a</sup>

Prior to summer field growth the significant variation in shoot-to-root ratio was associated with treatment. Mean shoot-to-root ratios for pruning treatment or canopy type were significantly different if the superscript does not contain the same letter.

cant (Figure 6). However, there were significant differences in seedling shoot-to-root ratio associated with the three canopies. Seedlings grown in the open had ratios averaging 0.95 while edge grown seedlings averaged 1.20. Seedlings grown under the closed canopy had the highest average shoot-to-root ratio at 2.99.

#### Shoot and Root Dry Weights

Shoot dry weights were significantly lower for top pruned seedlings than control or root pruned seedlings at the end of the experiment. No significant differences were associated with the different canopies. As shown in Figure 6, the mean root weight for top pruned seedlings was significantly lower than the control or root pruned seedlings. Means for the control, root, and top pruned seedlings were 2.2, 2.5, and 1.4 g, respectively. Canopy effects rested on lower root weights associated with seedlings grown under the closed canopy. Open grown seedlings had an average root weight of 2.76 g while edge grown seedlings averaged 2.71 g and seedlings grown under the closed canopy had an average root weight of 0.64 g.

#### Seedling Biomass

The total dry weight of shoot and root showed significant differences among treatments and canopies. Control seedlings had the highest average biomass at 5.28 g. Root pruned seedlings were lower, averaging 5.26 g. Top pruned seedlings were significantly lower than the other two at 2.82 g. Figure 6 also shows the canopy effects. Seedlings grown under the edge canopy had the greatest average total dry weight. Seedlings grown under the closed canopy had a significantly lower

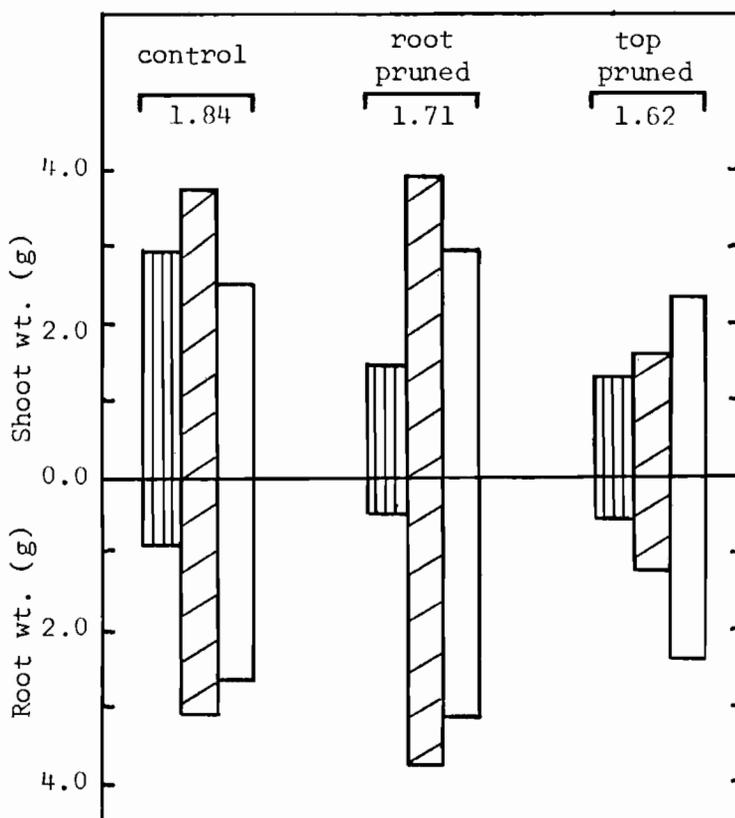


Figure 6. Shoot-to-root ratios of western hemlock seedlings grown in the field. Total weight of shoot and root of seedlings grown under the closed canopy ||| was significantly lower than the mean total weight of seedlings grown under the edge canopy // or in the open □. Top pruned seedlings had significantly smaller total weights than control or root pruned seedlings. Mean shoot-to-root ratios of the pruning treatments, bracketed numbers, were not significantly different. Shoot-to-root ratios were significantly higher and root weights significantly lower for seedlings grown under the closed canopy.

average biomass than those under the other canopies. The mean total dry weights of the open, edge, and closed canopy grown seedlings were 5.49, 5.80 and 2.52, respectively.

#### Needle Dry Weight

Analysis of the needle dry weights showed significant treatment differences. Control seedlings averaged 1.63 g, root pruned 1.32 g, and top pruned 0.80 g. Only the difference between control and top pruned seedlings was significant. The canopy differences were not significant.

#### Needle-to-Root Ratio

The ratio of needle dry weight to root dry weight showed significant differences associated with pruning treatment and seedling canopy (Table 3). Control seedlings had the greatest needle-to-root ratio with a mean of 1.01. Root pruned seedlings were intermediate averaging 0.90. Top pruned seedlings had a significantly lower ratio of .76 in comparison to the control seedlings. The open and edge canopies did not differ significantly, having mean ratios of 0.45 and 0.60, respectively. The closed canopy mean of 1.61, however was significantly higher than both of the other canopies. While a significant treatment by canopy interaction was present, this was primarily caused by an increasing difference of the top pruned seedlings from the control and root pruned seedlings in the closed canopy.

### Pruning Effects in Growth Chamber Environments

#### Bud Break

Treatment effects were apparent in the bud activity scores of the seedlings. Initially, as Figure 7 shows, control and root pruned

Table 3

Needle-to-Root Ratios of Western Hemlock  
Seedlings Grown Under Field Conditions

Pruning Treatment	Control	Root Pruned	Top Pruned	Mean
Canopy Type				
Closed	1.90	1.79	1.16	1.61 <sup>a</sup>
Edge	0.59	0.52	0.70	0.60 <sup>b</sup>
Open	0.54	0.37	0.44	0.45 <sup>b</sup>
Mean	1.01 <sup>a</sup>	0.90 <sup>ab</sup>	0.76 <sup>b</sup>	

Needle-to-root ratios of seedlings grown under the closed canopy were significantly higher than seedlings grown under the edge canopy or in the open. Mean ratios were significantly different at the 95% confidence level if the superscript does not contain the same letter.

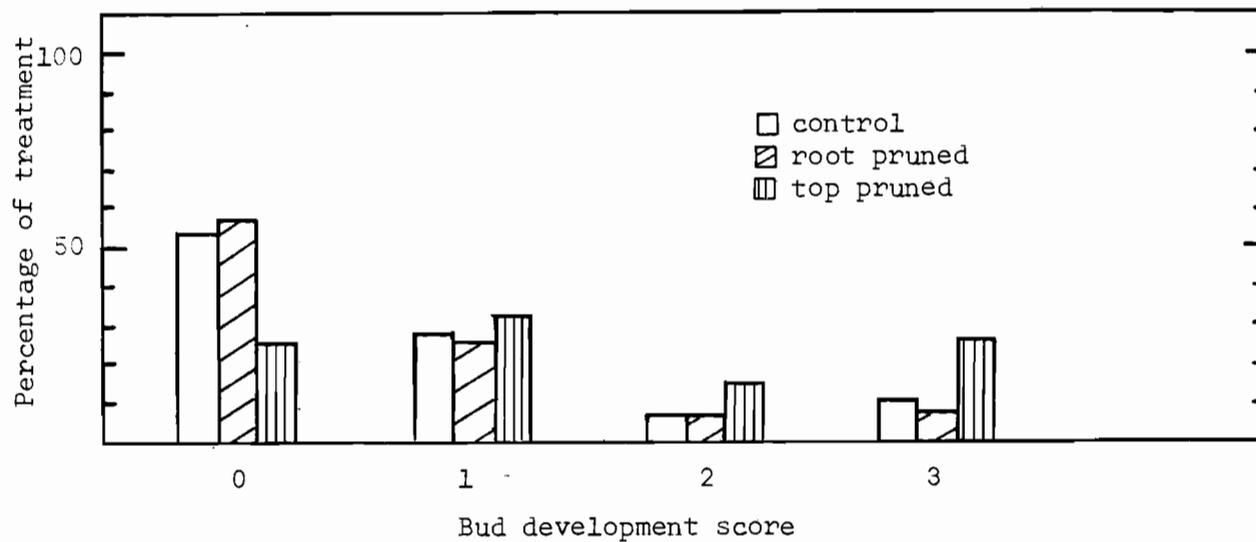


Figure 7. Score distribution of the western hemlock lateral buds on day 26. On this day the most developed lateral buds of the top pruned seedlings showed further development than the most developed lateral buds of control or root pruned seedlings.

seedlings had scores clustering around zero and one while the top pruned seedlings had scores more evenly spread from zero to three. Data in Figure 8 shows that after 25 more days in the growth chambers, all treatment classes scored the majority of their buds at level three. At the close of the experimental period, represented in Figure 9, control seedlings had 42% of the terminal scores as three and 49% scored four. Similarly, root pruned seedlings had 45% of their bud scored at three and 30% at four. In contrast, top pruned seedlings had 90% of their bud scores at three and only 9% at four.

