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Title: INFLUENCES OF SHOOT ORIGIN AND CERTAIN PRE- AND POST-SEVERENCE TREATMENTS ON THE ROOTING AND GROWTH CHARACTERISTICS OF DOUGLAS-FIR (PSEUDOTSUGA MENZIESII (MIRB.) FRANCO) STEM CUTTINGS

Abstract approved:
A. N. Roberts

Stem cuttings of current season shoots of seedling trees and clones of Douglas-fir were sampled periodically for two years to determine the influence of tree age, genotype, sampling date, shoot position, and foliage carbohydrate content on their rootability and subsequent growth characteristics. The potential for rejuvenating old clones was examined by determining the clones' response to repeated shearing of the stock plants, successive propagations--graft and cutting ramets, and cold storage of cuttings.

Depending on genotype, cuttings of juvenile trees under nine years of age had the potential for rooting 100 percent, declining rapidly after this age to less than five percent between ages 14 and 24 years. Genotypic differences in cutting rootability were greatest among the physiologically older trees. Rejuvenation of rooting in old trees and clones was achieved by shearing and successive propagations, but not by cold treatment of cuttings. Comparisons of sheared and non-sheared portions of old trees showed the rejuvenation effect to be localized in the sheared portion. Cutting ramets established from old clones produced cuttings which rooted 40 percent compared to six percent from grafted ramets and three percent from the ortet. Crown position (cyclophysis) had little influence on the rootability of shoots from trees under 24 years of age. Branch order positions (topophysis), however, were important in cutting selection with first order lateral (large), and second order terminal positions rooting better than first order terminal, first order lateral (small), or second order lateral cuttings. Seasonal fluctuations in shoot rooting potential were determined, with best rooting occurring in December, January, and February. Dormant November and December cuttings rooted as well after 60 days of 0° C storage as those receiving natural chilling on the tree; but they did not produce as much extension growth following rooting. Total and reducing sugars in the needles increased markedly during December and January, decreased in March and April, and were low during the summer and autumn. Starch levels were high in April, May, June, and July and relatively low at other times. Rootability did not always coincide with high needle carbohydrate content.

Cold storage reduced sugar levels, but increased rooting at a time when needle carbohydrate levels were lowest.

The natural transition of cutting ramets from the plagiotropic to the orthotropic growth habit was slow and was not hastened by staking, pruning, repotting, or unidirectional lighting. This transition occurs in some juvenile clones in three years, while others take seven or eight years or even longer for old clones. Preliminary observations of cutting ramets from different origins suggest that cuttings from young clones, terminal branch positions, top crown sections, and orthotropic shoots in sheared trees produce orthotropic trees more rapidly than cuttings from other sources.

Influences of Shoot Origin and Certain Pre- and Post-severance Treatments on the Rooting and Growth Characteristics of Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) Stem Cuttings

by

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Typed by Mary Jo Stratton for Darvil Kim Black

DEDICATION

This thesis is dedicated to

my wife and family

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TABLE OF CONTENTS

LIST OF TABLES

Table | GENERAL METHODS AND MATERIALS | Page

THE INFLUENCE OF TREE AGE AND REJUVENATION TREATMENTS ON THE ROOTING AND GROWTH OF DOUGLAS-FIR STEM CUTTINGS

THE INFLUENCE OF TOPOPHYSIS, CYCLOPHYSIS, AND GENOTYPE ON THE ROOTING AND SURVIVAL OF DOUGLAS-FIR STEM CUTTINGS

LIST OF APPENDIX TABLES

LIST OF FIGURES

THE INFLUENCE OF TOPOPHYSIS, CYCLOPHYSIS, AND GENOTYPE ON THE ROOTING AND SURVIVAL OF DOUGLAS-FIR STEM CUTTINGS

THE RELATIONSHIP BETWEEN CARBOHYDRATE CONTENT OF DOUGLAS-FIR NEEDLES AND ROOTING AND GROWTH OF STEM CUTTINGS

THE PROBLEM OF PLAGIOTROPIC GROWTH IN ROOTED DOUGLAS-FIR STEM CUTTINGS

LIST OF APPENDIX FIGURES

INFLUENCES OF SHOOT ORIGIN AND CERTAIN PRE- AND POST-SEVERENCE TREATMENTS ON THE ROOTING AND GROWTH CHARACTERISTICS OF DOUGLAS-FIR (PSEUDOTSUGA MENZIESII (MIRB) FRANCO) STEM CUTTINGS

INTRODUCTION

Douglas-fir is the most important timber species on the North American continent. It is also one of the best soft woods of the world. Nearly 30 percent of the commercial forest land in the West carries stands in which Douglas-fir predominates. Most of this timber area is on the Pacific Coast, mainly in the very productive area west of the Cascades, but Douglas-fir stands are also widespread in the Rocky Mountain states. This represents approximately 37, 352, 000 acres or 7. 3 percent of the total forest crop in the U. S. , and almost 25 percent of the saw timber produced, by far the most important species (38). It is also considered a leading timber species in Canada, where it is a native, and in New Zealand and Europe, where it has been introduced.

It is one of the most important Christmas tree species in those regions where it is adapted. Large Christmas tree plantations are common in the Pacific Northwest.

These facts give an indication of the importance of this species in meeting the soaring demands for wood products by a growing population and expanding economy.

In maintaining a supply equal to the demands for this species, there is a need in forest genetics, forest management, including Christmas tree management, for methods of propagating clonal lines of superior phenotypes and genotypes of Douglas-fir on their own roots. This would avoid problems of rootstock scion incompatability and rootstock influences common to grafted materials presently used in forest tree improvement. These trees would be useful in site performance evaluation, the establishment of seed orchards, and conceivably in the future for forest and Christmas tree plantations. One needs only look at the advancements made with horticultural plants by asexual propagation to see the potential with such an important timber, ornamental, and Christmas tree species. The problem of economically rooting cuttings of Douglas-fir, which will exhibit orthotropic growth, and doing this on a large scale must be solved. Solution of such problems with this species will have application to related species of forest and ornamental conifers.

Vegetative propagation has been employed by horticulturists for centuries to uniformly reproduce the desirable characteristics of selected individuals. The horticultural importance of this practice is appreciated when one realizes that nearly all tree fruits, nuts, cane fruits, strawberries, ornamental trees and shrubs, bulbous plants, potatoes, rhubarb, asparagus, and many florist materials are propagated in this manner. In recent years some forest trees have been propagated vegetatively.

Large scale propagation of many woody plants from cuttings is a relatively recent development. Prior to 1930, only the "easy-to-root" species and cultivars were so propagated. The discovery by Thimann and Went (147) that naturally occurring auxins contributed to root initiation led to the use of synthetic auxins in rooting many "difficult-to-root" species.

The conifers have been considered by some to be among the most difficult species to root (55, 105, 146). However, some conifers, largely ornamental forms used in landscaping, have been considered by others to be more or less easy-to-root from cuttings (55). Included in the latter classification are members of the following genera: *Taxus*, *Thuja*, *Juniperus*, *Chamaecyparis*, *Cryptomeria*, and some *Picea*. In spite of the considerable research in this country and abroad, relatively few timber species are propagated from cuttings (96).

The forest conifers which have been most widely studied and propagated from cuttings include *Pinus radiata* (14, 34, 35, 37, 62, 68, 90, 97, 149), *Picea abies* (21, 22, 31, 32, 51, 52, 146), *Pinus densiflora* (107, 108, 109, 110), and other species in the genus *Pinus* (115, 116, 148).

In this country vegetative propagation of forest species received little attention until the early 1940's. The significance of this approach to forest improvement was discussed by Shreiner (129) and more recently by Fielding (36). There has been a growing interest by foresters throughout the world in tree improvement through various means of vegetative propagation. Grafting scions from select trees onto chance seedling roots has been the most common procedure for seed orchard establishment with difficult to root species. An obstacle to the wider use of rooted cuttings has been the inconsistent results obtained and lack of knowledge concerning the many factors which interact and contribute to the rooting potential of these species. Progress in forest tree improvement must rely heavily on the identification and understanding of factors which contribute to the rooting of cuttings, because grafting and budding techniques have been plagued with poor graft unions, incompatability and rootstock influences. Grafting is not generally suited to the economic mass production of forest planting stock. The current interest in propagating forest species from cuttings has provided the research incentive to clarify the principles involved in rooting specific species.

Success in rooting Douglas-fir has been inconsistent and progress slow. Research has not been sufficiently detailed to obtain basic answers. However, research has shown the feasibility of rooting this leading timber species (1, 8, 9, 12, 95, 119, 120, 153). Some researchers have reported a problem with the growth behavior of rooted cuttings. McCullock (95) reported that after three years' growth in the field the trees had failed to develop well-defined leaders.

This same growth habit can be seen in pictures of rooted cuttings published by Griffeth (53). This plagiotropic growth habit has been considered by some researchers to be one of the most serious problems in using cuttings of coniferous species, particularly the firs.

This study was part of a team effort at Oregon State University to identify those factors involved in establishing Douglas-fir stem cuttings on their own roots. The objectives of these experiments were:

1. Determine the source of cutting material, with regard to cyclophysis and topophysis (physiological aging), giving the best rooting, survival, growth, and most importantly, established plants with symmetrical orthotropic growth habit.
2. Determine the influence of tree age on rooting and plagiotropic growth habit of cuttings. At what age do physiological changes occur which result in a rapid decline in rooting potential and increased incidence of the plagiotropic growth habit of cuttings?
3. Determine the feasibility of rejuvenating old clones by (a) heavy pruning of the stock plant, (b) successive propagation of cuttings from grafted ramets and rooted cuttings, and (c) cold storage of cuttings to achieve increased rooting and subsequent growth of cuttings.

4. Determine the relationship of needle carbohydrate levels in cuttings to their seasonal rooting response, clonal differences, trees' age, and rejuvenation treatment.

REVIEW OF LITERATURE

There is great variability among species and even genotypes within species in their ability to root from stem cuttings. The literature on propagation is voluminous and only those factors applicable to this study will be considered. Where possible, reference has been made to Douglas-fir, but conifers in general have been given consideration.

Genotype

Genotypic variation in rootability is common if not ubiquitous among forest tree species. Stack (137) feels that with Pinus strobus the genotypic variation in the stock plants is more critical to rooting than any other factor. Snow (135) noted clonal variations in rooting response of Acer rubrum from 17.5 to 97.5 percent. Achterberg (1), working with several forest species, but chiefly spruce and Douglas-fir, reported that differences between trees often obscured the effects of other treatments. Heinrich (58), also studying Douglas-fir, noted especially the variability in the rooting capacity of cuttings from different trees due to genetic differences. Lanner (83) reported clonal differences in rootability from 0 to 60 percent with Douglas-fir and two species of Abies. Fielding (34, 35, 37) reports that individual trees of Pinus radiata, particularly those over ten years old, exhibit large differences in the ease with which they are raised from cuttings, a behavior also reported by Duffield and Liddicott (29) for the hybrid Pinus attenuradiata, and by Ooyama and Toyoshima (110) for Pinus thunbergii and Pinus densiflora. These studies were repeated in successive years so probably represent true genetic differences. Thulin and Faulds (149) found less evidence of genetic differences in rooting potential with Pinus radiata.

This genetic difference, at least in part, may be due to a long lasting juvenile condition or a lack of aging in some clones. This is indicated by the work of Satoo et al. (125). Two clones of Cryptomeria japonica showed a decreased rootability with increasing age, while two other clones showed no significant change. This was also evident in Fielding's data.

This variation in rooting potential between clones is a general phenomenon and must be considered in all rooting studies.

Age of the Source Tree (Ortet)

The age of the seedling parent has been shown to effect the rooting potential of cuttings. Gardner (47) studied the rooting response of cuttings from seedlings of different ages from 21 species in 12 genera. A sample of his results show that rooting potential drops off rapidly at a very early age.

Species	Age of parent (years)	Rooting (%)
Pinus sylvestria	1	77
	2	8
	3	0
Pinus strobus	1	98
	2	51
	3	12
Pinus resinosa	1	62
	2	3
	3	7
Pinus taeda	1	46
	2	6
	3	0
Thuja occidentalis	2	100
	old	42
Picea exelsa	2	90
	old	50
Taxodium distichum	1	95
	2	30
	3	10

Similar responses have been noted in Larix (17). Cuttings from younger trees of Pinus strobus root better, respond more to hormone treatment and produce a greater number of roots than their older counterparts (113, 148). The rooting and early growth rate of cuttings from Pinus radiata has been reported to decline with seedling age (34, 35, 37, 68, 90, 97). Libby and Conkle (90) found that percent rooting and number of roots per cutting declined with age in Monterey pine, but more slowly than for most species. Thulin and Faulds (149) observed only a small decline in rooting with increasing age in this species. Murygina (101), working with Picea abies, found that the percent rooting, speed of rooting, root length and number, and the incidence of shoot growth in the year of rooting all decreased with increasing age of the parent tree. Similar results were reported by Girouard (49) for this species. Thimann and Delisle (146) studied the vegetative propagation of several "difficult plants," and placed the majority of forest conifers in this class. They considered the source tree age as the most important single factor affecting their rooting. Rooting dropped off rapidly with age, both with and without auxin. They found in some conifers, namely Tsuga canadensis and Picea pungens, that cuttings from old trees rooted only if given auxin. Franklin (43) noted that ortet age had a strong influence on the rooting of Pinus elliottii. Kleinschmidt (74), reporting on his results with Picea abies, found a marked change in physiological age occurred between the 15th and 20th year followed by a rapid decline in rooting.

Numerous attempts have been made to correlate the decrease in rooting with increased age with physiological changes in the plant. Many of these physiological changes have been associated with the transition of the plant from the juvenile to the adult phase. Changes other than the capacity to regenerate roots are distinguishable in conifer and deciduous species during this transition, such as changes in leaf shape, pigment production, phyllotaxy, shedding of leaves, thorniness, growth habit, and the onset of flowering, as well as many accompanying metabolic changes. In their efforts to explain changes in rooting potential accompanying aging, researchers have noted changes in food reserves, C/N ratio, vitamin B content, auxins, root promoting co-factors, gibberellins, cytokinins, and various inhibitors. The most commonly proposed explanation is the change in the growth promoter-inhibitor balance. In general, it has been shown that the growth promoters decrease and the growth inhibitors increase with increasing age (48, 60, 66, 108, 152). Ogasawara (108), working with Pinus densiflora, reported that with increasing age (from 1 to 15 years) the auxin content of one-year-old shoots decreased, while the inhibitor content increased. Yim (158) studied the growth substances in terminal buds of 1- to 17-year-old Pinus rigida trees. Concentrations of IAA were highest in the 1-year-old and very low in the 17-year-old trees. It was noted further that cuttings with flower primordia present rooted poorly compared with those having vegetative buds only, or those from which the flower buds had been removed.

The literature contains little information on the influence of age on the rooting of Douglas-fir cuttings. It is known that rooting potential declines with age, but whether a specific age is accompanied by a physiological transition reflecting changes in rooting potential has not been established.

Rejuvenation

As indicated in the previous section, one of the most consistent characteristics of juvenile trees is their relative ease of rooting when compared with cutting from older trees of the same species. Therefore, it would be highly desirable from the plant propagators' point of view to be able to restore in some degree this aspect of juvenility to old trees.

There is not complete agreement among plant physiologists regarding the possibility of rejuvenating or returning the adult plant to the juvenile phase. Excellent reviews have been presented on this subject by Schaffalitzky De Muckadell (127) and Robbins (117). The reversion of adult to juvenile forms has been accomplished with several techniques, including heavy pruning, grafting adult scions onto juvenile tissues, cold treatment, X-ray treatment, treatment with extracted products from the juvenile forms and treating the adult material with gibberellin (76).

Doorenbos (24) produced many reversions of Hedera helix to the juvenile form by grafting the mature fruiting form onto two- or three-year-old seedlings or on juvenile plants grown from cuttings. Reversion of adult Hedera canariensis tissues grafted on juvenile forms was influenced both by temperature and the amount of mature tissue initially present in the graft combinations.

Frank and Renner (42) noted that new shoots on adult Hedera helix cuttings reverted to the juvenile form when grown in a nutrient solution along with juvenile cuttings that rooted readily. They also showed that when adult ivy cuttings were exposed to a temperature of -10°C for a few hours the new branches that formed a few weeks later had juvenile characteristics. Similar reversions to the juvenile form have been observed in Hevea brasiliensis (102).

Chemical treatments have increased juvenility in a few cases. Robbins (118) induced reversion to the juvenile condition by spraying adult Hedera canariensis varigata plants with gibberellic acid 13 times over a period of 19 weeks. This was also achieved by Stoutmeyer et al. (143), especially at high temperatures.

Pruning has also been shown to enhance juvenility. It not only forces lower, and hence more juvenile meristems into growth, but appears to directly enhance the degree of juvenility expressed in growth (118).

It should be pointed out that many attempts at restoring "juvenility" to other species have been without apparent success. There is the possibility that the reversion is not complete and therefore not obvious, but there could well be a degree of reversion which is not apparent with the criteria used. The problem of aging has usually been studied by investigators interested in one or another specific characteristic. Robbins (117) considered the question from the viewpoint of the physiological aspects of aging in plants. This paper presents, in the writer's opinion, a workable hypothesis and one which current data seem to substantiate. He states that:

> Without attempting to deal with ultimate causes or to settle the question of juvenile vs adult meristems, I suggest as a working hypothesis that juvenility is an unstable metabolic state which exists in the meristem and which proceeds through a series of steps to a relatively stable metabolic state characteristic of the adult meristem. The change from unstable to stable may be associated with the loss in ability to synthesize physiologically important chemical substances and/or the development of the ability to synthesize others. . . . The steady state of the adult may be upset in the direction of the unstable metabolism of the juvenile by cold, X-rays, products from the juvenile, the formation of adventitious meristems and by the formation of zygotes or of asexual embryos (p. 292-293).

It seems likely that one might restore a degree of juvenility, such as the increased potential for rooting, without necessarily restoring other morphological and physiological characteristics, which have been commonly used as measures of juvenility.

As emphasized by Brink (11), the mechanism of phase change (rejuvenation), whereby some characteristics are maintained and perpetuated in somatic cells, remains an exceedingly important unsolved problem.

Among those studying the vegetative propagation of forest trees, results have differed concerning the possibility of restoring rooting in tissues from older trees by means of cuttings, grafting and pruning. Thomas and Riker (148), and later Patton and Riker (11~~5~~3), found no increase in percent rooting of cuttings from grafted ramets of mature trees. Similar results were obtained by Zak (159) in attempts to air layer Pinus echinata grafts made from ortet trees 35 to 40 years old.

Delisle (20) observed a progressive decline in rooting accompanying aging of Pinus strobus cuttings. Rooted cuttings that were planted in 1938 were grown for four years and then cuttings from these were rooted and planted. This procedure was repeated four times, every four years. The rooting was found to decrease with each successive series from first to last and some saplings produced cones toward the end of the study.

Fielding (35) found that Pinus radiata cuttings from cuttings did not root as well as cuttings from seedlings of the same age (this would not be the same physiological age). In five out of seven experiments the mean percentage of rooted cuttings was greater in the case of cuttings from seedlings. In the other two experiments they did not differ appreciably. In a more relevant comparison, however, he obtained 7 and 42 percent rooting from the seedling parent and first generation cuttings, respectively.