#### Elongation Rate

Treatment differences were significant for mean elongation rates as indicated in Table 4. During the two months in which elongation rates were computed, control seedlings had an average rate of 0.52 cm/wk, and root pruned seedlings average 0.40 cm/wk. Top pruned seedlings were significantly higher than seedlings of the other two treatments with a mean rate of 0.82 cm/wk. Data in Table 4 indicates that the elongation rate was not constant during the experimental period. For the five growth periods, the greatest mean elongation rate of the surviving seedlings was during the first three weeks. The subsequent periods showed a steady decline.

The differences between treatments for elongation rate showed a significant interaction with growth period as indicated in Figure 10. Control and root pruned seedlings roughly paralleled each other, control seedlings always maintaining a greater rate. The general pattern for these two treatments was a moderate initial rate of about 0.65

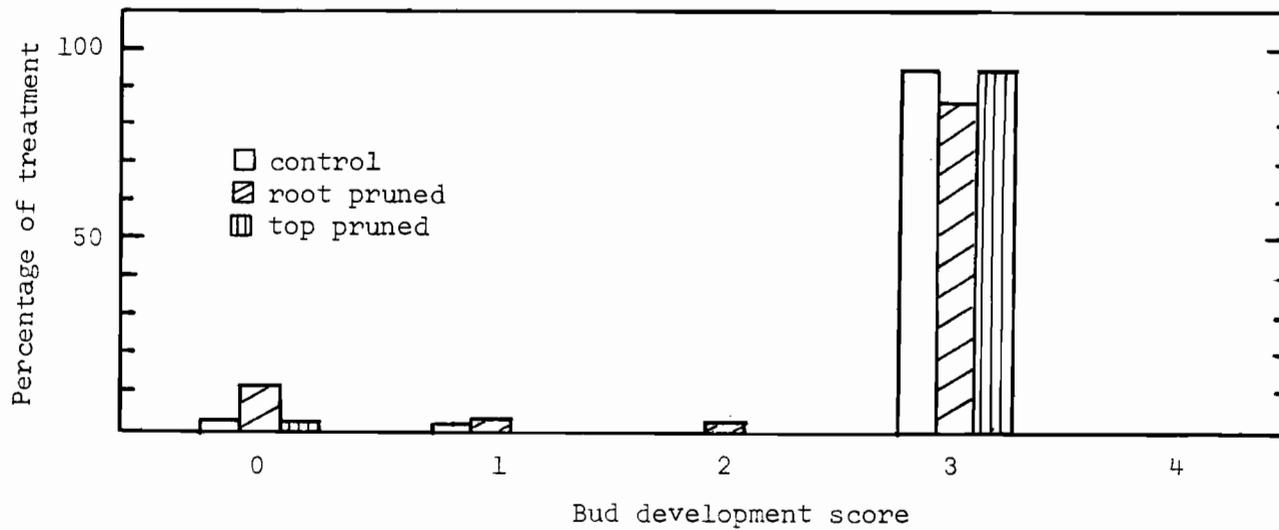


Figure 8. Score distribution of western hemlock lateral buds on day 51. Buds scores in all treatments indicated that shoots were generally elongating.

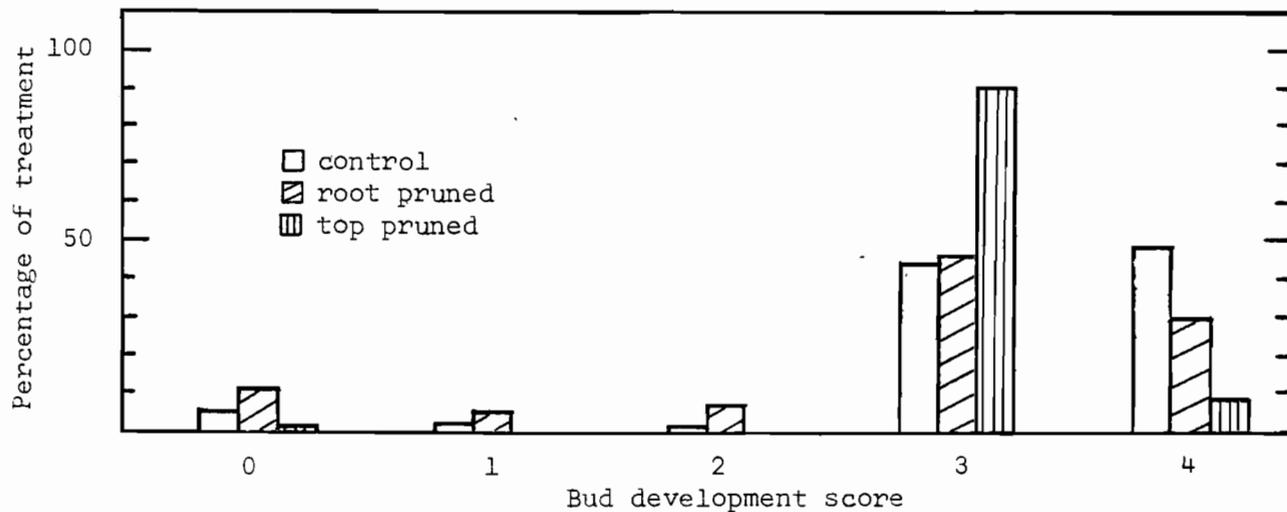


Figure 9. Score distribution of western hemlock terminal buds on day 96. The great majority of shoots of the top pruned seedlings appeared to be elongating while a large fraction of the control and root pruned seedlings had set terminal buds.

Table 4

Shoot Elongation Rates of Western Hemlock  
Seedlings Grown in Growth Chambers

Elongation (cm/wk) by pruning treatment		control	root pruned	top pruned	
		0.52 <sup>a</sup>	0.40 <sup>a</sup>	0.82 <sup>b</sup>	
Elongation (cm/wk) by growth period	day 40- day 51	day 51- day 61	day 61- day 79	day 79- day 89	day 89- day 96
	0.80 <sup>a</sup>	0.81 <sup>a</sup>	0.51 <sup>b</sup>	0.42 <sup>b</sup>	0.37 <sup>bc</sup>
Elongation (cm/wk) by growth chamber		15 C	20 C	25 C	
		0.80 <sup>a</sup>	0.40 <sup>b</sup>	0.82 <sup>b</sup>	

Mean shoot elongation was significantly greater for top pruned seedlings than control or root pruned seedlings. The first 21 days following bud break had significantly greater rates of shoot elongation than the following 35 days. The 15 C chamber had significantly greater rates of shoot elongation than the 20 C or 25 C chamber. Differences between elongation rates within one row are not significant if the superscript contains the same letter.

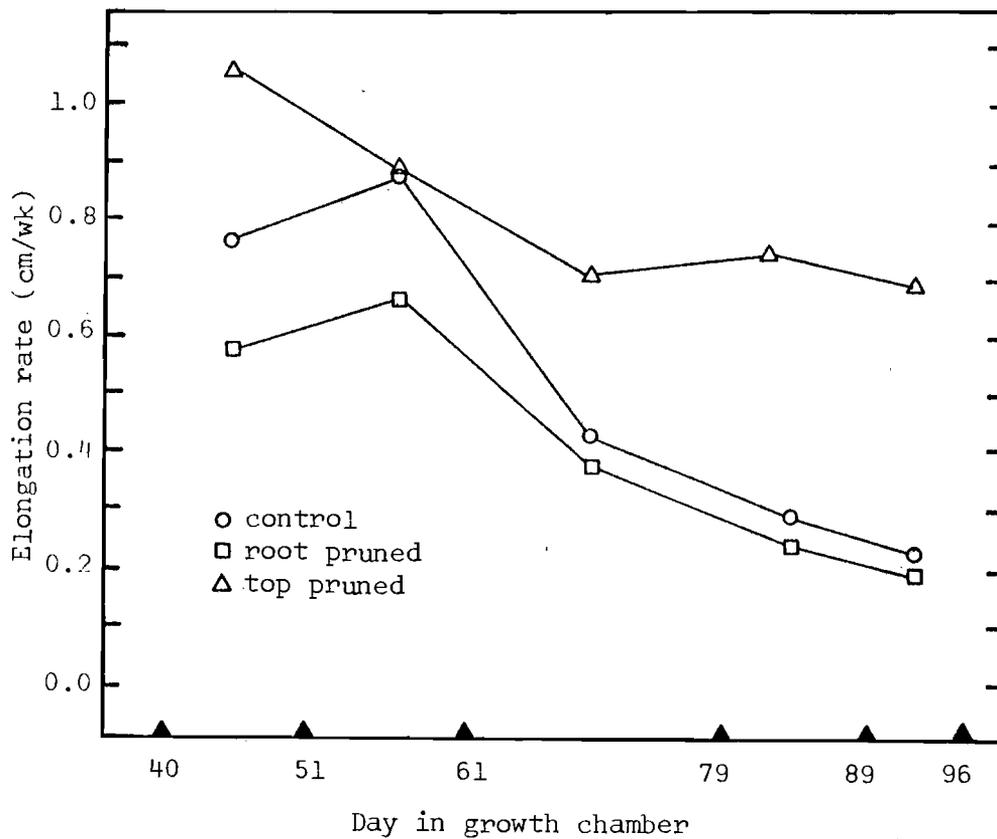


Figure 10. Pruning effects on shoot elongation rates of western hemlock seedlings grown in growth chambers. The mean shoot elongation rate of top pruned seedlings. The solid triangles on the horizontal axis indicate the dates of shoot measurement.

cm/wk increasing by 0.1 cm/wk during the second growth period and then declining during the last three growth periods to nearly 0.2 cm/wk. Top pruned seedlings showed a different pattern, however, Their peak elongation rate was during the initial growth period, averaging 1.05 cm/wk. This rate declined during the second and third growth periods then leveled out at approximately 0.70 cm/wk. This rate was well above control and root pruned seedlings.

#### Shoot and Root Dry Weights

The differences between treatments for shoot and root dry weights were significant. In both cases the significance rested with the lower weight of the top pruned trees. The differences between chambers among shoot weights were not significant. For root dry weight, the 15 C chamber was significantly lower (Figure 11). This lower weight was an average of 0.79 compared to 1.06 g and 1.24 g for the 25 C and 20 C chambers.

#### Shoot-to-Root Ratio

The treatment differences for this ratio were not significant at the conclusion of the growth trial. However, the differences between chambers were significant. The 15 C chamber had an average shoot-to-root ratio of 2.67. This ratio was significantly higher than the mean ratios in the 20 C and 25 C chambers which were 1.46 and 1.84, respectively. The difference between the 20 C and 25 C chambers was not significant.

#### Seedling Survival

Survival differences associated with treatments or chambers were not significant. A significant chamber by treatment interaction,

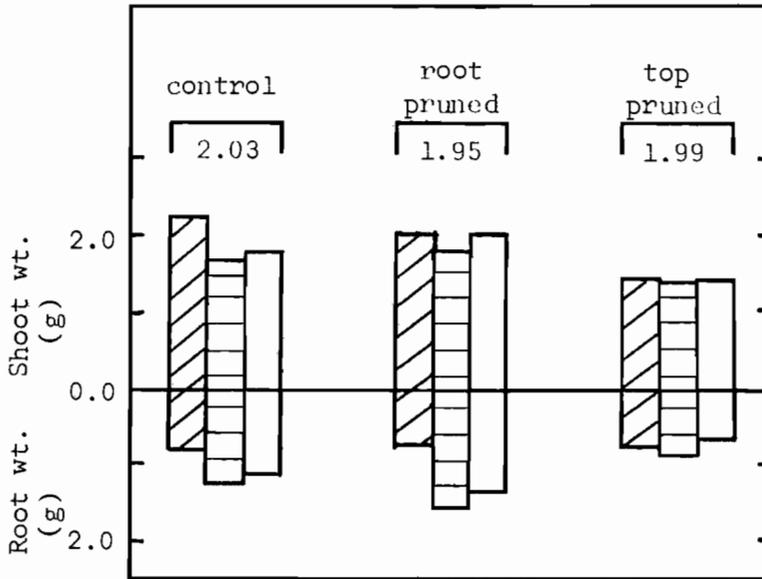


Figure 11. Shoot-to-root ratios of western hemlock seedlings grown in growth chambers. Mean root weights of seedlings grown in the 15 C chamber  $\square$  were significantly lower than the mean root weights of seedlings grown in the 20 C chamber  $\equiv$  or the 25 C chamber  $\square$ . Shoot weights were not significantly different between chambers. Top pruned seedlings had significantly lower shoot and root weights than control and root pruned seedlings. Mean shoot-to-root ratios of the pruning treatments, bracketed numbers, were not significantly different. The 15 C chamber had a significantly higher shoot-to-root ratio than the 20 C or 25 C chamber.

however, was shown (Table 5). This was brought on by a comparatively low survival rate of the top pruned seedlings in the 25 C chamber.

#### Root Disturbance and Storage Effects on Seedling PMS

##### PMS Variations of Western Hemlock Seedlings Grown in the Greenhouse

Analysis indicated an effect on plant moisture stress associated with the storage treatment (Table 6). Unstored seedlings had a significantly lower average PMS than the mean over all the stored seedlings. The average of the unstored seedlings was 11.7 bars versus 17.7 bars for the stored seedlings. The mean PMS values for the three storage periods were not significantly different. The differences associated with the root treatments were also significant. As seen in Table 6, control seedlings averaged 11.8 bars and were significantly lower than root abraded and root pruned seedlings which averaged 14.4 and 22.4 bars respectively.

The mean PMS readings for the four mornings examined showed significant differences (Table 6). Mornings 1, 3, and 15 were not significantly different from each other. However, morning 22 was significantly lower. A significant treatment by morning interaction was present (Figure 12). This was a function of a significantly lower mean PMS reading on the first morning after planting for the abraded seedling than would be anticipated for seedlings having a mean PMS equal to the average of the abraded seedlings.

New root percent, flushing percent, and survival all showed significant differences associated with root treatment, but not with

Table 5  
 Survival of Western Hemlock Seedlings  
 Grown in the Growth Chamber (%).

Treatment	Chamber Temperature			Mean
	15 C	20 C	25 C	
Control	81.8	77.5	100	86.4 <sup>a</sup>
Root Pruned	75.6	75.5	92.5	81.2 <sup>a</sup>
Top Pruned	94.7	79.4	37.5	70.5 <sup>a</sup>

Survival differences were nonsignificant between pruning treatment means and between chamber means.

Table 6

Plant Moisture Stress Levels of  
Western Hemlock and Douglas-fir Seedlings  
Grown in the Greenhouse

PMS (bars) by treatment	Control	Root Abraded	Root Pruned	
Western Hemlock	11.8 <sup>a</sup>	14.4 <sup>b</sup>	22.4 <sup>c</sup>	
Douglas-fir	8.8 <sup>a</sup>	11.5 <sup>b</sup>	12.5 <sup>b</sup>	
PMS (bars) by mornings from planting				
	1	3	15	22
Western Hemlock	16.3 <sup>a</sup>	16.9 <sup>a</sup>	17.2 <sup>a</sup>	14.5 <sup>b</sup>
Douglas-fir	9.3 <sup>a</sup>	10.8 <sup>b</sup>	12.4 <sup>c</sup>	11.2 <sup>b</sup>
PMS (bars) by months of cold storage				
	0	1	2	3
Western Hemlock	11.7 <sup>a</sup>	18.3 <sup>b</sup>	18.8 <sup>b</sup>	16.1 <sup>ab</sup>
Douglas-fir	9.3 <sup>a</sup>	10.8 <sup>a</sup>	12.4 <sup>a</sup>	11.2 <sup>a</sup>

Mean values in any one row were not significantly different at the 95% confidence level if the superscripts contain an identical letter.