Ooyama (109) and Ooyama and Toyoshima (110) reporting on several "difficult" species including Cryptomeria japonica, Pinus densiflora, Pinus thunbergii, as well as some other species, noted that cuttings from old trees were difficult to root; however, when "young" saplings were grown from these cuttings and used as parent trees, these secondary cuttings showed improved rootability and increased number of roots.

Sato (1968), as quoted by Fielding (35), showed the rooting ability of cuttings from grafts to be greater than that of cuttings from the same parent tree. Griffin (54) reports that in all cases tested, cuttings from grafts rooted better than their plantation grown ortets.

In general, research results indicate that the rooting potential in old clones of coniferous species can be increased through rejuvenation. Considering the premise of Robbins (117) and also considered by Goebel (50), rootability is closely associated with juvenility, it would appear that at least a degree of rejuvenation of old clones is possible. The possibility of this type of rejuvenation in Douglas-fir has not been previously established.

Cold Storage

Cold storage of conifer cuttings in the past has been largely a function of the time available to process the cuttings or the space available in rooting benches. Storage is common for as long as five months, but it has generally been thought that rooting gradually decreases with time in storage (3). Jacobs (68) found no effect on rooting from storing Monterey pine cuttings. More recently, however, Fielding (34) reported that storage of cuttings for two, three, and four months gave better than 90 percent rooting in each case as compared with 54 percent for cuttings not stored. Libby and Conkle (90) observed an increased rate of rooting with cold storage but no apparent effect on total rooting. Roberts (120) found cold storage of Douglas-fir cuttings to improve rooting during the most dormant period from mid-September to mid-November, but of no benefit at the other times and even detrimental at some times of the year.

Topophysis and Cyclophysis or Shoot Position Effects

The position on the tree from which cuttings are taken has long been recognized as influencing rooting potential, as well as growth habit (100, 131). Growth habit will be considered in more detail in the section following on plagiotropic growth. Molish (100) concluded "that it is not all the same in securing cuttings or scions from which part of the individual the cuttings or scions are taken" (p. 178). He further emphasized that contrary to appearances, foliage shoots of higher plants are not all alike qualitatively but are individually different. There is shoot and even bud individuality, and Hedera helix illustrates this quite well. Juvenile plants of this ivy produce plagiotropic shoots with two- to five-lobed leaves. The shoots of the mature plant, on the other hand, which are generally covered with flowers, grow upright, and have ovate pointed leaves. Cuttings from these two types of shoots behave quite differently. The juvenile cuttings root readily and retain their plagiotropic growth habit. Cuttings from the flowering portions root with difficulty and retain their upright growth habit and ovate leaf form (100).

Wareing (152) cites the classical set of experiments conducted by

Vochting in 1904 with *Araucaria excelsa*. These show the importance of shoot position when selecting cuttings. When apical shoots or an axillary bud from the main stem are selected for vegetative propagation, normal radially symmetrical plants were obtained. Rooted cuttings from first order side shoots grew horizontally as they would on the intact plant. Cuttings from second order shoots grew horizontally also, but only as string-like structures, without branching. Wareing states that there exists at the Munich Botanical Garden a plant from a side branch of *Araucaria excelsa* rooted by Goebel more than 50 years ago that is still growing horizontally. The phenomenon that cuttings or scionwood from different positions of a plant may perpetuate different growth habits in the propagules was given the term topophysis by Molish (100).

Horticulturists are well aware that if buds or cuttings are taken from the lower juvenile portion of seedling trees of many fruits such as apple, pear, or citrus and many of the ornamentals such as honey locust and beech, the plants produced therefrom will be vigorous, thorny, and slow to flower. Propagules obtained from the upper portion of the same trees produce nursery trees that are less vigorous, smooth-barked, thornless, and with a tendency to flower sooner. Another horitcultural example of topophysis is *Taxus cuspidata*. In order to obtain the desired upright habit, cuttings must be taken only from erect terminals or upright-growing laterals rather than horizontal side shoots (55). This phenomena is also seen in Taxus baccata (100) and Coffea arabica cuttings (55).

In forest species the phenomena of topophysis and cyclophysis has received some attention, but the results obtained are not consistent even within species. The position of the shoot in the crown (cyclophysis) has been reported to affect subsequent rooting potential in several species (1, 21, 22, 27, 34, 41, 51, 82, 111, 127, 130, 132, 133, 136). This appears to be especially true of trees past juvenile stage. In younger trees the difference may not be so great.

Grace (51), working with an 18-year-old Picea abies tree, obtained 86 percent rooting from the lower and 48 percent rooting in the upper part of the tree. Schrock (130) compared cuttings of an old Populus borolinensis tree taken from various heights in the tree crown. He found the growth and rootability of the cuttings increased with increasing distance from the stem base up to 4.5 meters and decreased from there on. Snow and May (136) found that with March cuttings of Pinus virginiana the lower section of the tree rooted 18.6 percent compared to 1.3 percent for the upper section. Acterberg (1), working chiefly with spruce and Douglas-fir, showed that, in general, shoots from the lower parts of the crown rooted slightly better. Kummerow (82) has shown with a 2.5-year-old Pinus radiata that in six zones from top to bottom of the plant, rooting was 5, 5, 58, 15, 2.5, and 10 percent, respectively. On the contrary, however, Fielding

(34) reports that cuttings of comparable type from 20-year-old trees of this species rooted equally well, regardless of their source in the crown. Ogasawara (106) and Komissarow (75) each reporting on results of several forest species, found little difference due to crown section. Frohlich (44) found no difference in rooting of cuttings from the upper and lower crown sections of Picea sp., and the same was found by Chandler (16) for larch.

The order of the shoot within the same crown section (topophysis), or the local position effect has been reported to have an effect on the rooting and growth habit of cuttings from several forest species (2, 32, 34, 44, 59, 73, 74, 97, 99, 132, 134, 146). Again the results have varied somewhat from one species to another and even from one test to another. The data are inconclusive. The reasons for these differences are not always clear, but considering that different authors took cuttings at different times from trees growing under different environmental conditions at different stages of physiological development and rooted them under different conditions, it is not surprising that the results obtained are often contradictory. Sherry (132) and Fielding (34) found that with Pinus radiata, the first order branches were best. Cuttings from second order branches were not as good, but rooted better than those from third order branches. With the same species Allsop (2) observed better rooting from the first laterals formed in the Spring. Mirov (97) reported that the farther the shoots were from the terminal, the better they rooted. Differing results have been obtained on topophysic studies by Russian researchers as reviewed by Komissarow (75). With Picea abies, Farrar and Grace (32) found little difference in the rooting of second-order terminals, second-order large laterals, second-order small laterals and third-order laterals, the average rooting being 90 percent. First-order terminal cuttings only rooted 67 percent, but these were described as having a superior shoot development following rooting. With this species Thimann and Delisle (146) also reported better rooting with laterals than terminals (146). Runquist and Stefansson (124) also had greater success with laterals. Yet, Levin (89) reported that laterals did not root as well as terminal shoots, and Ogasawara (106) in studying several forest species, found no consistent relationship between rootability and shoot position. Fielding (35) has suggested that the reported influences of crown section and shoot order may be largely a result of differences in shoot thickness and size. At best the results to date are inconclusive as regards the influence of shoot position on rooting potential.

Plagiotropism

The plagiotropic growth habit, characteristic of vegetative propagules in some conifers, presents as difficult a problem as adventitious root formation, in successfully establishing these trees from rooted cuttings. Some species, namely those in the genera *Pinus* and *Larix*, when propagated vegetatively soon develop a normal orthotropic, radially symmetrical growth habit, while those in the genera *Picea*, *Pseudotsuga*, and *Abies* retain their branch symmetry and plagiotropic habit for a considerable time (16, 22, 44, 59, 73, 74, 97, 134, 146).

Mirov (97) was one of the first to report the importance of shoot position on their subsequent growth habit when rooted as cuttings. Working with *Cunninghamia lanceolata*, he found cuttings taken from the upper part of the stem developed normal plants, while those taken from the lower side branches grew in a "staggering" manner.

Thimann and Delisle (146) observed that the plagiotropic growth habit was retained for a time in the lateral cuttings of spruce while terminal cuttings grew vertically.

Kleinschmit (74) reported differences in branching habit of four-year-old *Abies alba* rooted cuttings taken from different branch orders. After six years, cuttings rooted from first order laterals still resemble branches; first order terminals produced plants with normal tree form. Of interest is the fact that almost complete orthotropic plants were derived from first order laterals rooted from five-year-old *Picea abies*. He concluded that the lateral branch habit of a given parent tree becomes progressively more fixed with increasing age and development. Herrmann (59) reports similar differences between the growth habit of ten-year-old seedlings and cuttings or buddings of *Picea abies* and *Pseudotsuga menziesii*. He found the topophysic effect to be more pronounced in cuttings or scions collected from the flowering parts of the tree. Klaehn (73) has reported that several *Abies* species grafted on four-year-old *Abies balsamea* rootstock have performed quite differently with respect to growth habit. First-order laterals became orthotropic in the second year, while second and third order laterals retained their branch habit. This, in spite of the fact that all the scion-wood was from similar center crown sections of trees in the 20- to 30-year age class. He notes further that grafts from 25-year-old Douglas-firs, all from the top crown sections, exhibited orthotropic growth, whereas those from higher order laterals showed plagiotropic development. Frohlich (44) showed that the time required for orthotropic growth habit to develop in *Picea* cuttings was related to their original branch order position on the tree, taking longer with higher order branches. Within each branch order, material from younger trees made the transition faster than materials from older trees.

Though not relating it to position, McCulloch (95) recognized the plagiotropic growth habit as a possible problem in cuttings of Douglas-fir. He stated that

> . . . after three years' growth in the field the trees have failed to develop well-defined leaders. Laterals have developed well on the trees, and if growth continues to be

> concentrated here the experiment may conclude with a weeping type tree not suited for lumber production (p. 211).

The author has seen these trees at 30 years of age and sampled them as part of the thesis study. They have in time developed into uniform, symmetrical, orthotropic trees, well suited to timber production.

To summarize, there appears no serious problem with plagiotropism in cuttings of the genera Pinus, Taxus and Larix, although there are differences in cutting survival and vigor of growth. In contrast, there are marked differences reported for cuttings of various species of the genera Picea, Pseudotsuga, Abies, Auracaria and Cunninghamia, which all have similar formal growth habits. A partial solution to the problem of plagiotropic growth appears to be the proper selection of cutting material.

Physiological Condition of the Stock Plant

There is considerable evidence that the nutrition of the parent plant exerts a strong influence on the rooting of cuttings (30, 34, 55, 79, 92, 114, 138).

Kraus and Kraybill (79) observed that tomato cuttings high in carbohydrates but low in nitrogen produced many roots, while those containing ample carbohydrates but higher in nitrogen produced fewer but stronger roots. Green, succulent stems, very low in carbohydrates but high in nitrogen, all decayed without producing roots.

Grape studies (142) have shown that cuttings with high, medium and low starch content rooted 63, 35, and 17 percent, respectively.

It seems obvious that there must be an adequate supply of stored carbohydrates and proteins to satisfy the energy and tissue building requirements until the cuttings become established. A high C/N ratio has been found essential for rapid root formation. Cuttings low in carbohydrates and high in nitrogenous compounds root poorly. On the other hand, nitrogen may become so low that rooting will be poor in spite of high carbohydrate content (55, 75, 92). The nutritional status of the cutting depends upon the season of the year in which the cutting is taken as well as the vigor of the parent plant. Enright (30) found that three species of conifers responded more favorably to root inducing chemicals if the stock plant had been fertilized for a period prior to taking the cuttings.

Few attempts have been made to correlate stored food reserves with the rooting potential of conifers. Reines and Bamping (116) studied the carbohydrate level with seasonal rooting of cuttings of Pinus taeda and Pinus elliottii. They were unable to establish a relationship between rootability and carbohydrate level. They considered, however, that the balance of various organic components should not be ignored as a possible influence in rooting.

Though not correlated with rooting potential, several studies have been conducted on the seasonal and yearly variation of food reserves in conifers. Douglas-fir has been studied by Krueger and Trappe (81), Krueger (80), and Worley (154). The seasonal changes in carbohydrates in *Abies balsamea* recently observed by Little (91) are similar to those found in Douglas-fir. These studies all reveal wide fluctuations in the total food reserves during the season. Figure 1, composed from data in the literature, suggests correlations between periods of high sugar reserves and those of high rooting potential on stem cuttings of Douglas-fir (81, 120). The seasonal regeneration of roots in Douglas-fir seedlings reported by Stone *et al.* (141) lifted at different times of the year is similar to the rooting potential for stem cuttings.

The periodicity of rooting potential in conifers is well established with considerable variation among species, as well as within species. Species of conifers most thoroughly studied include: *Picea abies* (21, 31, 86, 101), *Juniperus* and *Taxus* species (84, 85), *Pinus radiata* (34, 68), *Pinus virginiana* (39, 136), *Auracaria excelsa* (93), and *Pseudotsuga menziesii* (120). In general, the best results were obtained with these species when cuttings were taken during the period from late autumn to late winter. However, many exceptions can be found for this generalization.

Thomas and Riker (148) obtained the best rooting with *Pinus strobus* when cuttings were taken from July to September. Mirov (98) noted that pine cuttings taken in spring did not root well, and suggested

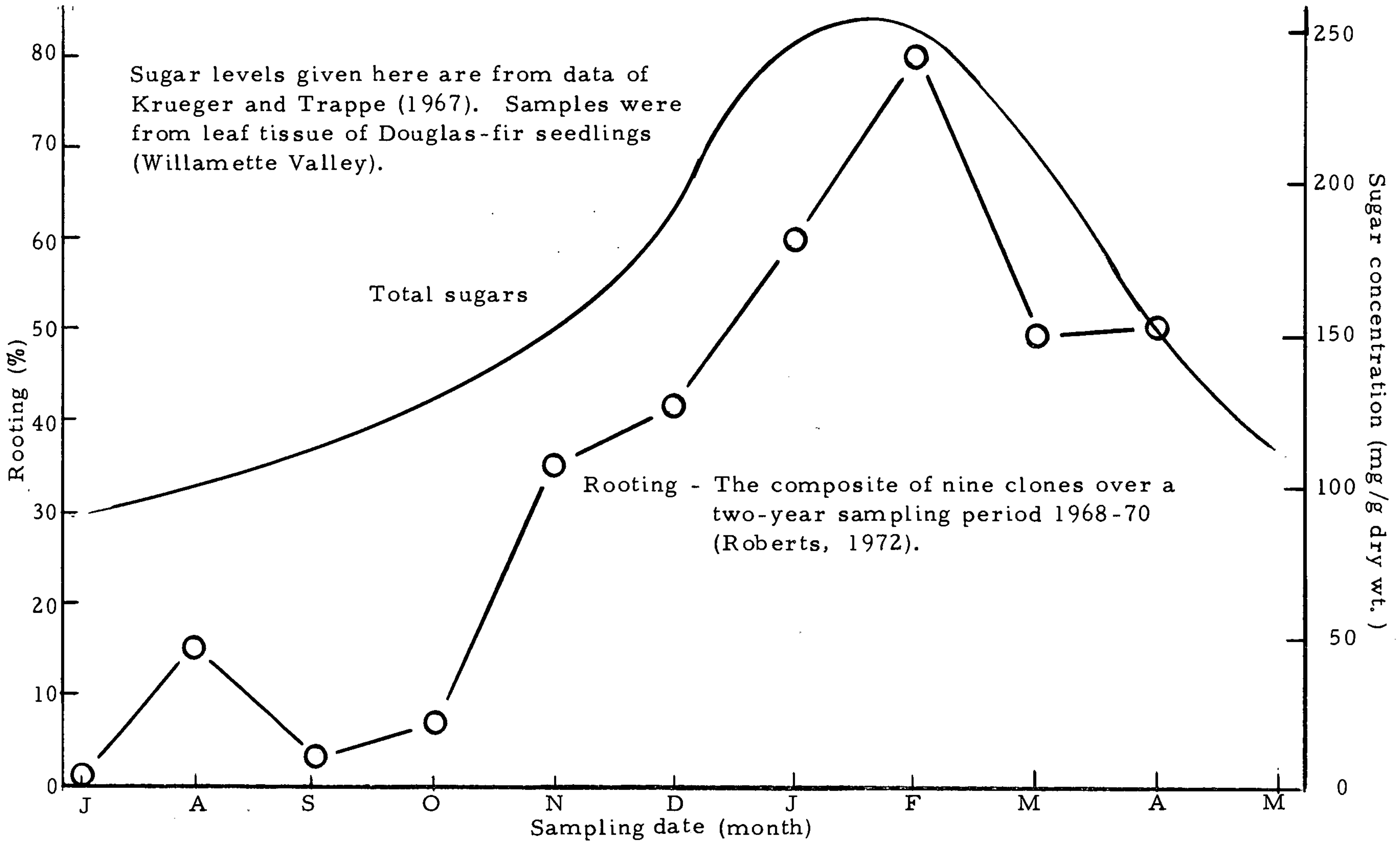

Figure 1. Seasonal fluctuations in shoot rooting potential and foliage sugar levels in Douglas-fir trees from reports in the literature.

that the failure was due to high greenhouse temperatures. Larsen (86) found best rooting in June and July with Picea abies and Picea sitchensis. Similar results have been reported for Picea glauca (31). With a few species, the season of collection does not seem as important as the environment and handling procedures during rooting (84).

There is some evidence that exposure of the stock plant to chilling temperature stimulates rooting (21, 90, 120).

There have been few attempts to correlate rooting with a specific physiological condition in the stock plant. The conflicting results reported by various researchers reflect the extreme importance of knowing the physiological status of the stock plant at the time the cuttings are taken. Too little attention has been paid to this consideration in the past. Rooting response no doubt depends on a multitude of factors not the least of which are the food reserves and the hormone balances within the stock plant at the time the cuttings are severed.

GENERAL METHODS AND MATERIALS

Procedures common to the several studies are outlined in this section, and will be referred to as standard procedures. Methods peculiar to a specific study will be included in the discussion of the experiment under consideration.

Source and Type of Cuttings

Plant samples were collected from trees growing at Corvallis, in McDonald Forest near Lewisburg, near Stayton, the David Mason seed orchard near Sweet Home, and from five select parent trees located near Sweet Home from which ramets in the seed orchard had been selected (Table 1). All plants used were growing in full sun and were selected on the basis of their age, or the degree to which they had been pruned. All stem cuttings were made from current season's growth. They were uniformly sized to approximately 15 cm in length with the exception of those cuttings from the basal portion of the older trees, which were often shorter than this due to lack of vigorous growth, but never less than 10 cm.

The cuttings were identified as to clone, crown section, branch order, and in the case of sheared trees, their growth angle on the tree. At the time of collection the cuttings were placed immediately into plastic bags and stored in an ice chest until transported to the greenhouse or laboratory at Oregon State University.

Table 1. Source plants for the cutting materials used in these studies. Age and size data, February 1972.

Clone	Age (yrs)	Height (m)	Diameter (cm)	Planting type and location
(a)				
30	9	3.0	6.5	field-grown near Corvallis
31	9	3.1	6.9	
32	9	2.9	6.9	
33	9	2.9	6.8	
34	9	3.0	6.9	
35	9	2.7	6.5	
36	9	3.6	7.0	
37	9	3.0	6.9	
38	9	2.8	6.8	
39	9	3.0	6.9	
55	15			forest-grown near Sweet Home
56	15			
57	15			
58	15			
59	15			
61	25	14.9	19.0	McDonald Forest near Lewisburg
62	24	14.0	17.0	
63	24	15.5	19.0	
64	25	15.8	21.0	
65	24	15.2	17.0	
66	24	16.8	20.0	
67	24	17.7	21.0	
(b)				
45	28	6.0	34.8	near Stayton
46	25	5.2	26.7	
47	26	5.2	30.7	
48	24	5.2	25.2	
49	27	6.0	30.0	
68	24	5.3	28.0	
40	35	7.9	55.0	Peoria Road
41	36	7.3	49.3	
42	37	8.2	46.0	
43	41	7.6	55.0	
44	42	7.9	58.0	

(Continued on next page)

Table 1. (Continued)

Clone	Age (yrs)	Height (m)	Diameter (cm)	Planting type and location
(c)				
70	61	26.0	78.0	near Stayton
71	56	28.0	100.0	
72	28	13.7	38.0	
73	25	12.8	33.0	
74	40	25.0	61.0	
75	32	14.6	42.0	
76	40	18.3	63.5	
77	38	22.0	62.0	

Clone	Parent age (yrs)	Plant location of		
		Ortet	Grafted ramet	Cutting ramet
(d)				
368	84	forest near Sweet Home	seed orchard	seed orchard
171	25			
173	34			
175	27			
176	25			

(a) Clones used in genotype X age X crown position X branch order study.

(b) Clones used in shearing study - fully sheared trees under utility lines.

(c) Clones used in shearing study - one-half sheared trees by the side of utility lines near Stayton.

(d) Clones used in successive propagation study.

Propagation Techniques and Facilities

The cuttings were all sized to 15 cm in length and the needles removed from the basal 5 cm. Cuttings to be cold stored were placed in 5 x 10 x 15 cm plastic bags by treatments, left unsealed and grouped in larger 10 x 15 x 38 cm plastic bags provided with a moist paper towel at the base. This prevented direct contact of moistener with the plant material. The larger bags were sealed at the top and the cuttings stored in an upright position at 0 ± 1° C for 60 days.

During the first year of these studies, immediately before benching, a vertical wound was made along two sides of the base of the cutting. This procedure was discontinued in later studies, when experiments showed no difference in total rooting or root quality as a result of this practice. Just prior to sticking, all cuttings were given a five-second basal dip in 10 percent Jiffy Grow[1] diluted with 95 percent ethanol.

The cuttings were placed in raised propagating benches located in greenhouses equipped with a pressurized intermittent misting system activated by a Solutrol with a percentage timer and light override. Bottom heat was provided with electric heating cable thermostatically controlled at 21 ± 3° C. The greenhouse temperature was maintained near 10 to 15° C. Evaporative coolers were used to

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[1] Jiffy Grow - a commercial preparation containing the following: 0.05 percent 2-naphthaleneacetic acid, 0.05 percent 3-indole-butyric acid, 0.0175 percent boron, 0.01 percent phenyl mercuric acetate.

moderate summer daytime maximums. No attempt was made to modify the daylength. The rooting medium consisted of five parts Delmonte El-8 white washed sand to one part Canadian sphagnummoss peat.

The cuttings were stuck in the propagation bench to a depth of 5 cm with an approximate spacing of 3 cm in the rows and 7 cm between rows. Cuttings which appeared dead during the rooting period were removed. The propagation beds were sterilized with steam at regular intervals between each series and the room periodically fumigated with Termil.

Cutting Response

Evaluation of rooting was made after the cuttings had been in the propagating bench for 90 days the first year and 120 days the second year of study. As the cuttings were removed, data were taken on number dead, number with callus but not rooted, rooting quality, and bud break.

Rooted cuttings were potted and evaluated on survival, and the amount and habit of growth.

Data Analysis

The data were placed on computer program cards and analyzed for significance with an analysis of variance program (157).

Definition of Terms

Topophysis (branch order) - The local position effect exhibited in vegetative propagules originating from different branch orders and/or terminals in contrast to laterals from within the same crown section of the tree (Figure 1, p. 69).

Cyclophysis (crown section) - The physiological age effect exhibited in plants originating from the same branch order in different crown sections of the same tree (Figure 1, p. 69).

Plagiotropic Growth - The horizontal or oblique angle with reference to the growth habit of the plant organ.

Orthotropic Growth - The vertical or upright growth habit of the plant organ.

Ortet - The one seedling plant from which members of a clone were originally derived by vegetative propagation.

Grafted Ramet - An individual member of a clone developed by grafting or budding of scionwood onto an established rootstock.

Cutting Ramet - An individual member of a clone developed from a rooted cutting. The source of clonal cutting wood may be from the ortet, the grafted ramet, or from a previously established cutting ramet.

Rejuvenation - Restoration of juvenile rooting potential to physiologically old clones. There may be other and perhaps more obvious morphological and physiological changes accompanying this dedifferentiation.

Sheared Trees - Trees located under utility lines, kept low by heavy and regular pruning (Figure 1, p. 42).

Growth Angle - Orientation of shoots within the crown section of sheared trees. Arbitrary classes used were (↑) 90-60°, (↗) 59-30°, (→) 0-29°, (↘) <0° from horizontal.

Root Quality - Arbitrary categories: (1) poor - one or two roots, (2) fair - three to five roots, (3) good - six or more roots (Figure 1).

Bud Break - Arbitrary categories: (1) no bud break to dark brown, (2) light brown to white, (3) first needle appearance to 2 cm, (4) needles extended more than 2 cm.

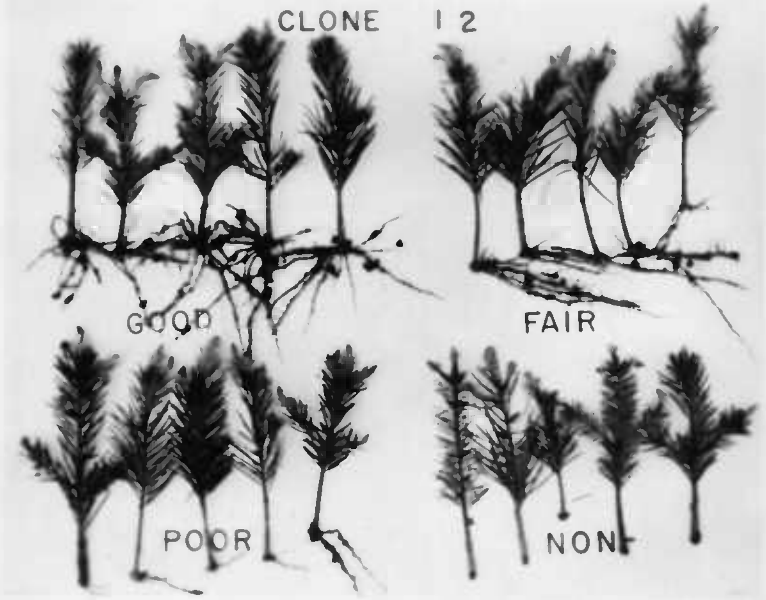

Figure 1. The root quality classes used in this study.

Prepared for Submission to Forest Science

THE INFLUENCE OF TREE AGE AND REJUVENATION TREATMENTS ON THE ROOTING AND GROWTH OF DOUGLAS-FIR STEM CUTTINGS

ABSTRACT. Over 15,000 cuttings were used during a two-year period to study the effects of tree aging and rejuvenation on the rooting of Douglas-fir stem cuttings. Up to 100 percent rooting, with little decline due to age, was observed in seedling trees up to nine years, declining rapidly after this age to less than five percent between ages 14 and 24. Rejuvenation of rooting potential in old trees was achieved by shearing and successive propagation. Old sheared trees rooted better than even younger non-sheared ones. Comparisons of sheared and non-sheared portions of the same old trees showed the rejuvenation effect of shearing to be localized in those portions of the tree. Cutting ramets established from old clones produced cuttings which rooted approximately 40 percent compared to 6 percent for grafted ramets and less than 3 percent for the ortet. Cold storage of cuttings was shown to stimulate rooting in old and young clones during the dormant period, but not above that achieved after natural chilling.

ADDITIONAL KEY WORDS. Pseudotsuga menziesii (Mirb.) Franco, successive propagation, cold storage.

FOREST conifers have generally been regarded as among the most difficult plants to grow from stem cuttings (146). However, investigations during the past 35 years have shown that many species once thought to be difficult-to-root are actually easy-to-root, particularly if the cuttings are taken from young trees. Parent tree age is one of the most important factors affecting the rooting and survival of conifer cuttings. It has made the rooting of cuttings impractical for many species, except the few which root readily. Though the young trees may be easy-to-root, the forester needs to root cuttings from older trees with well-established desirable characteristics.

The literature contains many studies revealing the influences of age on the rooting and growth of conifer species. Species most widely studied include *Pinus strobus* (47, 113, 148), *Pinus radiata* (14, 34, 35, 68, 90, 132), and *Picea abies* (22, 49, 101, 146). Other conifers have been studied to a lesser extent (16, 47). The studies show that rooting declines sharply as the trees progress from the early juvenile to the adult stage, rooting with limited success as the trees begin to flower. Although studies of aging in Douglas-fir are limited, reduced rooting potential and changes in growth habit associated with aging appear to be universal in woody species (127), and there are numerous examples of carryover of such changes into vegetatively propagated progenies.

Some factors affecting rooting of stem cuttings remain unsolved, especially the limitations imposed by tree aging. Little is known concerning the endogenous changes controlling this phenomenon. Numerous mechanisms of control have been proposed. Some recent studies have suggested that an inhibitor-promoter balance is the controlling factor (48, 66, 108, 158). These and similar studies indicate a build-up of inhibitors and a concomitant decline in promoters with age. It would be desirable to restore to old clones the rootability associated with juvenility. In spite of the lack of understanding concerning the internal mechanisms, it is clear that juvenile trees are often easy-to-root. Accounts of apparent complete rejuvenation of adult forms have been reported (24, 42, 118). Cases of complete morphological reversion to the juvenile form in forest trees are lacking, but several studies have shown some restoration of rooting potential by heavy pruning, grafting adult scions onto juvenile rootstocks, and by chemical and low temperature treatments (34, 35, 75, 109, 110, 125).

This study was conducted to (1) determine quantitatively the changes in rootability of Douglas-fir clones with increasing age, and to relate these changes to physiological events, and (2) determine if it is possible to restore rooting potential to old trees by heavy pruning, successive propagation, and cold treatment.

MATERIALS AND METHODS

Plant materials were collected from trees growing near Corvallis, Oregon, during 1970-72. All plants used were growing in full sun and were randomly selected within age classes. Sheared trees were growing along utility lines and kept low by annual shearing. Cuttings were 15 cm long except in lower crown sections of the old, non-rejuvenated trees where new growth was limited. Cuttings were identified as to clone, crown section, and branch order. Those cuttings receiving cold storage were sealed in plastic bags provided with a moistener, and kept at 0 ± 1° C for 60 days in an upright position.

Immediately prior to sticking, all cuttings were given a five-second dip in 10 percent Jiffy Grow[1]-95 percent ethanol solution. Greenhouse propagating benches were provided with pressurized intermittent misting, 21 ± 3° C bottom heat, and a five-part washed sand to one part sphagnummoss peat mixture. Room temperature was maintained near 10-15° C. Evaporative coolers were used to moderate summer daytime temperatures. The needles were removed from the basal 5 cm of the cuttings prior to benching and cold storage. The cuttings were stuck in the raised propagation benches to the depth of 5 cm with a spacing of 3 x 7 cm within and between rows. Between rooting series the rooting medium was steam sterilized and the room periodically fumigated with Termil for disease control.

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[1] Jiffy Grow - a commercial preparation containing the following: 0.05 percent 2-naphthalene acetic acid, 0.05 percent 3-indole butyric acid, 0.0175 percent boron, 0.01 percent phenyl mercuric acetate.

Evaluation of cutting survival, callusing, rooting, and bud break was made after the cuttings had been in the bench 90 and 120 days. Root quality classifications were as follows: Poor (one to two roots), Fair (three to five roots), Good (six or more roots).

Experiment 1. Age Effect

Age effects were studied using a factorial experiment with five seedling trees, four age classes and six sampling dates over a two-year period. The age classes represented were 4-5, 8-9, 14-15, and 24-26 years. In the 4-5 year age class, more than five trees were included due to the limited number of cuttings per tree. Sampling dates were December 1, 1970; February 1, August 1, October 1, and December 1, 1971; and February 1, 1972. On the December and August dates 80 cuttings per tree were used, whereas 20 per tree were used on the other dates.

Experiment 2. Rejuvenation

Rejuvenation in rooting potential of old trees was studied using the following approaches:

a. Old sheared trees (Figure 1) in two age classes, 25-28 and 35-42, were compared with the non-sheared trees used in Experiment 1. Again five trees in each age class were sampled with the number of cuttings per tree, collection dates, and treatment of cuttings the

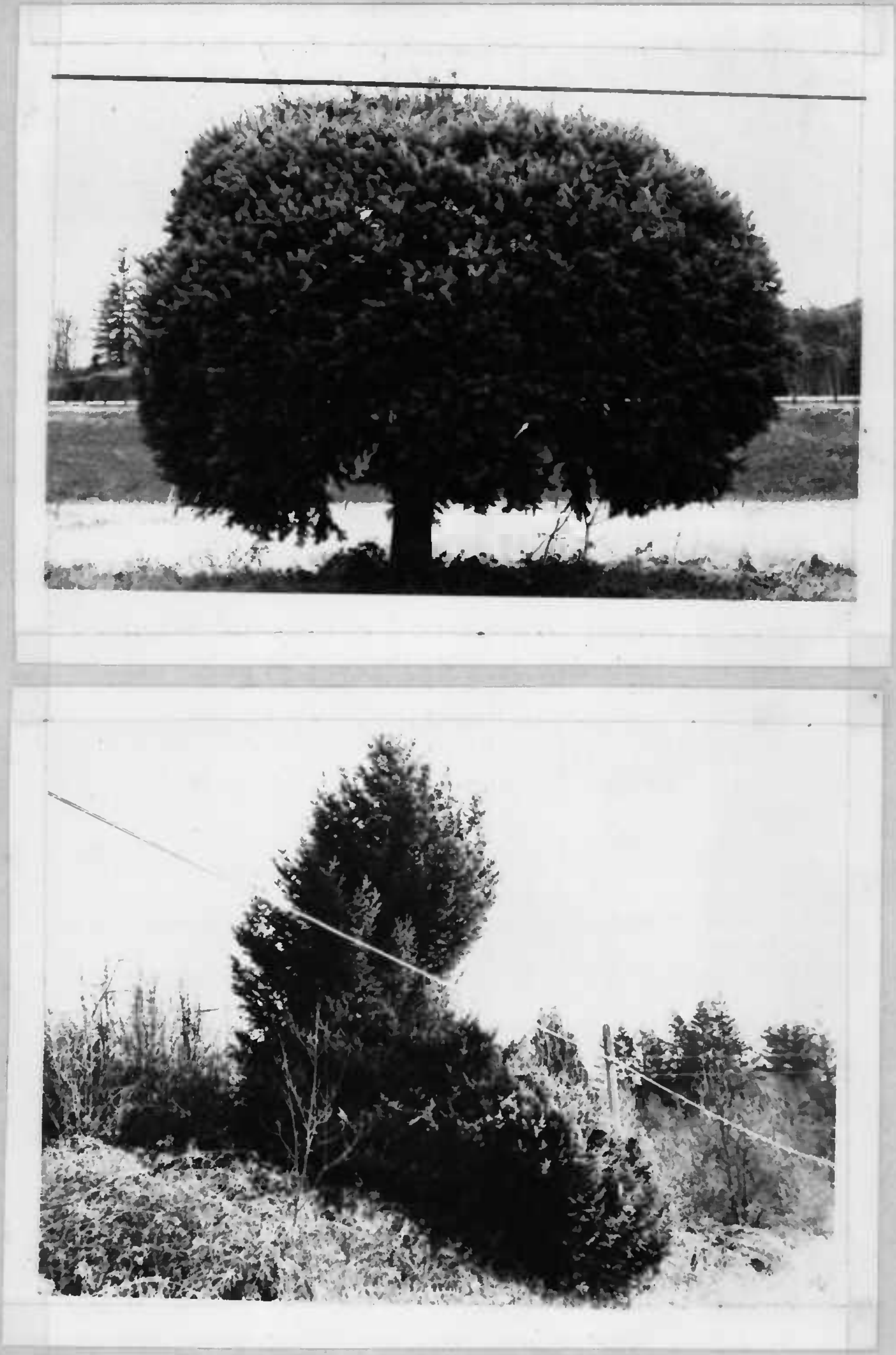

Figure 1. Typical of the sheared (top) and one-half sheared (bottom) trees described in Experiment 2, a and b. Shearing continued over extended period to keep the trees low.

same for both experiments. The trees used in these two experiments were growing in the Willamette Valley, but were not necessarily of the same seed source or growing at the same site.

b. The effects of shearing on rejuvenation were studied further by comparing sheared and non-sheared portions of the same old trees (Figure 1) ranging in age from 25 to 56 years. Three samples of 20 cuttings each from sheared and non-sheared portions of eight different trees were taken on two dates during 1971-72.

c. The effects of successive propagations on rejuvenation of rooting potential was studied by comparing cuttings from clonal material representing: (a) the parent ortet, (b) grafted ramets of the ortet, and (c) rooted ramet cuttings. These plants were all growing in the same area (b and c in the David T. Mason seed orchard near Sweet Home, Oregon, and a in the forests near Sweet Home). Twenty cuttings were sampled from each source on two dates during 1971-72. Five clones ranging in age from 25 to 84 years were included in this study. Rejuvenation resulting from successive propagations was further evaluated by comparing cuttings from 15- to 25-year-old seed orchard understock plants with three-year-old rooted cuttings of these same clones. Eleven clones with samples of 20 cuttings from each source, placed in the rooting bench on two dates, were included in the study.

d. Preliminary studies showed that cold treatment of cuttings was stimulating to rooting on a December 1 collection date. The rejuvenation of rooting potential as a result of chilling was studied by comparing standard 60-day $0^\circ \pm 1^\circ$ C cold storage of cuttings with no cold storage and a 60-day later collection date (winter chilling occurring naturally on the tree).

To further study the effects of cold storage, 4,200 cuttings (Experiment 1 - 20 cuttings/2 branch positions/4 crown positions/4 age classes/5 trees, and Experiment 2 - 20 cuttings/5 positions/2 age classes/5 trees) were harvested August 1, 1971, and December 1, 1971, with ten cuttings from each source being cold stored and benched on each of these dates.

Six-hundred cuttings (Experiment 1 - 10 cuttings/2 positions/ 1 crown level/4 age classes/5 trees, and Experiment 2 - 10 cuttings/ 2 positions/2 age classes/5 trees) from the trees described above, with ten cuttings from each source, were placed in the rooting bench on October 1 and February 1 for comparison.