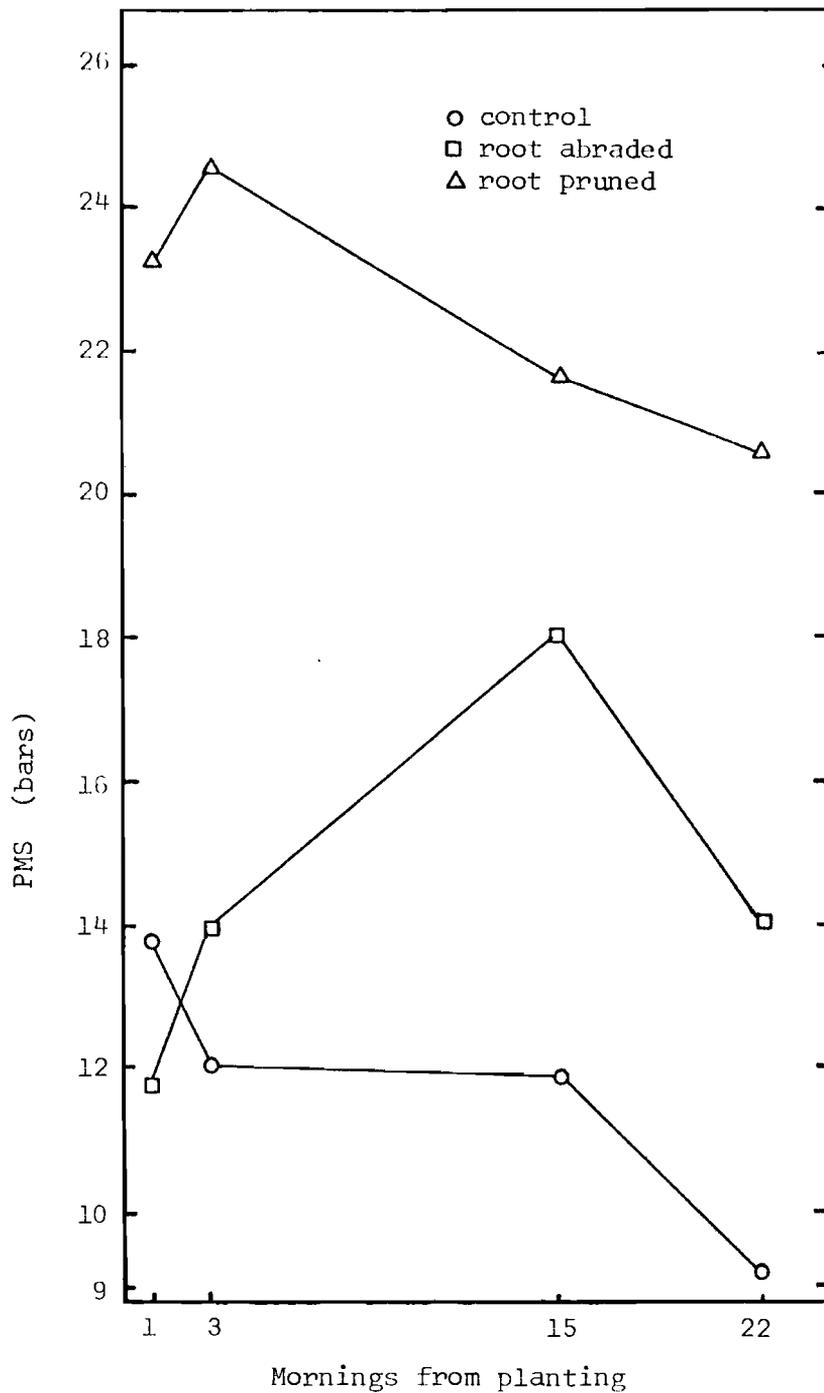


Figure 12. Root treatment effects on daily variation of plant moisture stress (PMS) of western hemlock seedlings planted in the greenhouse. This means of the control and abraded seedlings were unexpected similar on morning one considering the overall means. The standard error for the difference between two root treatment by day means was 3.21 bars.

storage treatment. Analysis of new root percentages showed the control seedlings had significantly greater scores than seedlings of either of the two root treatments as seen in Table 7, the difference between abraded and pruned seedlings was not significant.

Control seedlings also showed significantly higher percent flushing than seedlings of the two root disturbed treatments (Table 7). Root abraded seedlings were significantly higher in flushing percent than root pruned seedlings.

In addition, control seedlings showed significantly greater survival than the seedlings of the two root disturbed treatments (Table 7). Root abraded seedlings survived at significantly greater percentages than root pruned seedlings.

#### PMS Variations of Douglas-fir Seedlings Grown in the Greenhouse

The differences between mean PMS readings for the different periods of storage were not significant (Table 6). However, the mean reading for unstored seedlings was lower than those of stored seedlings.

There were significant differences associated with the root treatments. As shown in Table 6, control seedlings were significantly lower on the average than abraded or root pruned seedlings. The difference between abraded and root pruned seedlings was not significant.

Significant differences were associated with the four mornings that PMS readings were examined. Over all treatments the first morning after planting was significantly lower than mornings 3, 15 and 22 (Table 6). Morning 15 was significantly higher than readings on the other mornings.

Table 7

Growth Measures of Western Hemlock and Douglas-fir  
Seedlings Grown in the Greenhouse

Root treatment	Control	Root Abraded	Root Pruned
Western Hemlock	29.2 <sup>a</sup>	9.9 <sup>b</sup>	13.6 <sup>b</sup>
New roots (%)	29.2 <sup>a</sup>	9.9 <sup>b</sup>	13.6 <sup>b</sup>
Flushing (%)	87.3 <sup>a</sup>	55.0 <sup>b</sup>	24.7 <sup>c</sup>
Survival (%)	94.8 <sup>a</sup>	73.5 <sup>b</sup>	53.5 <sup>c</sup>
Douglas-fir			
New roots (%)	20.8 <sup>a</sup>	13.6 <sup>b</sup>	26.1 <sup>a</sup>
Flushing (%)	64.6 <sup>a</sup>	46.9 <sup>b</sup>	43.5 <sup>b</sup>
Survival (%)	98.8 <sup>a</sup>	94.1 <sup>b</sup>	93.7 <sup>b</sup>

Control seedlings generally had a greater proportion of the root mass as new roots, greater flushing, and greater survival than root disturbed seedlings. Differences between mean values for a given species and growth measure were statistically significant at the 95% confidence level if the superscript does not contain an identical letter.

Analysis indicated that a significant treatment by morning interaction existed (Figure 13). Control seedlings showed a relatively lower mean PMS reading on morning 22 than would be expected considering their mean PMS over all days. Root pruned seedlings showed a relatively high mean PMS reading on morning 1 considering their mean PMS.

For the Douglas-fir seedlings new root percent, flushing percent, and survival all showed significant differences associated with root treatment, but not with storage treatment. Control seedlings were significantly higher in their percentage of new roots than the root abraded seedlings. Controls were not significantly different from root pruned seedlings (Table 7). Control seedlings were significantly higher in percent flushing than the root treated seedlings (Table 7). The difference between the mean percent flushing for root abraded and root pruned seedlings was not significant. Seedling survival was also significantly greater for control seedlings than for the root treated seedlings (Table 7). The difference between the two root treatments was not significant.

#### PMS Patterns of Western Hemlock Seedlings Planted in a Cold Frame

Western hemlock seedlings showed no effect on plant moisture stress associated with storage period. However, differences in PMS associated with root treatment were significant (Table 8). Control seedlings were significantly lower than the root disturbed seedlings. Root abraded seedlings were significantly lower in their mean PMS reading than root pruned seedlings.