RESULTS AND DISCUSSION.

Aging

Rooting potential decreased with increasing age of the seedling trees. This response to aging is common to most, if not all, trees and shrubs. However, the results indicate that the decline in rootability of Douglas-fir may not be as rapid as in some other conifers. Differences in rooting potential of December cuttings from trees of different ages are typical of those sampled at other times (Figure 2). However, the best rooting was obtained with cuttings taken from December through February. The effects of tree age on the rooting of cuttings taken at other times were similar. These results show that excellent rooting can be expected from trees up to nine years of age. There was no significant difference in the rooting of cuttings from four- and nine-year-old trees. Up to 100 percent rooting was common in cuttings from some trees in these younger age classes, with an average of 60 percent rooting from December to February. Rooting declined rapidly at tree ages beyond nine years, and reached a very low level (less than five percent at all sampling dates) at 24 years. This decline in rooting does not appear to be due to the onset of flowering, since no evidence of strobili could be observed in the 15-year-old trees or any of the younger trees used in this study. Only the 24-25 year age class showed any evidence of cones or cone development.

Root quality was significantly better in cuttings from younger trees and dropped off rapidly with increasing tree age (Figure 3). The proportion of cuttings with good roots ranged from nearly 50 percent in the four- and eight-year-old trees to zero in the 24-year-old trees.

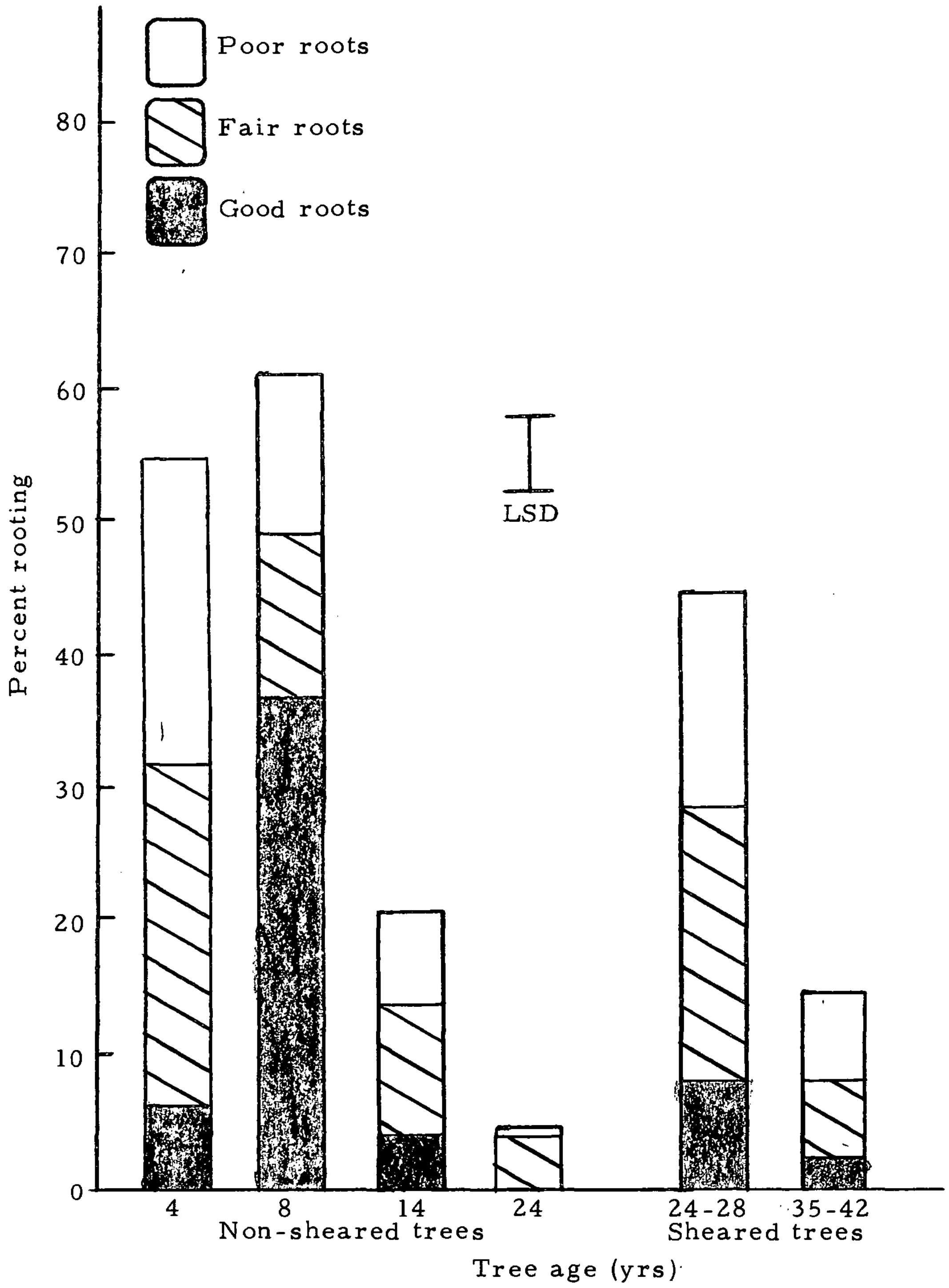

Figure 2. Percent rooting and quality of roots developing on cuttings from different age classes of sheared and non-sheared trees. Cuttings were harvested December 1, 1970, and given 60 days cold storage.

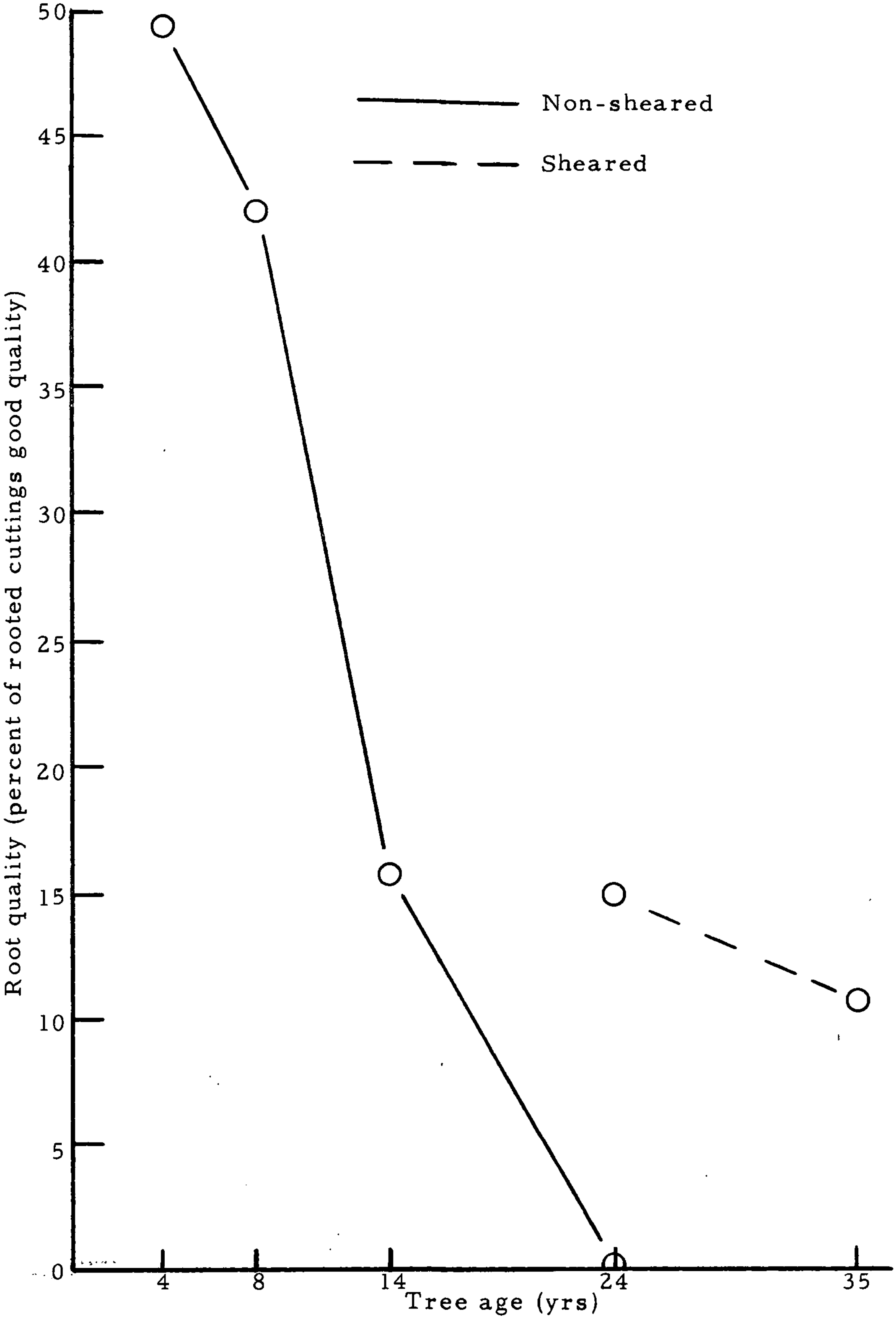

Figure 3. Percentage of cuttings with roots of good quality (more than five roots) from different age classes of sheared and non-sheared trees after 120 days in rooting bench. Dec. 1, 1971.

The effects of physiological aging were noted in other aspects of cutting behavior. There was a significant difference in time required for cuttings to root from trees of different age (Figure 4). In the four- and eight-year-old classes rooting was 75 to 80 percent complete after 90 days, but only 43 and 20 percent complete in the 14- and 24-year-old trees when compared with rooting at 150 days. Rooting was faster in cuttings from trees of all age classes when taken from February to April. This was also true for cuttings harvested in December and cold stored until February. Even during this period of rapid root development, the number of live and callused, but unrooted, cuttings was much higher in the older age classes (Figure 5). Thus, cuttings from old trees have a better chance of rooting if left in the rooting bench longer. The overall root quality was better, a greater number of the initiated roots elongated, if the cuttings were given 120 rather than 90 days in the bench. After 90 days, the rooted cuttings showed a distribution of 24 percent good, 28 percent fair, and 48 percent poor roots. During the longer period from 90 to 120 days the non-rooted, restuck cuttings developed roots of which 42 percent were good, 35 percent fair, and 24 percent poor. This suggests that an evaluation of the ultimate rooting potential in cuttings is not possible after only 90 days in rooting bench.

Earlier studies had shown a high correlation between rooting and bud break in cuttings (119). It was concluded that bud break contributed

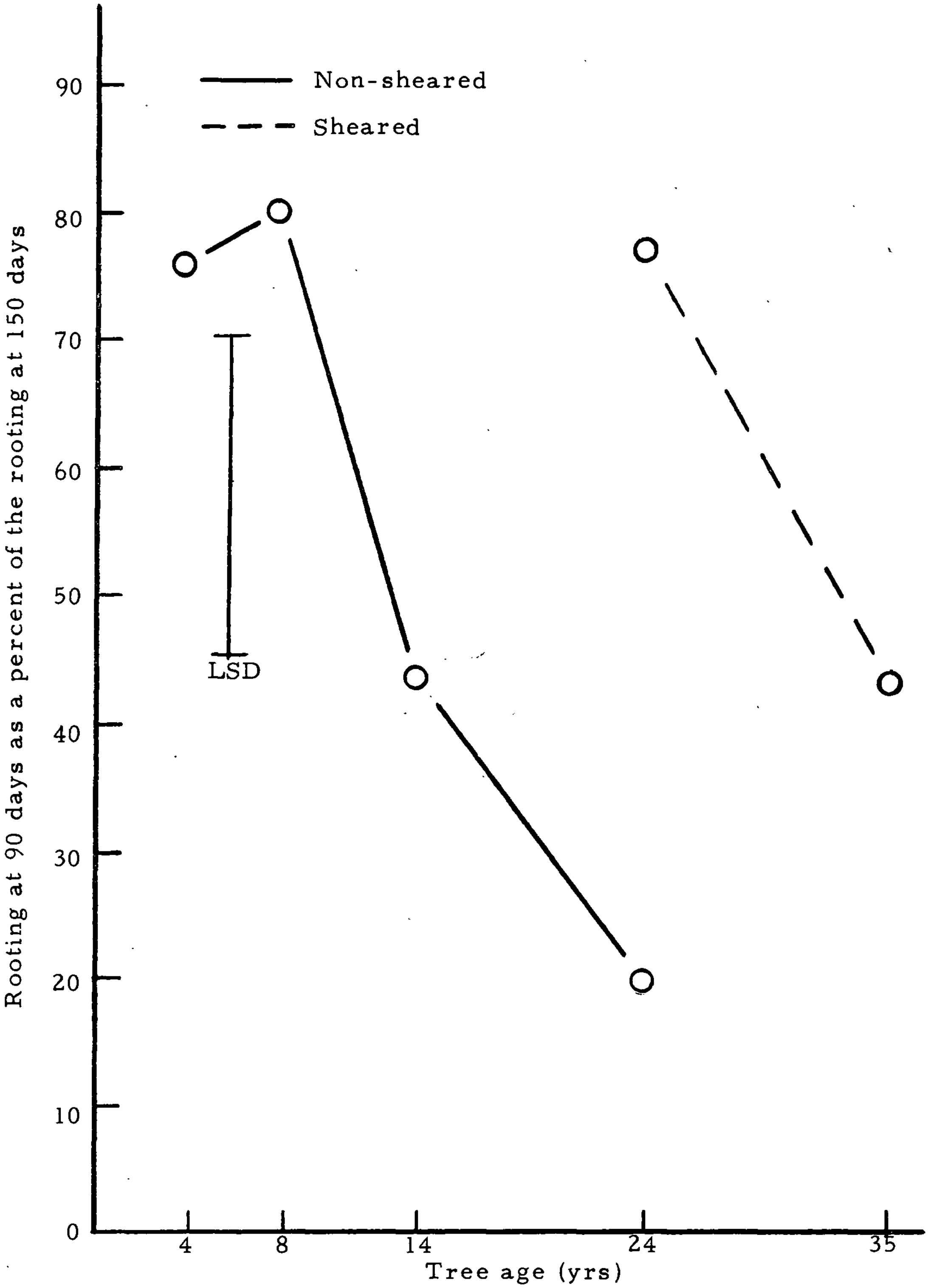

Figure 4. Rate of root development on cuttings from different age classes of non-sheared and sheared trees. Cuttings harvested 12-1-70 and stored 60 days at 0 ± 1° C. Rooting was evaluated after 90 days. Live unrooted cuttings were re-evaluated at 150 days.

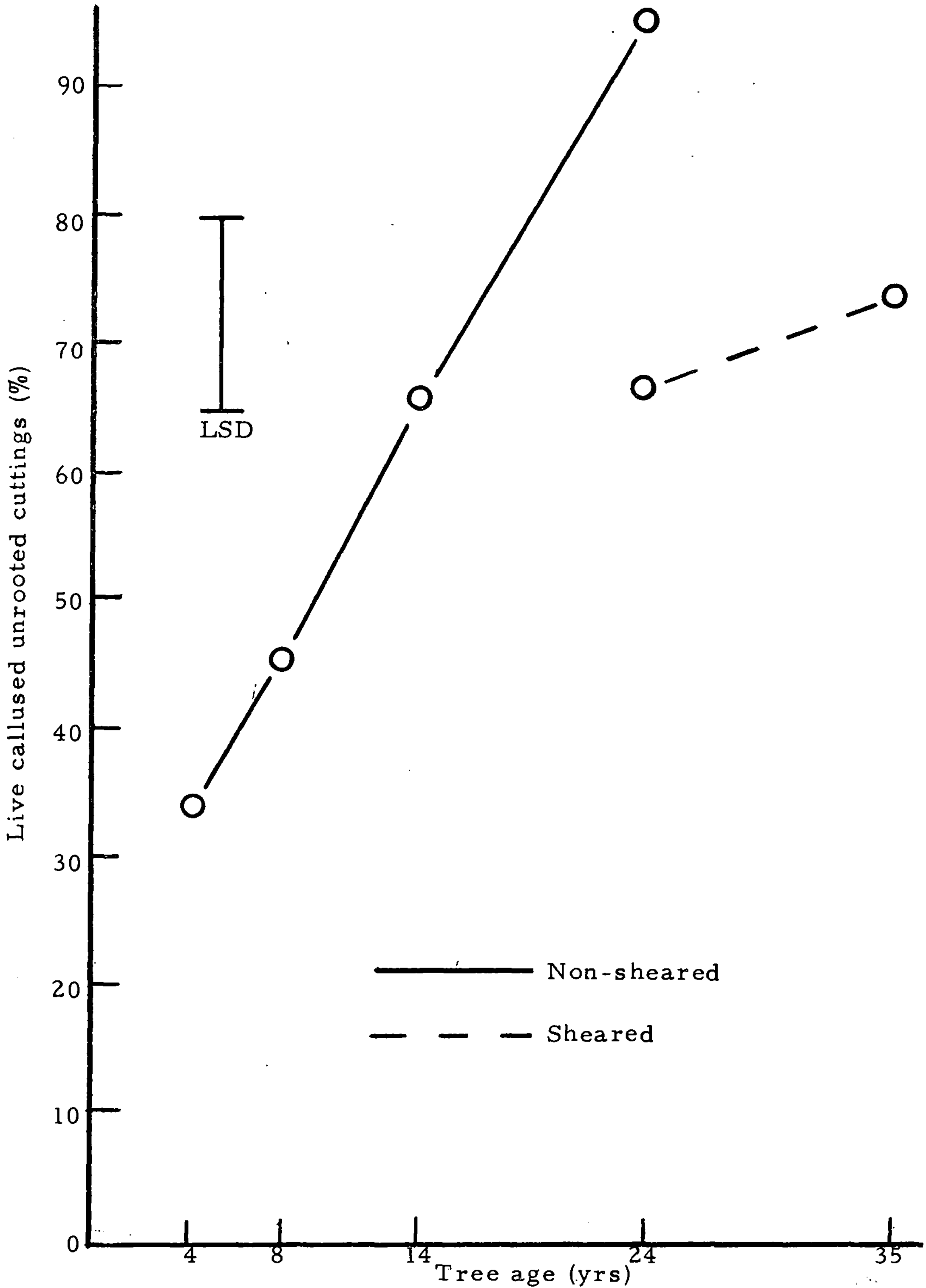

Figure 5. The percent of live, unrooted but callused cuttings after 120 days in rooting bench from sheared and non-sheared trees of different ages. December 1, 1971.

something to rooting. In this study, the December 1, non-stored cuttings rooted well with little evidence of bud break (Table 3). The chilling requirement for rooting is probably less than that for breaking bud dormancy. When the chilling requirement of the dormant bud is satisfied, bud break will often occur without any evidence of root development. There was, however, a significant relationship between tree age and the speed with which their cuttings broke bud in the bench (Figure 6). Since rooting was much better in cuttings from young trees, it is probable that early rooting was contributing to the rapid bud break in adequately chilled cuttings. Based on the length of the longest shoot, cuttings from younger trees always grew more vigorously the summer following transplanting from rooting bench (Figure 7). The amount of shoot growth made by the cutting was proportionately less with increasing age of parent tree. The differences were more evident in February cuttings, that were not stored than in those collected in December and given 60 days cold storage before benching. These differences could be the result of the lower accumulated food (sugars) reserves in the December cuttings. This was suggested by the fluctuations in carbohydrates in Douglas-fir stem cuttings observed seasonally and following cold storage (7). Mortality in the bench and during the first year following rooting was higher when the cuttings came from older trees (Figure 8). This is in agreement with the rooting percentages and quality of rooting obtained from cuttings in the different age classes.

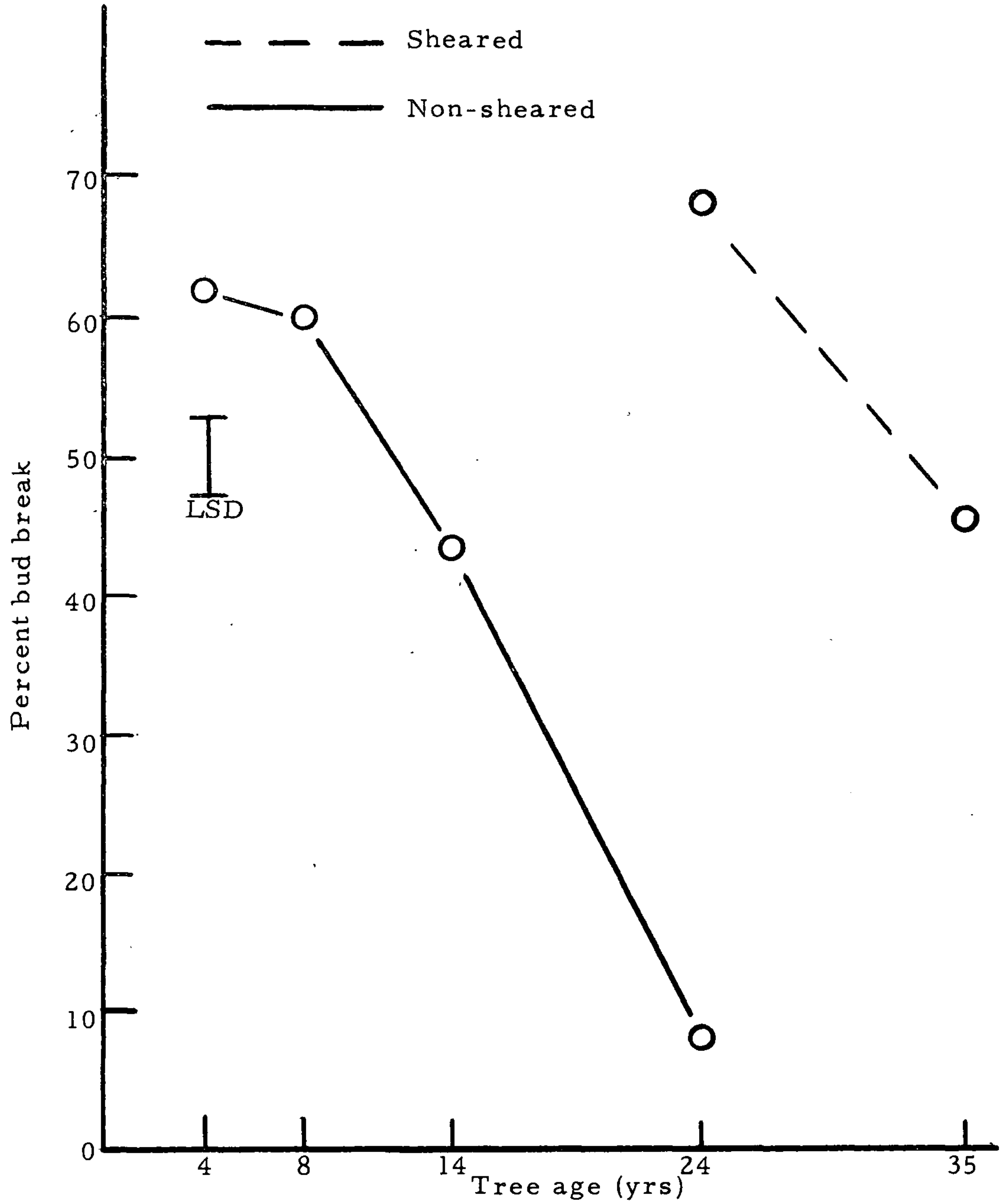

Figure 6. Percent bud break in cuttings from different age classes of sheared and non-sheared trees after 90 days in rooting bench. December 1, 1970 cold stored cuttings.

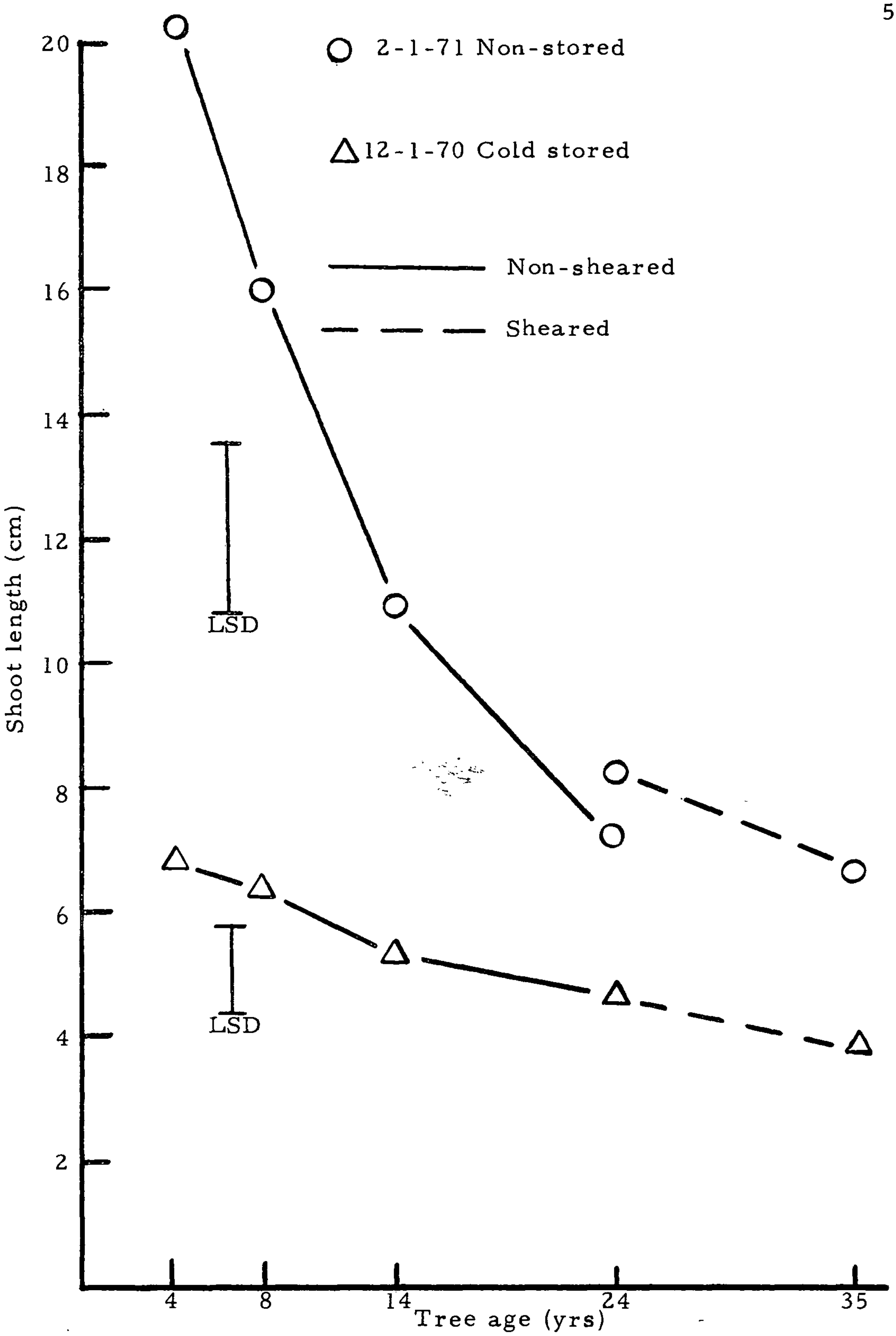

Figure 7. Shoot extension growth on rooted cuttings from sheared and non-sheared trees of different ages. Cuttings were harvested 12-1-71, given 60 days of cold storage and 2-1-71 non-stored. Values are mean length of longest shoot six months after rooting.

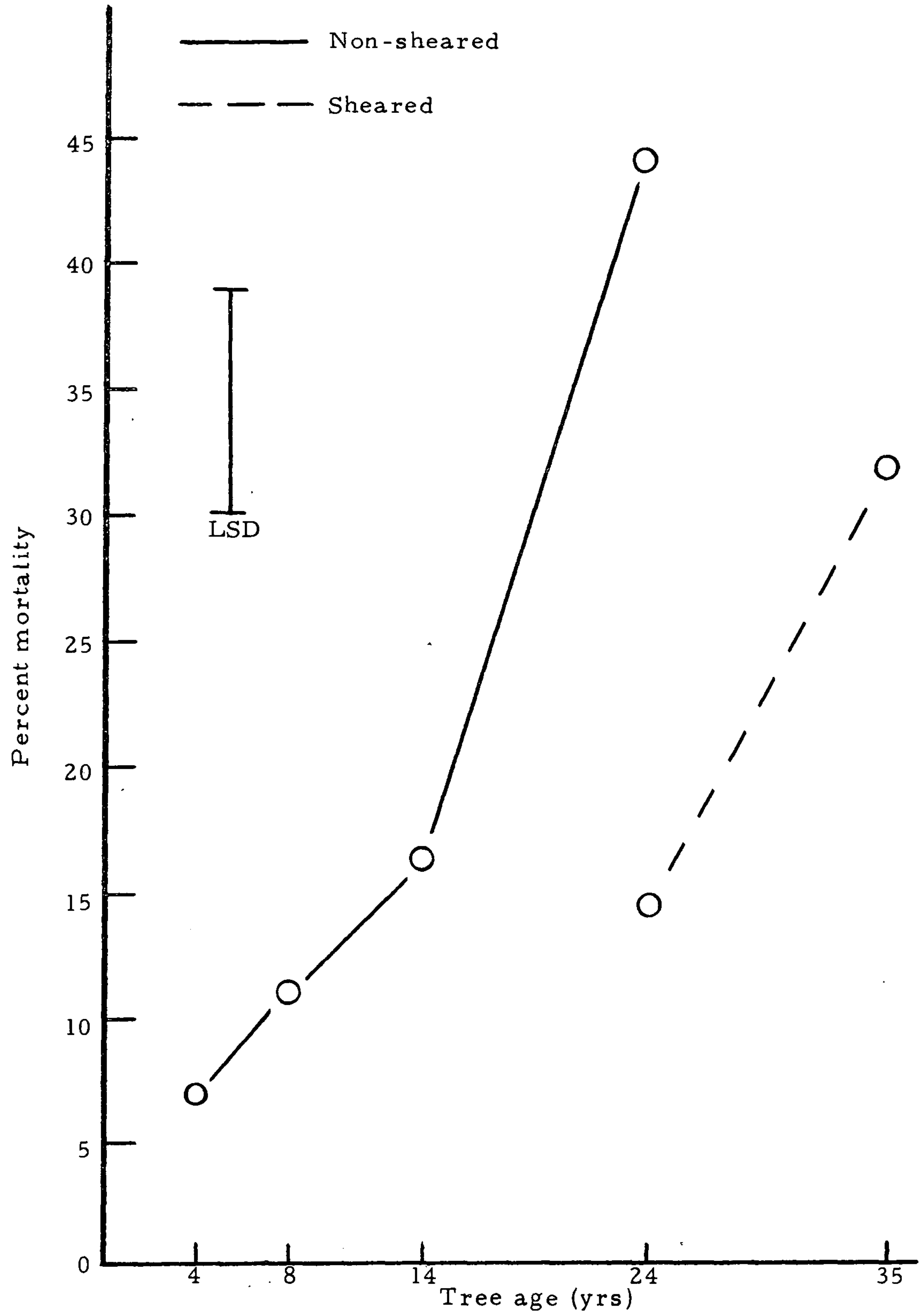

Figure 8. Percent mortality of rooted cuttings from sheared and non-sheared trees of different ages one year after rooting.

The morphological changes which accompany the aging of Douglas-fir trees strongly affect the availability of cutting material, as well as their rooting potential. The shoots low in the crown of older trees are not vigorous and generally lack adequate new growth to supply cutting material. Higher in the crown only the terminal shoots are free of male and female strobili. Fruiting shoots, such as these, have been found to have poor rootability in conifers (35, 158). Some fruiting shoots were rooted in this study, but the percentage was notably lower than for completely vegetative ones. The extreme top crown sections of the tree are often too vigorous to provide good cutting material, as well as being difficult to sample in older trees.

Rejuvenation

Various criteria have been used to distinguish between the juvenile and adult stages. "Most authorities consider that the greater ability of cuttings to root is most closely associated with juvenility" (117, p. 292). Significant rejuvenation in rooting and growth potential occurred as a result of shearing.

a. The Rejuvenation of Rooting by Shearing. At all sampling dates the 24-28 and 35-42 year-old sheared trees, some of which had numerous strobili in the crown, rooted better than the 24-year-old, non-sheared trees. Cuttings from the 24-28-year-old sheared trees generally rooted better than those from the 14-year-old non-sheared trees, but not as good as those from the 8-year-old class (Figure 2; Appendix Table 1). Shearing also improved root quality (Figure 3). The rate of root development was significantly faster in cuttings from sheared trees, being comparable to the much younger, non-sheared trees (Figure 4). Bud break occurred sooner and cutting mortality was lower in cuttings from sheared trees, again reflecting the rejuvenating effect of shearing (Figures 6 and 8).

A comparison of the rooting potential of cuttings from sheared and non-sheared portions of the same tree confirmed the possibility of rejuvenating old clones by shearing, and revealed the localized nature of the response (Figure 1; Table 1). In each of the eight trees, there was significantly better rooting in cuttings from the sheared portions of the tree. Even more significant perhaps was the greater number of trees that proved rootable when cuttings were taken from the sheared portions of the tree. When collected on August 1, 63 percent of the trees proved rootable from the sheared portions of the tree in contrast to only 12 percent when taken from the non-sheared portion. When the December 1 collection was given 60 days of cold storage, 62 and 25 percent of the trees provided cuttings which rooted from the sheared and non-sheared portions respectively. The significant rejuvenation due to shearing was also evident in a seasonal study of rooting as related to leaf carbohydrate levels in sheared and non-sheared trees (7).

Table 1. Percent rooting of cuttings from the sheared and non-sheared portions of the same old trees. Values are the average percentage rooting of 20 cuttings from each portion of eight trees (320 cuttings).

Sample (Date and cold) storage)	Sheared portion	Non-sheared portion	LSD .05
	(%)	(%)	
Aug. 1, 1971	9.3	1.2	7.0
Dec. 1, 1971	10.0	3.8	6.1
Dec. 1, 1971 (C.S.)[a]	20.0	9.4	11.2

[a] C.S. - Cuttings stored for 60 days at 0 ± 1° C.

b. <u>Rejuvenation Effects of Successive Propagation.</u> Stored and non-stored cuttings from cutting ramets rooted significantly better than cuttings from grafts or cuttings from the parent ortet of the same clone (Table 2-A). With the five clones included in this study and sampled December 1, 1 percent of the cuttings from the parent, 9 percent from the grafted ramet, and 45 percent from the cutting ramet rooted. Cuttings from only one of the parent trees rooted, while those from three of the grafted trees and from all five of the cutting trees rooted. The results were similar for cuttings taken on December 1 and cold stored 60 days at 0 ± 1° C and for February non-stored cuttings (Table 2-A). Since these trees were all growing in the same area under similar environmental conditions and represent comparisons within clones, it was evident that the cutting ramets were rejuvenated.

Table 2. A comparison of the rooting potential of cuttings of the same clone but from different sources. Values are percentage rooting.

A. Ortet versus grafted ramet versus cutting ramet (average of five clones.

Harvest date		Ortet	Source of cuttings		LSD .05
			Grafted ramet	Cutting ramet	
		(%)	(%)	(%)	
Dec. 1	Non-stored	1.0	9.0	45.0	10.3
Dec. 1	Cold-stored	7.0	6.0	48.0	12.0
Feb. 1	Non-stored	0.0	2.0	26.0	4.1
B. Ortet versus cutting ramet (average of 11 clones).					
Dec. 1	Non-stored	12.7		39.5	8.3
Dec. 1	Cold-stored	9.0		36.0	17.7

In a separate study, cuttings from cutting ramets rooted better than cuttings from parent seed orchard understock trees of the same clones (Table 2-B). Eleven clones were included in the study, with 12.7 percent of the parent ortet cuttings and 39.5 percent of the cutting ramet cuttings rooting from the December 1 non-stored samples. December 1 samples following cold storage gave comparable results (Table 2-B). Again a greater proportion of the clones were rootable as cutting ramets than they were as ortets. The quality of roots was also better from the cutting ramet trees with 30 percent of the rooted cuttings having good roots as compared to 11 percent from the ortet.

c. The Effects of Cold Storage. The literature suggested a rejuvenation stimulus due to cold (42, 76). Earlier reports (119) and a preliminary study (1970-71) showed that 60 days cold storage stimulated rooting, if given during the dormant period (Table 3). Cuttings taken on December 1 and given 60 days of cold storage rooted better than those not given the cold treatment and as good as those collected 60 days later, after having received natural chilling on the tree. The studies conducted during 1971-72 involved several collection dates and were designed to compare non-stored cuttings with those given 60 days cold storage or left on the tree to receive natural chilling (Table 3). Cuttings stored before bud dormancy was fully developed did not react favorably to the cold treatment. Thus, August

Table 3. Rooting response and bud break in cuttings from sheared and non-sheared Douglas-fir trees of different age classes receiving various cold treatments.

Treatment[a]	Tree age (years)						Mean
	Non-sheared trees				Sheared trees		
	4	8	14	24	24-28	35-42	
Percent rooting							
1	--	59	--	--	--	8	--
2	79	97	29	5	54	15	46.5
3	65	72	21	0	26	28	35.3
Mean	72	76	25	3	40	17	
1	57	56	16	0	14	12	25.8
2	92	56	44	0	16	4	35.3
3	95	68	32	14	14	6	38.2
Mean	81	60	31	5	15	7	
	L. S. D. .05		Age 6.23		Cold treatment 7.3		
Proportion of rooted cuttings good quality							
1	28	21	0	0	17	0	13.2
2	70	14	9	0	13	0	21.2
3	42	50	6	14	29	0	28.2
Mean	47	28	5	5	20	0	
Percent bud break after 120 days in bench							
1	32	20	20	0	26	12	18.2
2	100	36	96	26	94	76	71.3
3	100	62	98	72	72	66	79.7
Mean	77	39	71	33	67	51	
	L. S. D. .05		Age 7.97		Cold treatment 9.43		

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[a] 1 = harvested December 1, non-cold-stored; 2 = harvested December 1, given 60 days cold storage at 0 ± 1° C; 3 = harvested February 1, non-stored (receiving natural chilling on the tree).