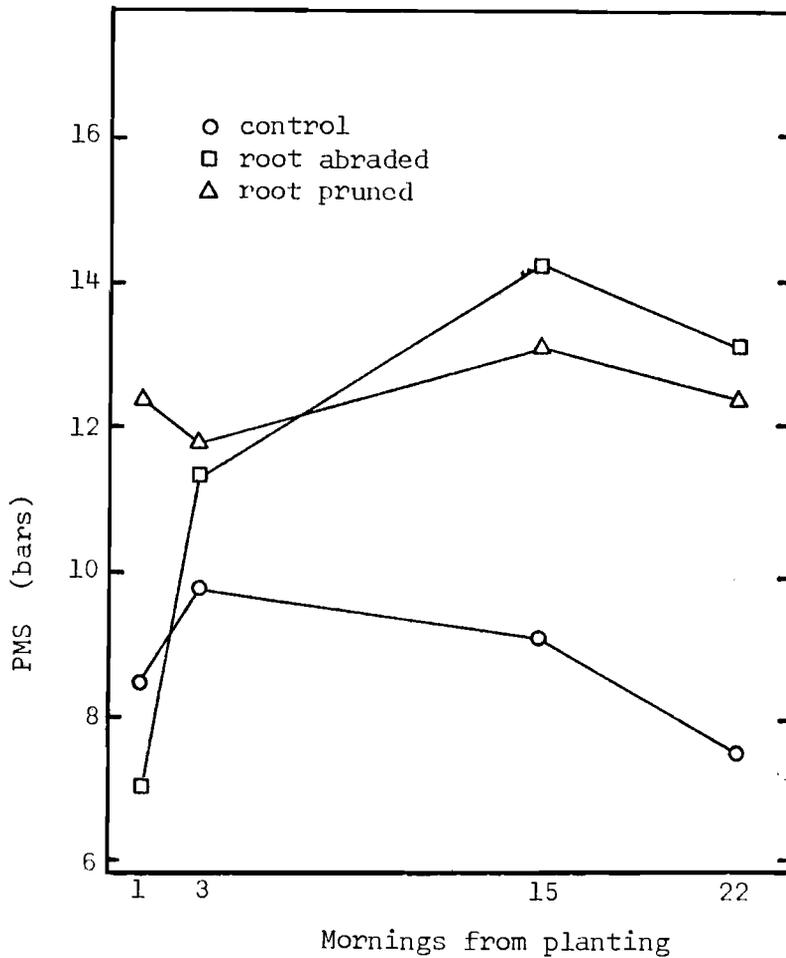


Figure 13. Root treatment effects on daily variation of plant moisture stress (PMS) of Douglas-fir seedlings planted in the greenhouse. Considering the mean PMS level for the abraded seedlings, the level on morning one was unexpectedly low. The standard error for the difference between two root treatment by day means was 1.88 bars.

Table 8

Levels of Plant Moisture Stress (PMS) and Terminal  
Bud Break of Western Hemlock and Douglas-fir  
Seedlings Grown in Shaded Cold Frames

Root treatment	Control	Root Abraded	Root Pruned
PMS (bars)			
Western Hemlock	5.5 <sup>a</sup>	7.8 <sup>b</sup>	12.1 <sup>c</sup>
Douglas-fir	5.2 <sup>a</sup>	6.6 <sup>b</sup>	7.6 <sup>c</sup>
Bud Break (%)			
Western Hemlock	97.8 <sup>a</sup>	72.8 <sup>b</sup>	42.7 <sup>c</sup>
Douglas-fir	34.7 <sup>a</sup>	11.5 <sup>a</sup>	8.4 <sup>a</sup>

Control seedling had more favorable PMS levels and bud burst percentages than the root disturbed seedlings. Mean values of PMS or bud break for a given species were significantly different if the superscripts do not contain an identical letter.

The analysis of bud activity for seedlings lifted in November or January and stored for 0, 1, or 2 months showed no significant differences between storage periods. However, differences associated with root treatment were significant. As seen in Table 8, control seedlings flushed at a significantly greater mean percentage than the root disturbed seedlings. Root abraded seedlings flushed at significantly greater rates than the root pruned seedlings.

#### PMS Patterns of Douglas-fir Seedlings Planted in a Cold Frame

For the Douglas-fir seedlings there were no significant differences associated with the storage treatments for PMS levels. The differences associated with root treatments were significant, however, As seen in Table 8, control seedlings were significantly lower in PMS than the root disturbed seedlings. Root abraded seedlings were significantly lower in their PMS readings than root pruned seedlings.

Bud break scores of the Douglas-fir seedlings that were planted in the cold frame did not show significant differences associated with storage treatment. The differences associated with root treatment were also nonsignificant (Table 8).

#### Seasonal and Root Disturbance Effects on Cytokinin Level

The activity levels of the zeatin and zeatin riboside fractions were closely linked. As Figure 14 indicates, only the control trees harvested on April 12 showed any obvious activity in the zeatin or zeatin riboside fractions. The samples taken in the months of August through early April had activity levels in the two cytokinin fractions

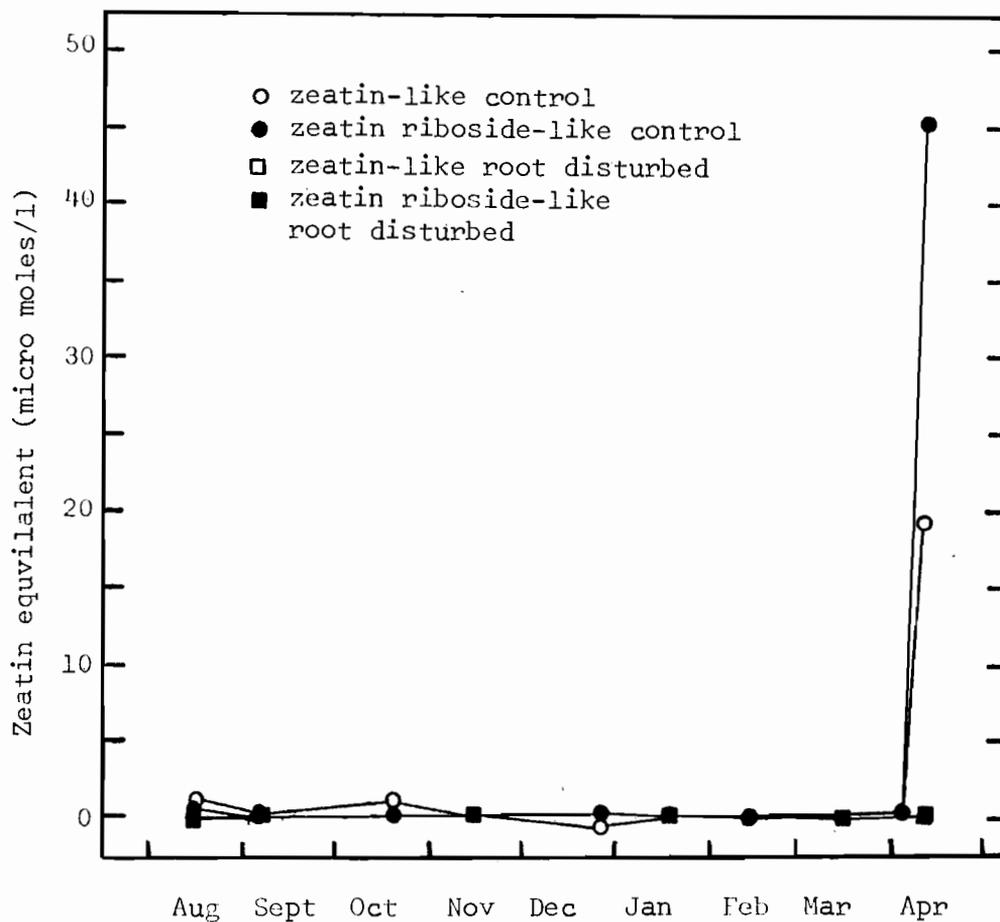


Figure 14. Seasonal content of zeatin-like and zeatin riboside-like compounds in the zylem sap of western hemlock samplings. The only major activity was found in samples taken April 14. High activity was not found in any of the root disturbed samples.

that were negligible. The xylem sap of the root disturbed trees never showed any great activity on any sample date including April 12.

## DISCUSSION

## In-bed Root Disturbance

Shoot dry weight was modified little by root disturbance. All seedlings showed a general weight increase until November followed by a plateau in mid winter and then a rise in April. Root weights of the control and July pruned seedlings also were not greatly different. The September treated stock, however, had consistently lower root weights. The real effect on shoot and root dynamics was found in the graph of shoot-to-root ratio. This graph showed two patterns. First, the unpruned controls had a seasonal pattern that would be expected considering the normal patterns of shoot and root development. Shoot-to-root ratios in spring and early summer were significantly higher as leader and laterals expanded, and then decreased in fall as the second peak in root growth occurred. Second, there was a pattern of shoot-to-root ratio recovery. Following pruning, the shoot-to-root ratios of treated seedlings were significantly higher than controls. However, the time required to match controls was faster in July than September. This difference in recovery rate is reasonable as during the summer months light, temperature, and with irrigation, PMS levels would be favorable for growth. However, metabolic activities would be slowed with the cooler weather of autumn. An interesting point is that all the seedlings tended to reach the same shoot-to-root ratio, despite pruning or differences in time of pruning.

## Pruning Effects Under Field Canopies

In looking at differences in elongation rate between root pruned, top pruned, and control seedlings there are several points of interest. First, for the unpruned seedlings elongation rates followed the expected pattern of greatest rates in the spring, declining in the summer and reducing to zero as buds set. Secondly, it was notable that root pruned seedlings generally had lower elongation rates than controls. For top pruned seedlings the apparent peak in elongation rate was delayed until July. Appreciable elongation rates for top pruned seedlings extended further than other treatments into the fall.