1, non-stored cuttings rooted better than those receiving cold storage. Dormant, October 1, non-stored cuttings did not root. Other studies have shown that September and October cuttings root better after cold storage (7, 117). The December cuttings without storage rooted 26 percent compared with 35 and 38 percent for those receiving 60 days cold storage or 60 days further chilling on the tree, respectively. Bud dormancy was broken in the latter two cases, and the cuttings appeared to be at the same stage of development in the bench, while the non-stored cuttings showed little sign of bud activity.

The post-severence cold storage of cuttings in which bud dormancy had developed was beneficial to rooting in all tree age classes whether sheared or not. Since rooting was not improved by cold storage of cuttings at sampling dates when the buds were not dormant, and cuttings from different tree ages did not respond differently, increased rooting of the cold stored dormant cuttings was probably due to some aspect of the removal of dormancy rather than any rejuvenation response. The chilling of the dormant shoot, whether in cold storage or in natural outside winter temperatures, probably stimulated rooting via a drop in inhibitor levels or a rise in promotive hormones that counteract the inhibitor(s). The more rapid and extensive bud break which accompanied the improved rooting of these cuttings suggests a removal of dormancy response (Table 3). It should be remembered, however, that the amount of chilling required to stimulate rooting may be less than that required to break bud dormancy. It was proposed that chilling stimulated rooting by causing a conversion of accumulated starch to sugar, but this was not confirmed in a study on the needle carbohydrate reserves before and after storage (7).

The nature, significance and complexity of aging and rejuvenation have puzzled plant physiologists for years. The physiological responses associated with rejuvenation are not clear. The findings reported here, however, are consistent with those for other conifers. Jacops (68) states that rooted cuttings undergo a degree of rejuvenation, and that sometimes juvenile leaves and buds are produced. Fielding (34) states that "hedged" trees are physiologically juvenile and root more readily than "unhedged" trees of a similar age, and that cuttings from cuttings root more readily than cuttings from seedlings; a finding also reported by Ooyama and Toyoshima (110). Patton (113), working with a Pinus sp., found that cuttings from cuttings rooted better than cuttings from seedlings up to nine years of age, at which time the differences between the two sources had disappeared.

The positive effects of repeated propagation by cuttings could be explained by the renewal of the root system which delays aging. Delayed aging may also account for the response observed in this study with sheared trees, since the shearing process was continued for several years. However, some already physiologically old, flowering trees were also shown to be rejuvenated by shearing and successive propagation, and the shearing response was found to be localized in the old tree, all of which suggest more than delayed aging. Increased availability of materials from the roots, however, may be contributing to improved rooting both for shearing and successive propagation.

SUMMARY AND CONCLUSIONS

In general, the results reported here for Douglas-fir resemble those for other conifers. The rooting potential remains high in young trees up to nine years of age, and then drops off rapidly, reaching a very low level between 14 and 25 years. It appears that individual trees of this species vary considerably in the rate at which cutting rootability declines with age. Trees over 15 years of age appear to differ much more than young trees in cutting rootability. The older trees are difficult to establish from cuttings, but the cuttings of a few of them develop roots quite readily. The rate of physiological aging thus appears to vary from clone to clone. Root quality, as well as rootability, declines with increasing age of the parent tree. The buds on cuttings from old trees are slower to break in the rooting bench, and produce less vigorous shoot growth in subsequent growing seasons. Mortality is hence greater in the cutting bench and nursery row in cuttings from old trees.

While it can be concluded that rooting and root quality decrease with age of the ortet, there is considerable evidence that old clones can be rejuvenated by heavy shearing, and successive propagation. Cuttings from established cutting ramets show the greatest potential for rejuvenation even for clones up to 84 years from seed. Genetically identical trees (clone) with identical chronological age appear to be quite different physiologically, as evidenced by the increased rooting potential of cuttings from grafted and more particularly of cutting ramets. Cutting ramet plants show more juvenile morphological characteristics, such as differences in needle and bud form and lack of flower production, than the ortet or grafted ramet trees.

Cold storage of dormant cuttings was shown to improve rooting, but this was true for cuttings of both young and old trees.

Prepared for Submission to Forest Science

THE INFLUENCE OF TOPOPHYSIS, CYCLOPHYSIS, AND GENOTYPE ON THE ROOTING AND SURVIVAL OF DOUGLAS-FIR STEM CUTTINGS

ABSTRACT. Over 12, 000 stem cuttings were taken at different sampling dates over a two-year period to study the effects of cutting origin on the rooting of Douglas-fir shoots. Crown position (cyclophysis) was shown to have little influence on rootability of trees up to 24 years of age. Branch order positions (topophysis) were found to be important in rooting, with first order lateral (large) and second order terminal positions rooting better than first order terminal, first order lateral (small), or second order lateral cuttings. Terminal and lateral cuttings taken at random showed little difference in rooting potential with the former rooting best in the pre-dormant state and the latter best in the post-dormant period. Cuttings from imposed upright or downward branches did not root as well as horizontal controls. Cuttings from different growth angles on sheared trees were not significantly different in percentage rooting after 120 days. Of the variables studied, genotype had the greatest influence on cutting rootability with the greatest differences occurring among the physiologically older clones.

ADDITIONAL KEY WORDS. <u>Pseudotsuga</u> <u>menziesii</u> (Mirb.) Franco, branch position, crown level.

THE GENETIC variability in shoot rooting potential, like other plant characteristics found in mixed seedling populations, is as evident in forest conifer species as those under cultivation. Differences in rooting potential between seemingly similar trees can often obscure the effects of other treatments. This has been reported in Douglas-fir (1, 58), as well as other forest species (29, 34, 35, 135, 137).

Positional effects due to different crown sections, or branch orders within the same crown section, are also evident in the rooting and growth of cuttings from woody species. Classical examples of position effects are found in the studies of Hedera helix and Araucaria excelsa. In the case of ivy, Molish (100) reported that young plants had a plagiotropic growth habit with two- to five-lobed leaves, whereas older plants had orthotropic growth, ovate leaves, and were covered with flowers. Cuttings rooted from these two types of plants or different sections of the same plant retained their respective plagiotropic or orthotropic growth habits, and showed great differences in rooting potential, the mature phase being nearly impossible to root. The work with Norfolk-Island-Pine, conducted by Vochting in 1904 and discussed by Wareing (152), also shows the importance of shoot position in selecting cuttings. Cuttings from apical shoots or axillary buds along the main stem gave normal orthotropic radially symmetrical plants, whereas cuttings from side branches grew plagiotropically.

The influence of cyclophysis on rooting and growth habit has been demonstrated for several forest conifers (27, 51, 106, 130, 133). Results differ, but generally cuttings from the lower part of the crown have rooted best. In younger trees these differences may not be as important (29). Fielding (34), however, found cuttings from trees over 20 years of age gave uniformly low rooting percentages irrespective of their source in the crown.

Topophysis has been reported to significantly influence rooting and growth of forest conifers. Lateral shoots have generally rooted best (32, 59, 73, 74, 134, 146). Other studies, however, show no differences (106) or show the terminal shoots to root best (35). It has been suggested that difference due to crown section and shoot order may be largely a result of shoot thickness and size (35).

This study was done to determine the rooting and survival of Douglas-fir stem cuttings from: (a) genotypes within different tree age classes ranging from the juvenile to the mature, (b) different crown sections, and (c) different branch orders within the same crown section of the tree, at different sampling dates.

MATERIALS AND METHODS

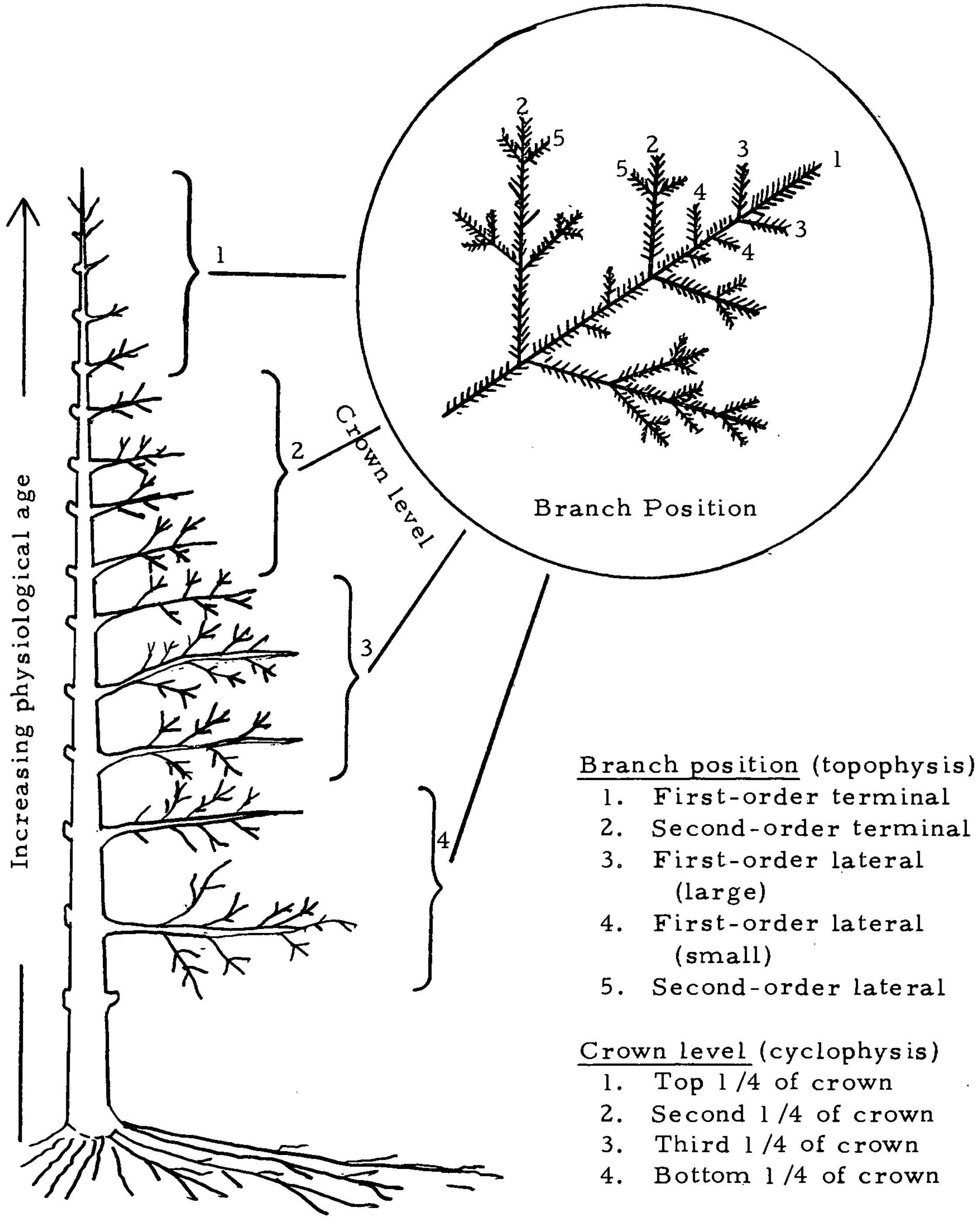
Stem cuttings were harvested from Douglas-fir trees growing near Corvallis, Oregon, during 1970-72. A description of the plant materials, cutting preparation, and propagation procedures has been previously described (7).

Genotypic and positional effects were studied using a factorial experiment with cuttings selected from at least five trees in each of four age classes (4, 8, 14, and 24 years), two branch order positions (terminal and lateral), and four crown levels (Figure 1). Five samples of ten cuttings from each position of each tree were included in this study during 1970-72. Each sample was analyzed separately.

Branch order effects were studied in more detail by comparing shoot rootability within five branch orders of three 9-year-old, field-grown trees. Branch order positions compared were (1) first order terminal, (2) second order terminal, (3) first order lateral (large), (4) first order lateral (small), and (5) second order lateral (Figure 1). Three samples of ten cuttings from each position of each tree were analyzed in this experiment.

Old sheared trees growing under utility lines and sheared annually provided an abundance of cuttings representing different growth angles and positions. In a separate experiment, terminal cuttings were selected according to their growth angle on the tree and identified in degrees from horizontal (0 degrees) as follows: (1) 90-60°, (2) 60-30°, (3) 30-0°, and (4) less than 0°. Laterals were taken at random. Six samples of ten cuttings were taken from each position on ten sheared trees in two age classes (24-28 and 36-41 years) during 1970-72.

The influence of imposed position on rooting was determined in



Branch position (topophysis)
1. First-order terminal
2. Second-order terminal
3. First-order lateral (large)
4. First-order lateral (small)
5. Second-order lateral

Crown level (cyclophysis)
1. Top 1/4 of crown
2. Second 1/4 of crown
3. Third 1/4 of crown
4. Bottom 1/4 of crown

Figure 1. Topophysis and cyclophysis. Diagram showing the physiological age levels and branch order positions used in this study.

another experiment by tying whole branches from throughout the crown into an upright or down position. Terminal and lateral cuttings from these positions were compared with controls in each crown section. In August, 1970, these positions were imposed on branches in five 14-year-old trees. In March, 1971, these positions were imposed on branches in three 15-year-old and three 9-year-old trees. In each year the cuttings were collected December 1 and cold stored before rooting. In the second year, the main leader of the tree was removed in an effort to better maintain the imposed position.

RESULTS AND DISCUSSION

Based on differences observed in physiological development of shoots at different positions within the tree, and reports in the literature, differences in rooting and growth behavior were expected of cuttings from different parts of the crown and positions within a given crown section. The differences in rooting potential due to cyclophysis were not as great for Douglas-fir as has been reported for other species. Topophysis, however, significantly affected rooting.

Topophysis

Differences were small, but the influence of branch order on rooting potential appears dependent on the stage of shoot development. Figure 2 gives the results of a comparison between terminal and lateral

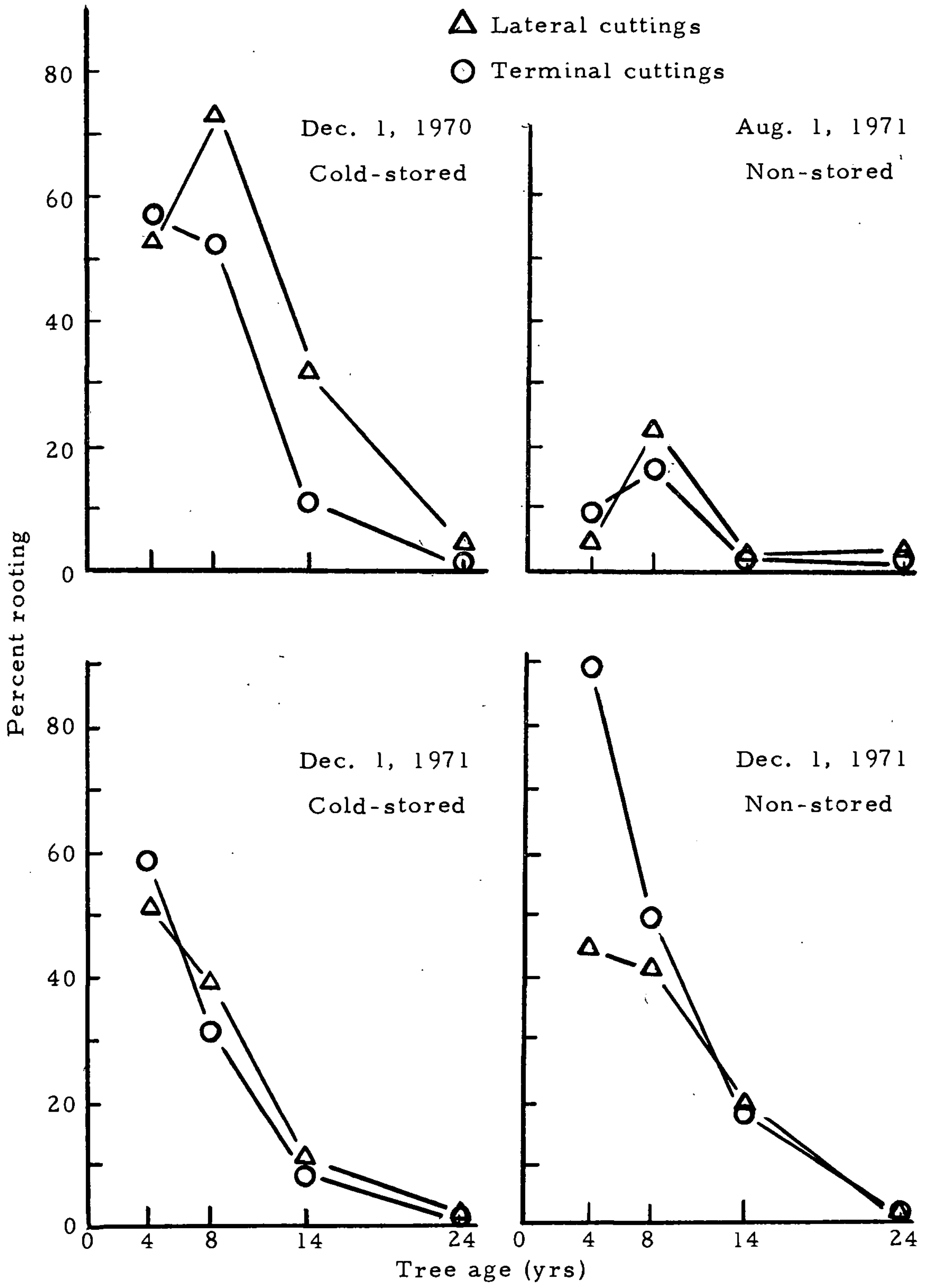
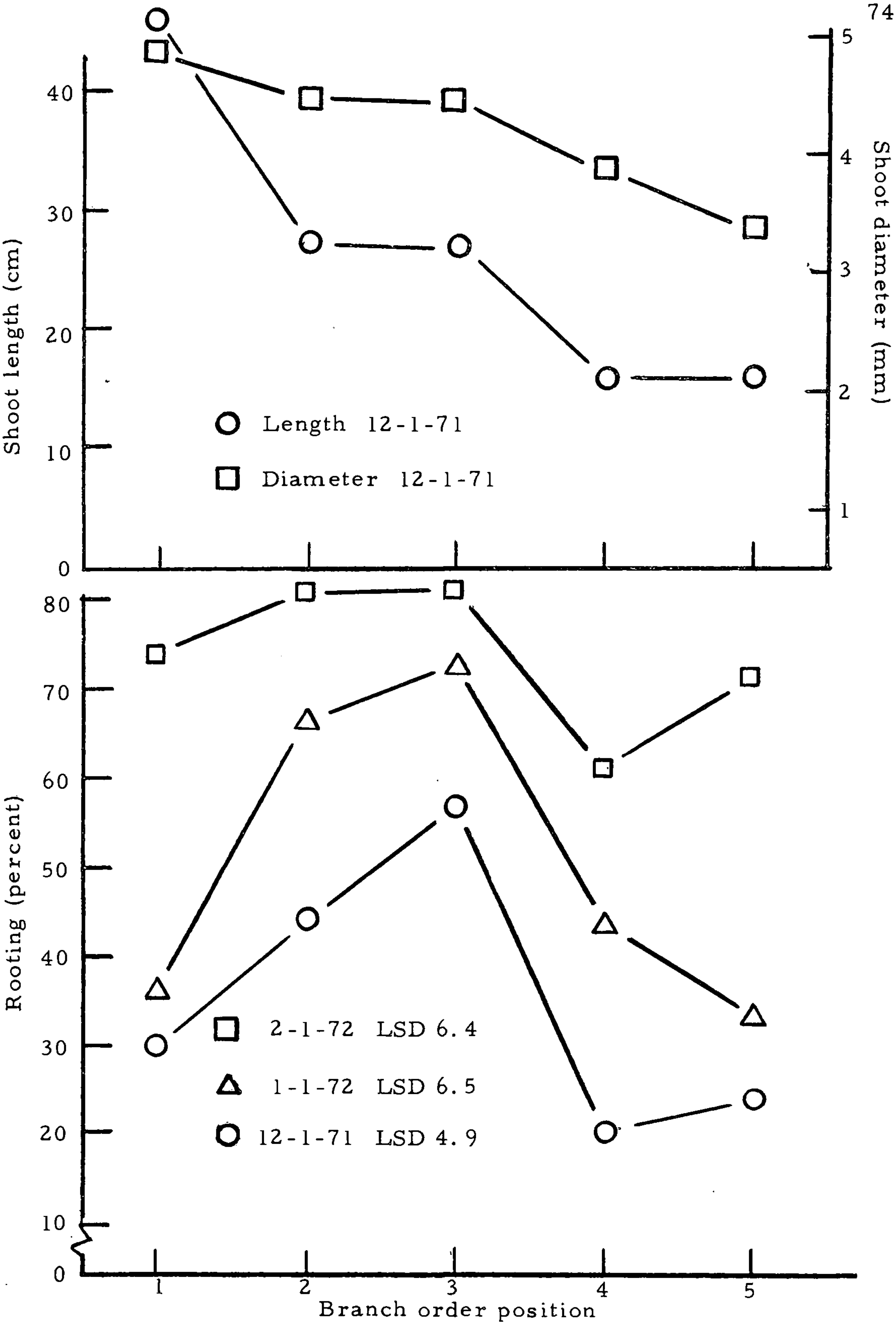


Figure 2. The effects of branch order position on the rooting of cuttings of Douglas-fir trees of different ages. Difference due to branch order position was not significant at .05 level.

cuttings at different sampling times and age classes with and without post-severence cold storage. Eight hundred cuttings from each of these positions, and representing various crown positions, were compared in each of these four samples. The August 1 cold-stored cuttings are not included since this treatment proved detrimental, with less than one percent rooting. Terminal cuttings rooted slightly better than laterals when taken in the pre-dormant condition (August 1), but the difference was not significant. Cuttings taken in December gave slightly better rooting from lateral positions. Terminal cuttings from four-year-old trees rooted better than laterals at all dates sampled, whether stored or not. Other differences noted between terminal and lateral cuttings sampled in December included a significantly greater proportion of the rooted lateral cuttings with more than five roots (good quality). A greater percent of the terminal cuttings were callused but not rooted and more terminal cuttings were dead in the bench with a higher mortality rate the growing season following rooting. In general, however, terminal cuttings showed greater extension growth the summer following rooting, the principal shoot averaging 9.3 cm in length compared with 7.2 cm for lateral cuttings. The younger clones grew more than the older ones. These and other observations suggest that terminal cuttings may recover an orthotropic growth habit sooner than laterals.

The more refined study of five different branch order positions revealed that random selection of terminal and lateral cuttings is inadequate for determining the influence of branch order on rooting (Figure 1). A comparison of these five positions showed that cuttings from position three (first order lateral - large) rooted best on the three winter sampling dates (Figure 3). From three trees, over 80 percent rooting was obtained from positions two (second order terminal) and three when cuttings were harvested February 1. Position one cuttings (first order terminal) were considerably more vigorous, as indicated by the current season's shoot length and diameter at 15 cm from the tip, but cuttings from this position did not root as well, and had a higher mortality rate. Cuttings from positions four (first order lateral - small) and five (second order lateral) were least vigorous and gave the poorest rooting. Root quality was also best in cuttings from positions two and three. The proportion of the total rooted cuttings with good root quality was 21, 36, 35, 6, and 6 percent from positions one, two, three, four, and five, respectively.

By combining the terminal and lateral cuttings of this study into separate categories, the differences due to position are erased. Terminals from the December 1 harvest gave 36.6 and laterals 36.6 percent rooting, respectively. January samples rooted 51.7 and 51.1, and February 1 samples 71.6 and 63.3 percent, respectively, for terminals and laterals. These results show the necessity for identifying branch order position in comparing terminal and lateral cuttings.


Shoot length (cm)
0
10
20
30
40
Shoot diameter (mm)
1
2
3
4
5
Length 12-1-71
Diameter 12-1-71
Rooting (percent)
0
10
20
30
40
50
60
70
80
2-1-72 LSD 6.4
1-1-72 LSD 6.5
12-1-71 LSD 4.9
1
2
3
4
5
Branch order position

These differences coupled with the interaction due to season of collection (that is, terminals generally rooting best in autumn and laterals best in winter), and the variability due to age and genotype, not to mention the differences due to environmental conditions, indicates why researchers have often appeared to obtain conflicting results.

Cyclophysis

Randomly selected terminal and lateral cuttings from various crown sections of trees of different age showed no significant difference in rootability (Figure 1). A total of 1,600 cuttings from each crown level were compared, 400 for each of the four samples illustrated in Figure 4. Slightly better rooting was observed in cuttings collected from the lower crown sections in December, but these differences were not significant at the .05 level. In the August 1 collection the top level rooted slightly better than those from the other sections. There was no significant interaction of crown level with the age classes or branch order positions included in this study, with regard to total rooting, root quality, dead cuttings, callusing, or bud break. None of these factors were significantly different from one crown level to the other. These results are not in agreement with those reported previously for this and similar species. Achterberg (1) reported that spruce and Douglas-fir rooted best if shoots were taken from the lower parts of the crown, but that large individual

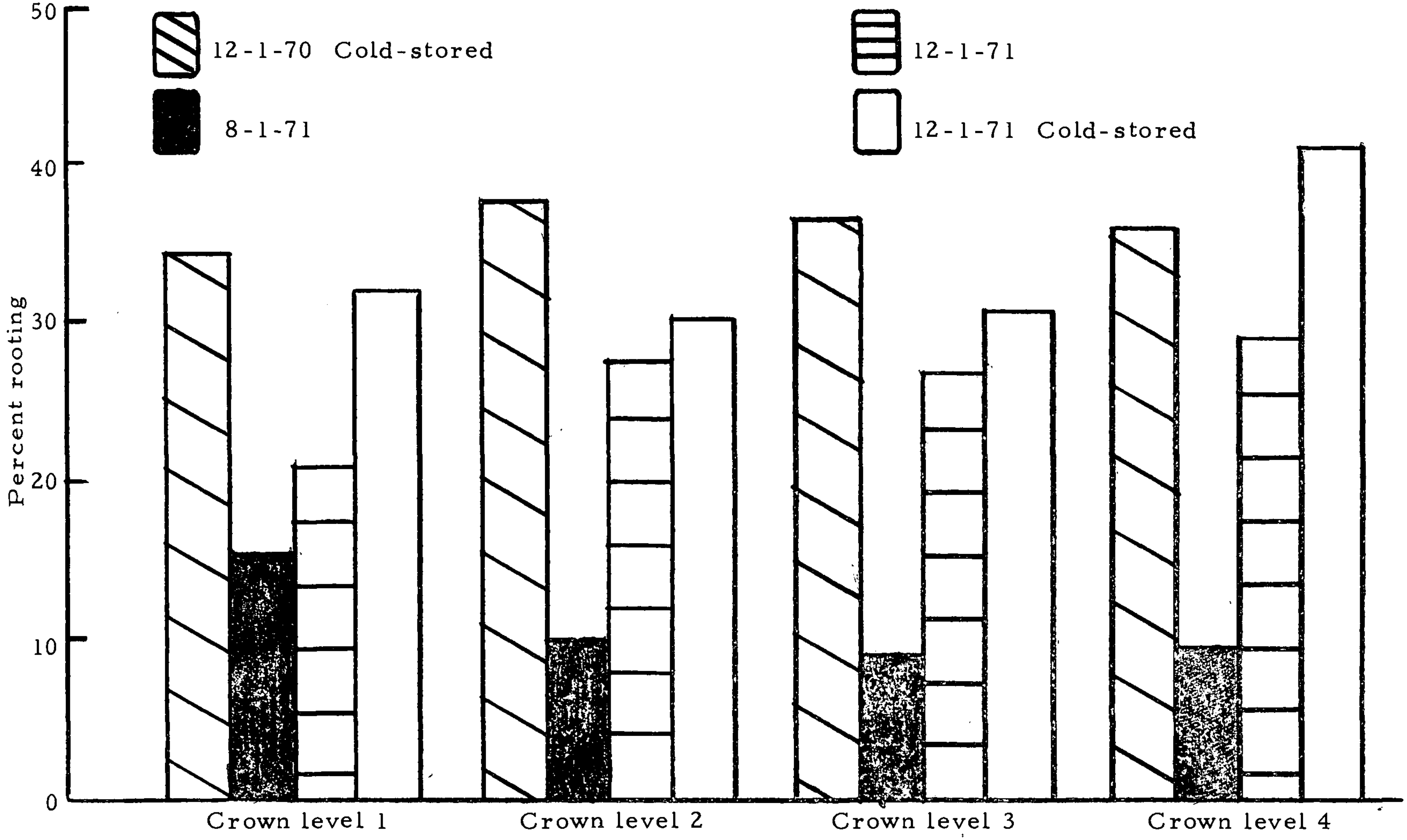


Figure 4. The percentage of cuttings rooting from different crown sections of Douglas-fir trees. Four samples are included for comparison. Dates are for time of harvest. Values are the mean of four age classes. Differences due to crown level are not significant at .05 level for any harvest date or cold storage treatment.

differences between trees often obscured the effects of other treatments. Grace (51) showed that Norway spruce gave rooting percentages of 48 and 86 for the upper and lower crown sections respectively. These results, however, were obtained from one 18-year-old tree. The lack of agreement in results reported here and those obtained in similar studies may be due to differences in sampling date, sample sizes, tree age, or genetic differences in parent trees. The large number of cuttings, representing more than 20 trees, used in this study is convincing evidence that there is little or no difference in cutting rooting potential due to crown level in Douglas-fir trees up to 24 years from seed.

Imposed Positions

Cuttings from imposed branch positions did not root as well as those from unaltered control branches (Table 1). Terminal and lateral cuttings from upright, downward and control branches were compared. The reason for this response is not known, but could be due to a change in the hormone balance during the nine-month period when these branches were tied into these abnormal positions. How these treatments will affect the growth of the ramet plants therefrom is not known at this time.

Table 1. The effect of an imposed orientation of parent shoots on their subsequent rooting potential. 1971.

Imposed position of parent shoot	Percent rooting Terminal	Percent rooting Lateral	Mean
Upright (↑) vertical	10.0	2.5	6.3
Down (↓) vertical	8.3	5.0	6.7
Control - horizontal	25.0	26.6	25.8
LSD .05	Imposed position -		14.8

Growth Angle on Sheared Trees

Growth angles of terminal shoots taken throughout the crown from old sheared trees (24-42 years) did not influence cutting rootability during a 120-day rooting period (Table 2). The more orthotropic the shoot was on the tree, however, the greater was the mortality of its cuttings. Rate of rooting was determined to be slower in cuttings from older trees (7), thus the ultimate rooting of cuttings would probably have been greater in the plagiotropic shoots, if the rooting period had been longer. The most orthotropic shoots were most vigorous, had thicker bark, a larger pith section, and fewer needles per centimeter, which probably accounts for the higher mortality and poorer root quality in these cuttings in comparison to their more plagiotropic counterparts. The more orthotropic the shoot growth was on the ortet tree, however, the more orthotropic the cutting ramet plant was 18 months after rooting (7). The latter may prove to

Table 2. The effects of shoot growth angle within the crown of sheared Douglas-fir trees on their rooting potential. All cuttings were harvested December 1. Three samples are included for comparison: (1) 1970 cold-stored, (2) 1971 non-stored, (3) 1971 cold-stored.

Sample number	Angle of terminal shoot growth (degrees from horizontal)				
	60-90° (↑)	30-59° (↗)	0-29° (→)	<0° (↘)	Lateral
Shoot length (cm)					
1	42	36	27	16.5	14.1*
Shoot diameter (mm)					
1	6.3	5.6	4.0	3.4	3.3*
Percent rooting					
1	30.3	35.0	27.7	28.0	--
2	10.0	13.0	13.0	14.0	13.0
3	23.0	18.0	10.0	14.0	15.0
Percent dead in bench					
1	43.0	32.0	17.3	10.0*	--
2	10.0	10.0	8.0	1.0	3.0
3	27.0	3.0	6.0	0.0	4.0*
Percent callused - live unrooted					
1	29.0	30.0	52.0	61.0*	--
2	72.0	71.0	78.0	66.0	68.0
3	47.0	79.0	83.0	86.0	81.0*
Percent bud break in bench					
1	35.7	50.3	70.0	40.7*	--
2	3.0	11.0	19.0	25.0	24.0
3	79.0	56.0	85.0	85.0	83.0

*Difference due to growth angle or position significant at .05 level.

be a more important consideration in the establishment of cutting ramet trees than the small differences observed in rooting of cuttings from these different growth angles.

Genotype

Throughout these studies, genotype was observed to be a most important factor affecting cutting rootability. Trees growing side-by-side under apparently identical environmental conditions, and of the same chronological age, showed extreme variability in rooting potential (Table 3). These differences were not as great in younger trees as older ones. This confirms the findings reported earlier (125) that seedling differences in rooting potential become more exaggerated with age, and suggests that the aging process proceeds more rapidly in some genotypes.

CONCLUSIONS

The variation in rooting potential due to genotype, especially among physiologically old trees, should be considered in all rooting studies. When few genotypes are used in a given experiment, variation due to genotype may obscure other effects. This should also be kept in mind when studying the literature on rooting this and similar species. Only a small difference in rooting was found in cuttings from different crown sections or between randomly selected terminal and lateral cuttings.

Table 3. Percent rooting of cuttings at different harvest dates over a two-year period for individual trees of different ages.

		Date and treatment						
Clone	Age (yrs)	Dec. 1 '70 CS[a]	Feb. 1 '71 NS[b]	Aug. 1 '70 CS	Dec. 1 '71 NS	Dec. 1 '71 CS	Feb. 1 '70 NS	Mean
		Number of cuttings /clone						
		80	20	80	80	80	20	
young	4	55	53	6	57	68	95	55.6
30	8-9	35	87	50	31	68	80	58.4
31		56	40	15	5	44	90	58.4
32		55	73	10	36	36	40	41.6
33		73	60	0	51	14	40	39.6
34		95	67	40	53	74	90	69.7
55	14-15	24	20	0	2	24	20	15.1
56		3	7	0	0	8	10	4.5
57		9	0	0	0	8	50	6.0
58		42	27	0	33	20	30	25.4
59		30	53	12	11	40	50	32.8
61	24-25	3	0	5	1	1	10	3.3
62		0	0	0	0	0	0	0.0
63		16	0	5	1	1	0	3.9
64		0	0	0	0	0	40	6.7
65		6	0	0	1	3	20	1.5

(Continued on next page)

Table 3. (Continued)

Clone	Age (yrs)	Date and treatment						Mean
		Dec. 1 '70 CS	Feb. 1 '71 NS	Aug. 1 '70 CS	Dec. 1 '71 NS	Dec. 1 '71 CS	Feb. 1 '70 NS	
		Number of cuttings/clone						
		80	20	80	80	80	20	
Sheared								
45	24-28	49	23	0	20	18	40	25.0
46		25	3	5	8	22	30	15.5
47		57	43	0	24	58	0	30.3
48		41	43	5	4	30	0	20.5
49		44	20	0	0	2	0	11.0
40	35-42	5	0	1	8	0	0	2.4
41		6	0	0	22	2	0	5.0
42		10	3	0	6	2	0	3.5
43		33	37	3	8	4	0	14.1
44		16	50	1	26	22	30	24.2
Mean		30.2	27.2	6.1	15.8	21.8	29.4	

LSD .05 date and cold storage = 4.44

LSD .05 clone = 7.9

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[a] CS = cold storage for 60 days at 0 ± 1° C.
[b] NS = non-stored

Therefore, when selecting cutting material, a consideration of more importance than rooting would be the influence of cutting origin on the subsequent growth habit of the rooted cutting. Terminal cuttings from the top crown section will apparently produce an orthotropic plant more rapidly (7). Comparisons of rooting of randomly selected terminal and lateral cuttings are not meaningful, however, since it was shown that significant differences in rooting occur within each of these groups. First order lateral (large) and second order terminal cuttings root best, but the subsequent growth habit of plants from the five branch orders studied have not been established.

Shearing regenerates old trees and provides an abundance of cutting material with differing shoot orientation. Upright shoots root as well and appear to produce an orthotropic plant more rapidly than shoots oriented more horizontally on the tree (7). Since crown position does not affect rooting in the tree age classes studied, repeated shearing of the upper crown sections would make available an abundance of rootable cuttings capable of more rapidly producing orthotropic ramets.

Prepared for Submission to Forest Science

THE RELATIONSHIP BETWEEN CARBOHYDRATE CONTENT OF DOUGLAS-FIR NEEDLES AND ROOTING AND GROWTH OF STEM CUTTINGS

ABSTRACT. Differences in rooting and growth of Douglas-fir shoots were compared with sugar (total and reducing) and starch content of needles to determine possible relationships. High shoot rootability did not coincide with high levels of total sugars, reducing sugars or starch. A seasonal study showed cutting rootability increased from September to a peak in February, with no rooting in October, May, June, and July. The seasonal high for starch occurred in April, May, June, and July and was relatively low at other times. Total and reducing sugars were highest during January and February. Cold storage of cuttings produced high shoot rootability, without increasing total or reducing sugars, at a time when needle carbohydrate levels were lowest (October and November). Cutting rootability was shown to be significantly influenced by genotype, tree age (up to 25 years), branch order position, successive propagations, and shearing but none of these variables were found to have differences in sugar (total or reducing) levels. Low sugar levels in needles, however, were followed by reduced shoot growth of the rooted cutting.

ADDITIONAL KEY WORDS. Pseudotsuga menziesii (Mirb.) Franco, cold treatment.

SEASONAL fluctuations in the quality and quantity of carbohydrates in leaves and stems of conifers have been studied in detail (76, 81, 91, 115). These fluctuations appear to be predictable in those species studied. There are numerous reports that the nutrition of the plant influences the rootability of cuttings taken from it (30, 34, 55, 79, 92, 114, 138). The role that various carbohydrates play in rooting of conifers is not clear, however, and there are conflicting reports in the literature (54). Studies with Douglas-fir show that the total and reducing sugar content is high in winter and low in summer, whereas starch content is highest in early spring just before bud burst. The late winter and early spring decrease in sugar content coincides with a buildup of starch (81).