There is, however, a difficulty in interpreting the elongation rates for the first growth period. The elongation rate is an average over 77 days. During the early portion of the period there was presumably little or no growth and thus later elongation rates must have been higher than the reported figures. A further problem is encountered with the top pruned seedlings. As elongation was computed from previous height, the actual elongation of the lateral branch would have been greater than computed in the cases where the bud of the dominant branch was below the pruning height. Growth must have occurred to simply achieve initial height.

Differences in elongation rate between the canopies are also interesting. A contrast is immediately apparent between open grown seedlings and seedlings grown under the closed canopy. Elongation rates in the open are more erratic than the relatively smooth curve found under the closed canopy. This variability found in the open

is attributable to the lack of a vegetative canopy which functioned to moderate extremes of temperature and transpiration demand. Thus open grown seedlings suffered extreme temperature, and tip mortality in August, which reduced height increment to zero. Lack of moisture and light took their toll as seedlings grown in the open or under the closed canopy had significantly lower average elongation rates than those under the edge canopy.

The net results of the shoot and root growth dynamics are apparent in the bar graphs of the shoot-to-root ratio. Initially in April, the significant variability was associated with the pruning treatment. In contrast, December dry weights showed no significant differences associated with pruning treatment. As in the earlier experiment, all seedling treatments within a particular canopy produced uniform shoot-to-root ratios. However, shoot-to-root ratios did vary between canopies. Considering the light, temperature and associated moisture demands of the different canopies the relative ratios are reasonable. The lowest growth of shoot relative to root was in the open where transpiration demands were probably the greatest. Similarly, highest relative growth of the shoot was found under the closed canopy where moisture demands are probably the least. Brix (1970) also found that western hemlock seedlings had increasing shoot-to-root ratios as light intensity decreased from full sun to ten percent. Like Brix, this study found the total dry matter production for control seedlings was greatest under intermediate light conditions, where neither moisture stress nor lack of light severely limited growth.

## Pruning Effects in Growth Chamber Environments

The effects of pruning treatments were apparent in the bud burst scores of the seedlings. As top pruning removed the terminal bud from the seedling, a comparison of lateral buds is more appropriate. An acceleration in bud burst is apparent on day 26 for the top pruned seedlings. While over 50% of the control and root pruned seedlings appeared inactive and under 20% had burst bud, the top pruned seedling had only 25% with buds appearing inactive and 50% had broken. The increased activity of the lateral buds of the top pruned seedlings can be interpreted as a hormonal effect. Suppression of lateral bud development or apical dominance is effected by production of auxin in the shoot apex and transport down the stem. Removal of the terminal shoot allows growth of the lateral bud. Apparently the shoot exerts an influence even during a simulated spring bud break.

Later bud scores on day 51, showed not only increased development, but also a possible root pruning effect. Control and top pruned seedlings had only 4 and 3% of their seedlings with lateral buds inactive, but root pruned seedlings had three times that proportion. A greater percentage of inactive buds for the root pruned trees indicates slower shoot development. This is reasonable if the seedling is expending relatively more energy in root production.

Terminal buds for control and root pruned seedlings showed the same pattern as lateral buds though development was slower. Activities of terminal buds were scored for all treatments at the close of the experiment on day 91. For top pruned seedlings the bud stage of the

tallest branch was scored as the terminal. Interestingly, the increased growth propensity of the top pruned seedlings was still evident. Ninety percent of the top pruned seedlings were apparently elongating, while control and root pruned seedlings were about evenly split between expansion and bud set phases.

Considering the patterns of the bud scores, the results of the analysis of the elongation rates are not surprising. Top pruned seedlings had significantly greater elongation rates than the control and root pruned seedlings. Unlike the control and root pruned seedlings, those top pruned showed only a minor decline in average elongation rate towards the end of the experiment. Although the average difference in rates of elongation for root pruned and control seedlings was not significant, the root pruned seedlings were consistently lower during each growth period. This result suggests that root pruning lowered growth potential of the shoot.

Dry weight measures of these growth chamber grown seedlings further parallel the field study. Top pruned trees had smaller weights though the ratios of shoot-to-root did not differ significantly between any of the treatments in a given growth chamber. However, significant differences were associated with the three chambers. Again, the highest shoot-to-root ratios were found in the presumably lowest moisture stress condition, the 15 C chamber. While the shoot-to-root ratios of the 20 C and 25 C chambers were not significantly different, it is puzzling that the ratios were higher where the temperature was lower. Possibly the expected increase in evaporative demand associated

with temperature was counteracted by an apparent lower relative humidity in the 25 C chamber. Seedlings in the 20 C chamber did appear to require more water than the 15 C or 25 C chambers to maintain moist soil.

#### Root Disturbance and Storage Effects on Seedling PMS

Root disturbance had a significant effect on plant moisture stress following planting. For hemlock seedlings grown in the greenhouse, the average level of stress was higher for the two disturbance treatments than controls. While pruning caused the largest increase in PMS levels, the abraded seedlings provided the most intriguing pattern of stress over time. The morning after planting PMS readings were below the level of controls. Although on subsequent mornings the PMS levels of the abraded seedlings were above controls. While root damage would be expected to increase PMS levels relative to controls, the lower average PMS for abraded seedlings on the first morning provided a puzzle. It is suggested that the abrasion resulted in an increased level of abscisic acid (ABA) or its precursor in the roots, and as the water stored in the roots were transpired ABA levels increased in the needles leading to reduced stomatal aperture and water conservation. Therefore, PMS levels of the abraded seedlings could be lower relative to controls. Presumably, however, transpiration losses during the first full two days were not recouped with the damaged root system as well as the controls. Therefore the PMS levels were comparatively higher. In all three root treatments there is a decline in PMS between mornings 15 and 22, indicating an increased efficiency and recovery of the root system. The

consequences of increased PMS levels were apparent in terms of the formation of new roots, bud flushing, and survival. In all three categories, control seedlings were higher. Pruned seedlings, which had the highest average PMS, had the lowest percentage of surviving seedlings flush, as well as the lowest survival rate.

For Douglas-fir seedlings grown in the greenhouse the same pattern was found among the root treatments for the PMS levels. The most striking difference between the two species was the difference in general level of PMS. The hemlock seedlings had consistently higher average levels than the Douglas-fir. Further, an individual morning's data showed the greatest variability with the hemlock seedlings. This variability difference may be an effect of the size difference between the two species or possibly it indicates a deeper dormancy on the part of the Douglas-fir.

Similar effects were found for the two species in percent flushing and survival. The general level of seedling survival was considerably higher in the less stressed Douglas-fir however. The percentage of new roots in the Douglas-fir did not show any decrease for pruned seedlings. Presumably, the less severe PMS levels combined with a smaller root system following pruning allowed the percentage, if not the mass, of new roots to equal controls.

Storage effects on seedlings grown in the greenhouse were statistically significant only for the western hemlock. Apparently any long term storage of one to three months is detrimental for that species in terms of keeping PMS low after planting. For the Douglas-

fir seedlings there was an apparent storage effect in the November lifted stock. However, the uniformity of PMS level associated with storage treatment in January and March lifted seedlings prevented identification of a trend (Table 9). A difference in the storage character of early and late lifted Douglas-fir seedlings has been noted earlier (Hermann et. al. 1972).

Disturbance effects for hemlock and Douglas-fir seedlings grown in the cold frame paralleled those in the greenhouse for average PMS and percent flushing. Again Douglas-fir had lower average PMS levels and less variability. Although PMS levels in the cold frame were generally lower, it is again apparent that the minimization of root disturbance is advisable to reduce harmful impacts on survival and growth.

#### Seasonal and Root Disturbance Effects on Cytokinin Level

The pattern of cytokinin content of the xylem sap of young western hemlock trees provided an interesting basis for speculation. The lack of any detectable levels of cytokinin activity during the dormant season is consistent with other reports noted above (Alvim et. al. 1976, Domanski and Kozlowski 1968). Further, the occurrence of zeatin-like and zeatin riboside-like compounds in the sap paralleled identifications in other conifers. In contrast to several reports of broad-leaved species, (Hewett and Wareing 1973, Brown and Dumbroff 1974, Taylor and Dumbroff 1975) the increase in cytokinin activity did not coincide with the beginning of bud swell. Swollen buds were noticed in March, yet no activity was detected. Activity was first

Table 9

Lifting Date and Storage Effects on Plant  
Moisture Stress (PMS) of Douglas-fir  
Seedlings Grown in the Greenhouse

Month of lifting		PMS (bars)	Range (bars)
November	Months of Storage	0	
		1	7.93
		2	12.46
		3	16.18
			14.76
January	Months of Storage	0	
		1	9.24
		2	8.87
		3	11.41
			2.54
March	Months of Storage	0	
		1	10.46
		2	11.19
		3	9.58
			2.67
			8.52

Although the mean PMS values over all lifting dates showed no significant effect of storage, there may be an effect in November lifted stock. The range in PMS levels was greatest in seedlings lifted then.

found in mid April, well after initial bud swell. However, Owens and Molder (1973) reported that mid April was the period that the bud apices began to initiate axillary buds. It remains for future investigation whether the coincidence in time of cytokinin activity in the xylem sap and axillary bud initiation is real and significant. However, application of a synthetic cytokinin was found to promote axillary bud development in Douglas-fir seedlings (Zaerr personal communication).

The absence of any detected cytokinin activity in the root disturbed trees lends some basis to the notion that cytokinins are one of the messengers integrating shoot and root growth. Judging from previously described experiments, root disturbance shifts growth toward regeneration of the lost portion, generally at the expense of the shoot. If increased levels of cytokinins indicate increased development of the shoot, a lack of cytokinin activity would seem to indicate a slowdown in shoot development as would be necessary for the root disturbed trees in the process of adjusting shoot and root balance.

## CONCLUDING THOUGHTS

In looking at the series of experiments as a whole, several conclusions become apparent. The unifying finding of the first three experiments on growth dynamics of the seedlings was that pruning had no season-long effect on the shoot-to-root ratio. The relative balance of shoot and root dry weight appeared to be a function of the seedling environment and was unaffected by pruning treatment. Additionally, the recovery of the shoot-to-root ratio from in-bed disturbance was found to be faster in July than in September. Pruning experiments showed that top pruned seedlings broke bud earlier and had greater average elongation rates during the growth period than either root pruned or control seedlings. Root pruned seedlings had apparent decreases in shoot growth potential as they were slower to break bud and set bud earlier than control or top pruned seedlings.

Turning to internal parameters of seedling growth, it was found that root disturbance has an effect of raising PMS levels of planted seedlings for at least the first month of growth. Storage of western hemlock seedlings for periods of one month or longer increased PMS levels compared to seedlings stored one day only. This detrimental effect of storage may also occur in November lifted Douglas-fir seedlings. Higher and more variable PMS levels were found in hemlock seedlings relative to the Douglas-fir seedlings. Smaller size of the hemlock or physiological differences from the Douglas-fir seedlings may account for the differences in PMS.

Examination of xylem sap extracted from young western hemlock trees found negligible cytokinin activity from August to early April. In mid April, after the beginning of bud swell, zeatin-like and zeatin riboside-like substances were found. Root disturbance appeared to have decreased cytokinin levels in the xylem sap.

These results are of importance to those who deal with seedlings. For the regeneration forester it is important to note that not all seedlings will be successfully planted on all sites. The universal hemlock seedling does not exist because planting sites are not uniform. There is even a caution for harvesting stands and then presuming the development of seedlings already present. The seedling which is growing under a dense canopy has a high shoot-to-root ratio, and will not be adjusted to the rapid changes in light, temperature, and moisture stress levels. If root area does not increase sufficiently shoot or needle mortality appears inevitable.

For the nursery operator these findings suggest that cultural practices should be implemented to produce seedlings of several shoot-to-root ratios in order to fit the variety of planting sites. Further a continued effort is necessary in reducing root damage in order that PMS levels are kept as low as possible.

For the research scientist there are further questions to be resolved. It was found that some pruning promoted root growth, and yet severe pruning increased PMS and mortality. Is there some middle ground where some degree of root pruning can be exploited to enhance seedling establishment? Does this middle ground of pruning intensity alter seasonally as does root regenerating capacity. Hormonal aspects

are also to be considered. To what degree are abscisic acid levels in the needles affected by root abrasion? What are the **auxin fluxes** in shoots of top-pruned seedlings? For western hemlock, the question of cytokinin content of xylem sap relative to bud burst and later developmental stages of the shoot is still open.

## BIBLIOGRAPHY

- Alvim, R., E. W. Hewett, P. F. Saunders. 1976. Seasonal variation in hormone content of willow I; changes in ABA content and cytokinin activity in the xylem sap. *Plant Physiol.* 57:474-476.
- Brix, H. 1962. The effect of water stress on the rates of photosynthesis and respiration in tomato plants and loblolly pine seedlings. *Physiol. Plant.* 15:10-20.
- \_\_\_\_\_. 1970. Effect of light intensity on growth of western hemlock and Douglas-fir seedlings. *Bimonthly Res. Note, Department of Fisheries and Forestry.* 26:34-35.
- Brouwer, R. 1962. Distribution of dry matter in the plant. *Neth. J. Agric. Sci.* 10:361-367.
- Brown, C. J. 1977. Patterns of growth and seasonal changes in the concentration of abscisic acid and indoleacetic acid in roots of western hemlock. MS thesis, Oregon State University, Corvallis, Oregon. p. 97.
- Brown, D. C. and E. B. Dumbroff. 1974. Root growth inhibitors and cytokinin-like activity in sugar maple seedlings during the dormant season. *Plant Physiol. Suppl.* p. 71.
- Carlson, L. W. 1977. The effect of defoliation on conifer seedling root initiation. *Bimonthly Res. Notes, Northern Forest Res. Centre.* 33:1.
- Cleary, B. D. 1970. The role of moisture stress and temperature. *in* The growth of seedlings in the regeneration of ponderosa pine. K. K. Hermann ed. Oregon State University, Corvallis, Oregon. pp. 64-68.
- \_\_\_\_\_. and R. Greaves. 1977. Determining planting stock needs. *in* Tree planting in the inland northwest. D. M. Baumgartner and R. Boyd ed. Washington State University Cooperative Extension Service. 22 unnumbered pages.
- \_\_\_\_\_. and R. D. Greaves and P. W. Owston. 1978. Seedlings. *in* Regenerating Oregon's Forests. B. D. Cleary, R. D. Greaves and R. K. Hermann eds. Oregon State University Extension Service. pp. 63-97.
- Domanski, R. and T. T. Kozlowski. 1968. Variations in kinetin-like activity in buds of Betula and Populus during release from dormancy. *Can. J. Bot.* 46:397-403.
- van Dorsser, J. C. and D. A. Rook. 1972. Conditioning of radiata pine seedlings by undercutting and wrenching: description of methods equipment and seedling response. *New Zeal. J. For.* 17:61-73.

- Dykstra, G. F. 1974. Undercutting depth may affect root regeneration of lodgepole pine seedlings. *Tree Planters Notes* 25:21-22.
- Edgren, J. W. 1974. Wrenching recent developments in an old technique. in *Proceedings of Western Forestry Nursery Council Meeting*. Portland, Oregon.
- Eis, S. 1968. Lateral root pruning - a promising forest nursery practice. *For. Chron.* 44:12-13.
- \_\_\_\_\_ and J. R. Long. 1972. Lateral root pruning of sitka spruce and western hemlock seedlings. *Can. J. For Res.* 2:223-227.
- \_\_\_\_\_. 1973. Root pruning in the nursery. *Tree Planters Notes* 24:20-22.
- Fletcher, R. A. and D. Mc Cullagh. 1971. Cytokinins induce chlorophyll formation in cucumber cotyledons. *Planta* 101:88-90.
- Greaves, R. D., R. K. Hermann and B. D. Clearly. 1978. Ecological Principles. in *Regenerating Oregon's Forest*. B.D. Clearly, R. E. Greaves and R. K. Hermann eds. Oregon State University Extension Service. pp. 7-26.
- Gross, K. 1976. Dependence of rates of net photosynthesis and transpiration of *Picea abies* seedlings on water potential of the root medium and on air humidity with different CO<sub>2</sub> content. *Forstwissenschaftliches Centralblatt* 95:211-225.
- Hermann, R. K. 1962. The effect of short term exposure of roots on survival of 2-0 Douglas-fir stock. *Tree Planters Notes* 52:28-30.
- \_\_\_\_\_. 1964. Importance of top-root ratios for survival of Douglas-fir seedlings. *Tree Planters Notes* 64:7-11.
- \_\_\_\_\_. 1967. Seasonal variation in sensitivity of Douglas-fir seedlings to exposure of roots. *For. Sci.* 13:140-149.
- Hewett, E. W. and P. F. Wareing. 1973a. Cytokinins in *Populus x robusta*: qualitative changes during development. *Physiol. Plant.* 29:386-389.
- \_\_\_\_\_. 1973b. Cytokinins in *Populus x robusta*: changes during chilling and bud burst. *Physiol. Plant.* 28:393-399.
- \_\_\_\_\_. 1973c. Cytokinins in *Populus x robusta*: a complex in leaves. *Planta* 112:225-233.
- Horgan, R., E. W. Hewett, J. M. Horgan, J. G. Purse, P. F. Wareing. 1975. A new cytokinin from *Populus x robusta*. *Phytochemistry* 14:1005-1008.

- Humphries, E. C. 1960. Effect of mutilation of the root on subsequent growth. *Sci. Hort.* 14:42-48.
- Itai, C., A. Richmond and Y. Vaadia. 1968. The role of root cytokinins during water and salinity stress. *Israel J. Bot.* 17:187-195.
- \_\_\_\_\_ and Y. Vaadia. 1971. Cytokinin activity in water stressed shoots. *Plant. Physiol.* 47:87-90.
- Jones, J. R. 1972. Moisture stress in Arizona mixed conifer seedlings. U.S.D.A. For. Serv. Res. Paper, Rocky Mtn. For. and Range Exp. Stat. No. RM 86.
- Keinholz, R. 1934. Leader, needle, cambial and root growth of certain conifers and their interactions. *Bot. Gaz.* 96:73-92.
- Keller, A. and E. B. Tregunna. 1976. Effects of exposure on water relations and photosynthesis of western hemlock in habitat forms. *Can. J. For. Res.* 6:40-48.
- Koon, K. B. and T. O' Dell. 1977. Effects of wrenching on drought avoidance of Douglas-fir. *Tree Planters Notes Spring 1977:* 15-16.
- Krueger, K. W. 1967. Light intensity and amount of foliage influence root production of Douglas-fir seedlings. *J. For.* 65:222.
- \_\_\_\_\_ and J. M. Trappe. 1967. Food reserves and seasonal growth of Douglas-fir seedlings. *For. Sci.* 13:192-202.
- Larson, M. M. 1975. Pruning northern red oak nursery seedlings: effects on root regeneration and early growth. *Can. J. For. Res.* 5:381-386.
- \_\_\_\_\_ and G. H. Schubert. 1969. Effect of osmotic water stress on germination and initial development of ponderosa pine seedlings. *For. Sci.* 15:30-36.
- \_\_\_\_\_ 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 Oregon State University For. Res. Lab.* p. 20.
- \_\_\_\_\_ and P. F. Wareing. 1972. Effect of day length and chilling on the responses of Douglas-fir (*Pseudotsuga menziesii*) seedlings to root damage and storage. *New Phytol.* 71:1055-1067.

- Leshem, B. 1965. The annual activity of intermediary roots of Aleppo pine. *For. Sci.* 11:291-298.
- \_\_\_\_\_. 1970. Resting roots of Pinus halipensis: structure function and reaction to water stress. *Bot. Gaz.* 131:99-104.
- Lopushinski, W. 1969. Stomatal closure in conifer seedlings in response to leaf moisture stress. *Bot. Gaz.* 130:258-263.
- \_\_\_\_\_ and T. Bebee. 1976. Relationship of shoot-root ratio to survival and growth of outplanted Douglas-fir and ponderosa pine seedlings. U.S.F.S. Res. Note PNW-274.
- Lorenzi, R., R. Horgan and P. F. Wareing. 1975. Cytokinins in Picea sitchensis; identification and relation to growth. *Biochem. Physiol. Pflanz.* 168:333-339.
- Luckwill, L.C. 1960. The physiological relationships of root and shoot. *Sci. Hort.* 14:22-26.
- McGee, C. E. 1976. Differences in bud break between shade-grown and open-grown oak seedlings. *For. Sci.* 22:484-486.
- Meidner, H. 1967. The effect of kinetin on stomatal opening and the rate of water intake of carbon dioxide in mature primary leaves of barley. *J. Exp. Bot.* 18:556-561.
- Miller, C. O. 1956. Similarity of some kinetin and red light effects. *Plant Physiol.* 31:318-319.
- \_\_\_\_\_. 1961. A kinetin like compound in maize. *Proc. Nat'l Acad. Sci.* 47:170-174.
- Morris, R. O., J. B. Zaerr, R. W. Chapman. 1976. Trace enrichment of cytokinins from Douglas-fir xylem extrudate. *Planta* 131:271-274.
- Mozes, R. and A. Altman. 1977. Characteristics of root to shoot transport of cytokinin 6-benzylaminopurine in intact seedlings of Citrus aurantium. *Physiol. Plant.* 39:225-232.
- Mullen, R. E. 1966. Root pruning of nursery stock. *For. Chron.* 42:256-264.
- \_\_\_\_\_. 1973. Root and top pruning of white spruce at the time of planting. *For Chron.* 49:134-135.
- Owens, J. N. and M. Molder. 1973. Bud development in western hemlock I annual growth cycle of vegetative buds. *Can. J. Bot.* 51:2223-2231.

- Nelson, E. A. and D. P. Lavender. 1978. Dormancy of western hemlock seedlings. in Western hemlock mgmt. W. A. Atkinson and R. J. Zasoski eds. College of For. Resources, University of Washington. Seattle, Washington.
- Purse, J. G., R. Horgan, J. M. Horgan and P. F. Wareing. 1976. Cytokinins of sycamore-maple spring sap. *Planta* 132:1-8.
- Reid, D. M. and W. J. Burrows. 1968. Cytokinin and gibberellin-like activity in the spring sap of trees. *Experientia* 24:189-190.
- Richmond, A. E. and A. Lang. 1957. Effect of kinetin on protein content and survival of detached *Xanthium* leaves. *Sci.* 125:650-651.
- Rook, D. A. 1969. Water relations of wrenched and unwrenched *Pinus radiata* seedlings on being transplanted into conditions of water stress. *New Zeal. J. For.* 14:50-58.
- \_\_\_\_\_. 1971. Effect of undercutting and wrenching on growth of *Pinus radiata* seedlings. *J. Appl. Ecol.* 8:477-490.
- Sands, R. and R. L. Correll. 1977. Water potential and leaf elongation in radiata pine and wheat. *Physiol. Plant.* 37:293-297.
- Staden, J. van. 1976. Occurrence of a cytokinin glucoside in the leaves and honey dew of *Salix babylonica*. *Physiol. Plant.* 36:225-228.
- \_\_\_\_\_. 1978. A comparison of the endogenous cytokinins in the leaves of four gymnosperms. *Bot. Gaz.* 139:32-35.
- \_\_\_\_\_ and N. A. C. Brown. 1977. The effect of ringing on cytokinin distribution in *Salix babylonica*. *Physiol. Plant.* 39:266-270.
- Stone, E. C., J. L. Jenkinson and S. L. Krugman. 1962. Root regenerating potential of Douglas-fir seedlings lifted at different times of the year. *For. Sci.* 8:288-297.
- \_\_\_\_\_ G. L. Schubert, R. W. Benseler, F. J. Baron, S. L. Krugman. 1963. Variation in the root regenerating potentials of ponderosa pine from four California nurseries. *For. Sci.* 9:217-225.
- Sutton, R. F. 1967. Influence of root pruning on height increment and root development of outplanted spruce. *Can. J. Bot.* 45:1671-1682.

- Tanaka, Y., J. D. Walstad, and J. E. Borrecco. 1976. The effect of wrenching on morphology and field performance of Douglas-fir and loblolly pine seedlings. *Can. J. For. Res.* 6:453-458.
- Taylor, J. S. and E. G. Dumbroff. 1975. Bud, root, and growth-regulator activity in *Acer saccharum* during the dormant season. *Can. J. Bot.* 53:321-331.
- Todd, G. 1964. Douglas-fir seedlings have roots? *J. For.* 62: 561-563.
- Waring, R. H. and B. D. Cleary. 1967. Plant moisture stress: evaluation by pressure bomb. *Science* 155:1248-1254.
- Weiss, C. and Y. Vaadia. 1965. Kinetin-like activity in root apices of sunflower plants. *Life Sci.* 4:1323-1326.
- Wilcox, H. E. 1955. Regeneration of injured root systems in noble fir. *Bot. Gaz.* 116:221-234.
- Zaerr, J. B. 1970. Effects of plant moisture stress on growth of Douglas-fir trees. in *Tree ring analysis with special reference to Northwest America*. University of British Columbia Faculty of Forestry Bull. No. 7, pp. 3-6.