The seasonal rooting responses of conifer cuttings are well known. Some important conifers which have been studied include *Picea abies* (22, 31, 86), *Pseudotsuga menziesii* (119, 120), *Juniperus* and *Taxus* species (39, 84, 85), *Pinus radiata* (14, 35), *Larix* sp. (17), *Pinus virginiana* (162), *Araucaria excelsa* (93), and others (69). Douglas-fir rooted best when cuttings were taken during the period from late autumn to late winter (119, 120). Rooting of Douglas-fir cuttings has also been shown to be strongly affected by clonal origin, tree age, shoot position, and shearing of the stock plant (7).

There is evidence that exposure of the stock plant to chilling temperatures stimulates rooting (90, 120). This chilling treatment of the intact plant is also known to increase the sugar content of the needles (76).

The seasonal fluctuations in sugar content of Douglas-fir shoots reported by Krueger and Trappe (81) suggest a positive correlation with the rooting potential of cuttings observed by Roberts et al. (119, 120. Both studies were made at Corvallis, Oregon, but admittedly on trees of different age. This apparent correlation prompted further consideration of stored carbohydrates as a factor in determining shoot rootability.

The experiment was designed to determine if variations in carbohydrate levels in the needles of cuttings could be correlated with (1) the best rooting periods, treatments, and sources of cutting material, and (2) shoot growth potential of the rooted cutting.

MATERIALS AND METHODS

Douglas-fir stem cuttings were collected monthly from six trees (two 9-year-old and two 24-year-old non-sheared, and two 24-year-old sheared) growing near Corvallis, Oregon, during the period April 1971 to May 1972. Shoot and bud growth was measured on ten random shoots of each of these six trees. Twenty cuttings per tree were given standard preparation and auxin treatment as reported previously (7), and rooted in a controlled environment chamber with 500 foot candle incandescent/fluorescent lighting with long days (14 hrs), 10 ± 2° C air temperature without misting and 21 ± 2° C bottom heat. Evaluation of rooting response was made after 120 days in the rooting bench. Leaf samples were taken from similar cuttings on each of these dates and analyzed for total and reducing sugars and starch.

Sugar levels were considered further in the following study: Cuttings were collected the first of August, October, and December 1971, and February 1972, auxin treated, and rooted in a mist propagating house. Cuttings from 54 trees growing in the same area but not necessarily of the same site or seed source were included in the study representing the following: (1) Ten sheared trees - five in each of two age classes (24-28 and 36-41) with age and growth angle of cuttings compared, (2) eight one-half sheared trees with sheared and non-sheared portions compared, (3) 20 non-sheared trees - five in each of four age classes (4, 9, 15, and 25 years) with age, crown level and branch position compared, and (4) 16 clones including parent tree ortet, cutting ramet and grafted ramet trees compared (7). A minimum of ten cuttings / position / tree were placed in the propagating bench without storage, and leaf samples from each treatment were analyzed for total and reducing sugars. An equal number of cuttings in each of the August and December treatments were given 60 days cold storage at 0 ± 1° C before rooting and leaf analysis.

Leaf samples from each treatment were dried for 48 hours in a

55° C drying tunnel, ground to 40 mesh in a Thomas grinder before analysis (81). The sugar and starch content of the stem tissue was not measured in this study. It has been shown that fluctuations of these organic nutrients follow a similar pattern in stems and leaves of conifers (116).

Sugar Analysis

The method for total and reducing sugar analysis involves a modification of the procedure for determining glucose as proposed by Hoffman (63) and adapted by the Technicon Company for glucose analysis (145). A complete description of procedures, including the Technicon flow diagram used in this procedure, has been previously described (7). The procedure involves a color change in the potassium ferricyanide-potassium ferrocyanide, oxidation-reduction reaction. The yellow solution of potassium ferricyanide is reduced to the colorless ferrocyanide. The color is measured at 420 mμ using a flow cuvette with a 15 mm light path. The procedure is not specific for glucose so any reducing material is determined by first making an acid hydrolysis of the extract, then determining the reducing substance present.

Starch Analysis

Starch determinations were made using the anthrone colorimetric method (56). Soluble sugars are extracted from the dried plant tissue with 80 percent ethanol. The sugar-free residue is treated with perchloric acid solution; the extracted starch is precipitated with iodine, the starch-iodine complex is decomposed with alkali and the starch determined colorimetrically at 620 mμ with anthrone reagent.

Growth Analysis

Shoot extension and bud size were measured on terminal shoots of the source plants. Shoot length was measured from the bud scale scars of the previous season to the tip of the current-season shoot. Terminal bud size included the length from the base of the basal bud scale to the bud tip and the greatest diameter along this length was measured.

RESULTS AND DISCUSSION

A superficial comparison of the data on seasonal rooting and sugar level fluctuations gives the impression that the potential for root initiation is positively correlated with high sugar levels. Although rooting and total sugar content appears to be correlated, this apparent relationship does not hold true since cuttings rooted well during September, November and December when starch and sugar levels were lowest (Figure 1).

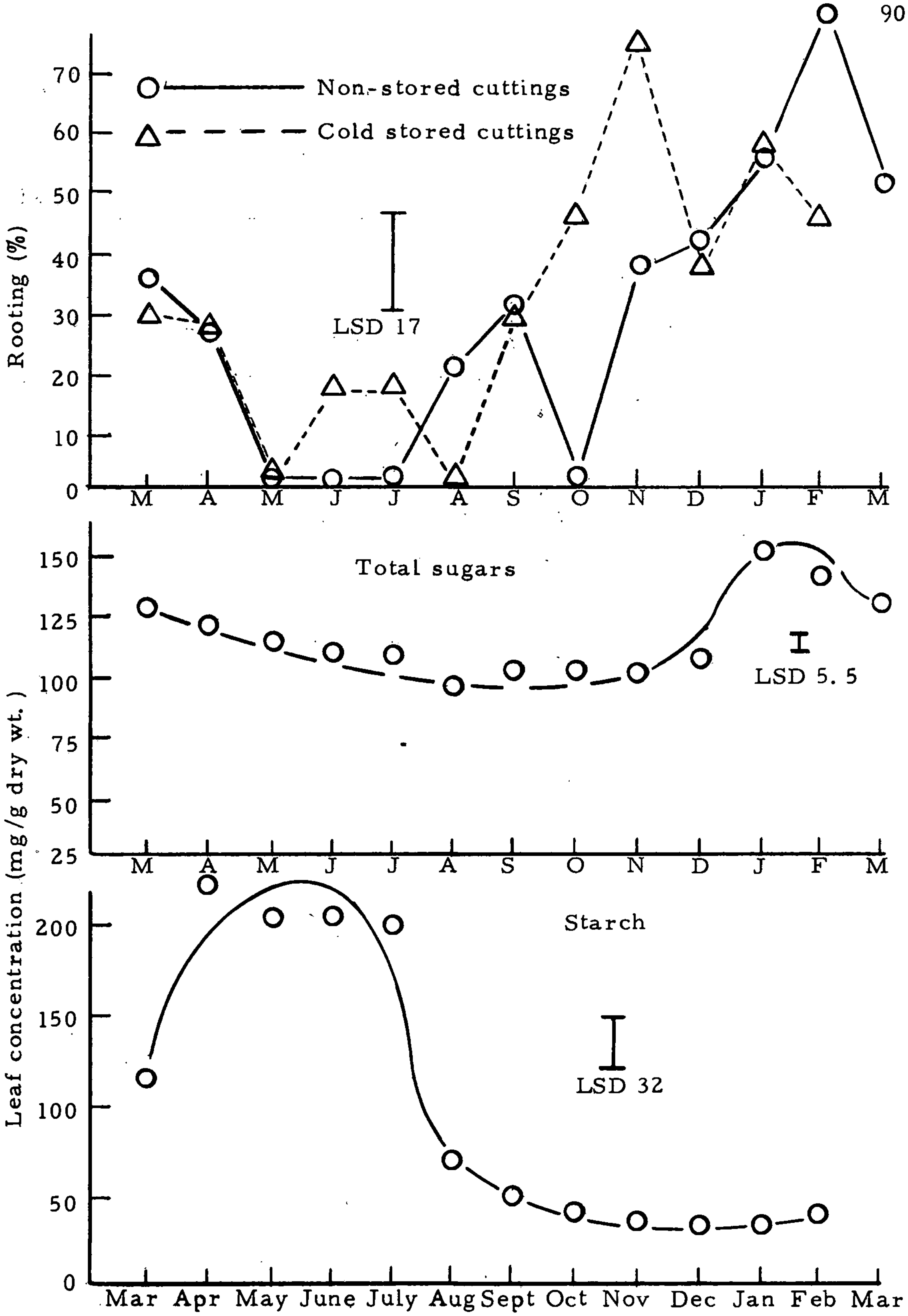

Figure 1. Rooting potential (cold stored and non-stored) and sugar and starch levels in leaf tissue of Douglas-fir stem cuttings sampled at mid-monthly intervals (1971-72).

Following shoot extension, rooting began in August, increased monthly to a peak in February, except for an extremely low level in October. Following the peak in February, rooting dropped off gradually to another low in May which extended through the summer. This rooting pattern is consistent with other seasonal studies on Douglas-fir except for the better rooting obtained during September in this study (119, 120).

Carbohydrate levels like rooting followed a seasonal pattern. Total and reducing sugar levels reached a peak during January and February, and were relatively low from June to November. The seasonal curves for these two classes of sugars followed an identical pattern. Starch levels were highest during April, May, June and July, decreased rapidly during August, and reached the lowest levels from September to February, the period of maximum rooting (Figure 1). These results on seasonal fluctuations in starch and sugars are consistent with previous studies on Douglas-fir (81, 154). Thus, it is clear that high starch accumulation is not contributing to rooting.

Further evidence that high sugar levels were not necessary for good rooting was the improved rooting response obtained when dormant cuttings were cold stored (Figure 1). October and November harvested cuttings rooted significantly better with 60 days of $0 \pm 1^{\circ}$ C cold storage (C.S.).

This improved rooting response from cold-storage has been observed in other studies on Douglas-fir (7, 119, 120). This cannot be explained on the basis of a conversion of accumulated starch to sugar in the leaf tissue during the storage period. Sugar levels in needles of cuttings before and after storage on two dates did not show an increase in sugar as a result of the cold treatment (Table 1). On the contrary, the August 1 and December 1 cuttings had 46 and 14 percent less needle sugar, respectively, after cold storage than before. Therefore, the 74 percent rooting of the November cold-stored cuttings, which is as high as the best non-stored sample, occurred when sugar and starch levels in the needles were lowest. The reduction in needle sugar levels during storage suggests a high respiration rate in the pre-dormant (August) cuttings, with reduced respiration in the somewhat dormant (December) ones. This, however, does not exclude the possibility that these reserves were translocated into the stem tissue during the storage period. The fact that sugar reserves were affected more by cold storage in the August cuttings suggests that enzyme activity is different at these times.

Total and reducing sugars did not differ significantly as a result of tree age, genotype, successive propagation, shearing, or branch position (Tables 1 and 2). These were all shown to influence rooting potential (Tables 1 and 2), and a more detailed report of their affects on rooting was reported previously (7). This is further evidence that total and reducing sugars are not limiting rooting.

Table 1. Sugar levels (total and reducing), and percent rooting of cuttings from different age classes of non-sheared and sheared trees with different sampling dates and cold treatments. Sugar levels in mg/g dry wt. leaf tissue. Age and shearing differences are not significant at .05 level.

	Age	8-1-71 (NS)[a]			8-1-71 (CS)[a]			12-1-71 (NS)			12-1-71 (CS)			2-1-72 (NS)			Means		
	class	RS[b]	TS[b]	PR[b]	RS	TS	PR	RS	TS	PR	RS	TS	PR	RS	TS	PR	RS	TS	PR
		mg/g	mg/g	%	mg/g	mg/g	%	mg/g	mg/g	%	mg/g	mg/g	%	mg/g	mg/g	%	mg/g	mg/g	%
Non-sheared	4	90	95	6	56	57	0	99	106	57	90	90	92	146	150	92	96	100	49
	8-9	93	108	23	44	45	0	84	97	56	84	86	56	151	154	68	91	98	41
	14-15	107	117	3	63	66	0	92	96	16	89	91	44	142	146	32	99	108	19
	24-25	103	117	2	58	60	0	88	95	0	77	80	0	35	138	14	92	98	3
Sheared	28-30	90	103	2	52	55	0	91	101	14	84	86	16	136	141	14	91	98	9
	35-42	87	92	1	51	53	0	82	92	6	71	74	14	137	143	16	86	90	7
	Mean	95	105	6	54	57	0	89	98	26	82	84	35	141	145	38			

LSD .05 - Sugar levels = 21.6, Percent rooting = 4.3, Date and cold storage = 4.2

[a] NS = non cold stored cuttings
CS = 60 days 0 ± 1° cold storage

[b] RS = reducing sugars
TS = total sugars
PR = percent rooting

Table 2. Reducing and total sugar content (mg/g dry wt.), and percent rooting of current season Douglas-fir needles from different clonal origins--ortet, grafted ramet, and cutting ramet. December 1, 1971. Differences in sugars due to genotype or clonal origin are not significant at .05 level.

Clone	Ortet (parent)			Grafted ramet			Cutting ramet		
	R.S.	T.S.	P.R.	R.S.	T.S.	P.R.	R.S.	T.S.	P.R.
	mg/g	mg/g	%	mg/g	mg/g	%	mg/g	mg/g	%
368	78	85	0	88	100	0	83	92	10
171	99	103	5	97	102	0	90	91	60
175	85	92	0	76	81	10	72	80	40
176	88	95	0	108	108	25	77	85	100
173	85	94	0	82	83	10	74	76	25
Mean	85	94	1	90	95	9	79	84	45
1	76	77	25				60	90	30
2	100	100	5				95	100	20
3	86	102	0				86	98	20
4	90	90	15				87	104	75
5	82	92	5				85	88	85
6	76	90	0				70	85	10
7	92	93	5				85	90	50
8	85	93	35				85	93	40
10	80	83	10				72	85	30
12	80	85	25				83	100	35
13	70	76	0				80	92	40
15	75	80	-				67	80	-
Mean	83	88	13				80	92	40

LSD .05 rooting = 8.3

R.S. = Reducing sugar
T.S. = Total sugar
P.R. = Percent rooting

These results show that stored carbohydrate levels are present in sufficient quantities for rooting throughout the season and in the cutting sources studied. The competition of the expanding shoot for these reserves does not explain the poor rooting at different times and from different sources, since measurements showed that shoot extension and major bud growth occurred during a two-month interval in the spring (Figure 2). Thus, the stimulus for root initiation and development must come from some other, yet undetermined, source. Auxins, rhizocaline, ABA, GA, ethylene, and other promoters and inhibitors have been proposed to influence rooting (5, 19, 61, 142). It is most likely a balance between several of these known and perhaps some yet unknown promoters and inhibitors. The minimum level of, and/or balance of, specific carbohydrate components, however, cannot be ignored as a possible influence on the rooting potential in cuttings.

Contrary to rooting response, the amount of extension growth following rooting appears to be affected by leaf sugar content. Rooted cuttings, sampled February 1 and having relatively high sugar levels (145 mg/g), had an average shoot growth of 11.7 cm compared to 5.3 cm for the December 1 samples stored for two months and having a resulting low sugar content (84 mg/g) (Figure 3). This more than two-fold greater shoot growth from cuttings with high accumulated sugar levels would merit more extensive research.

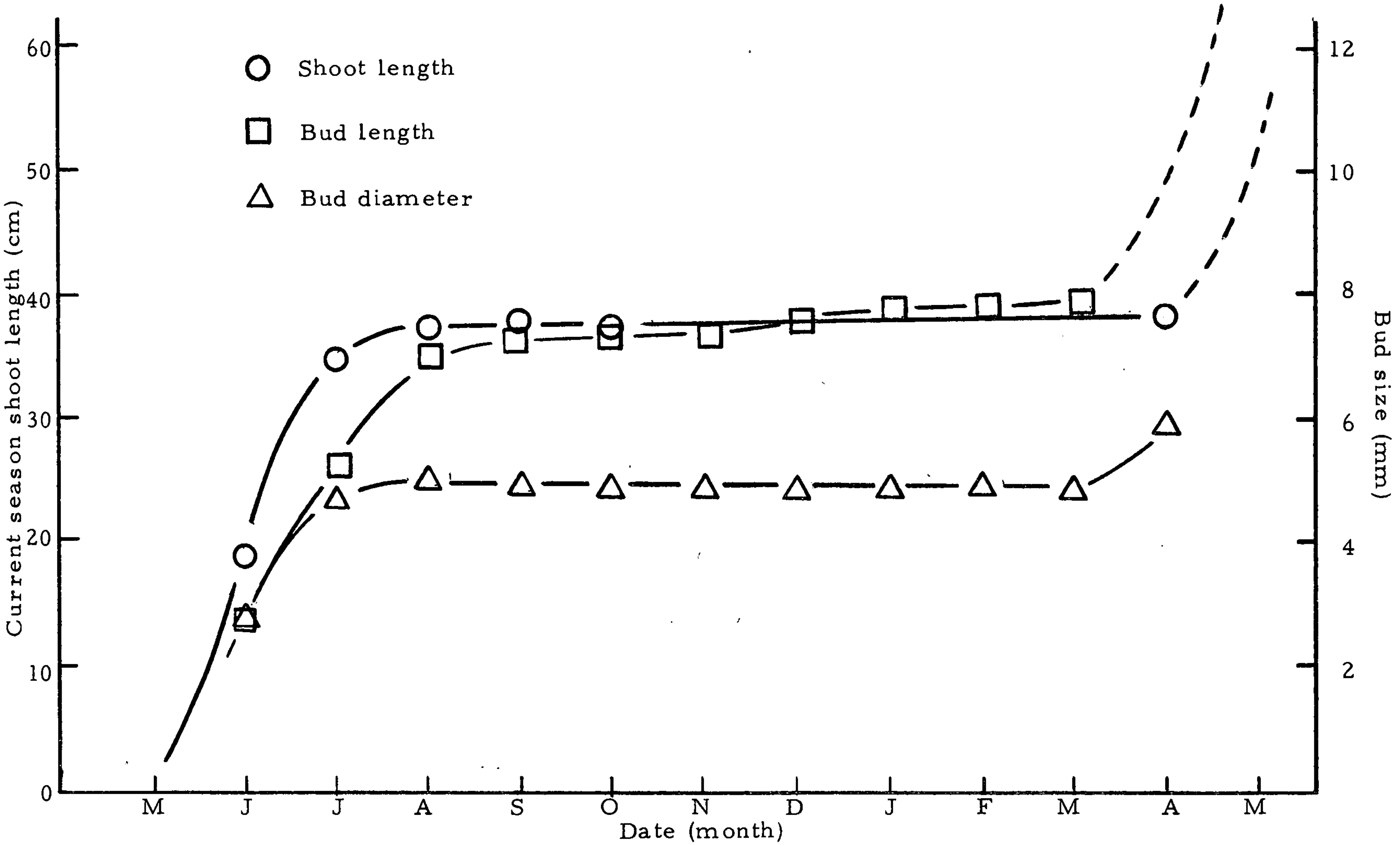

Figure 2. Seasonal extension growth, bud length, and bud diameter. Based on the mean of ten randomly sampled terminal shoots from six trees sampled mid-monthly. Dotted line indicates bud break.

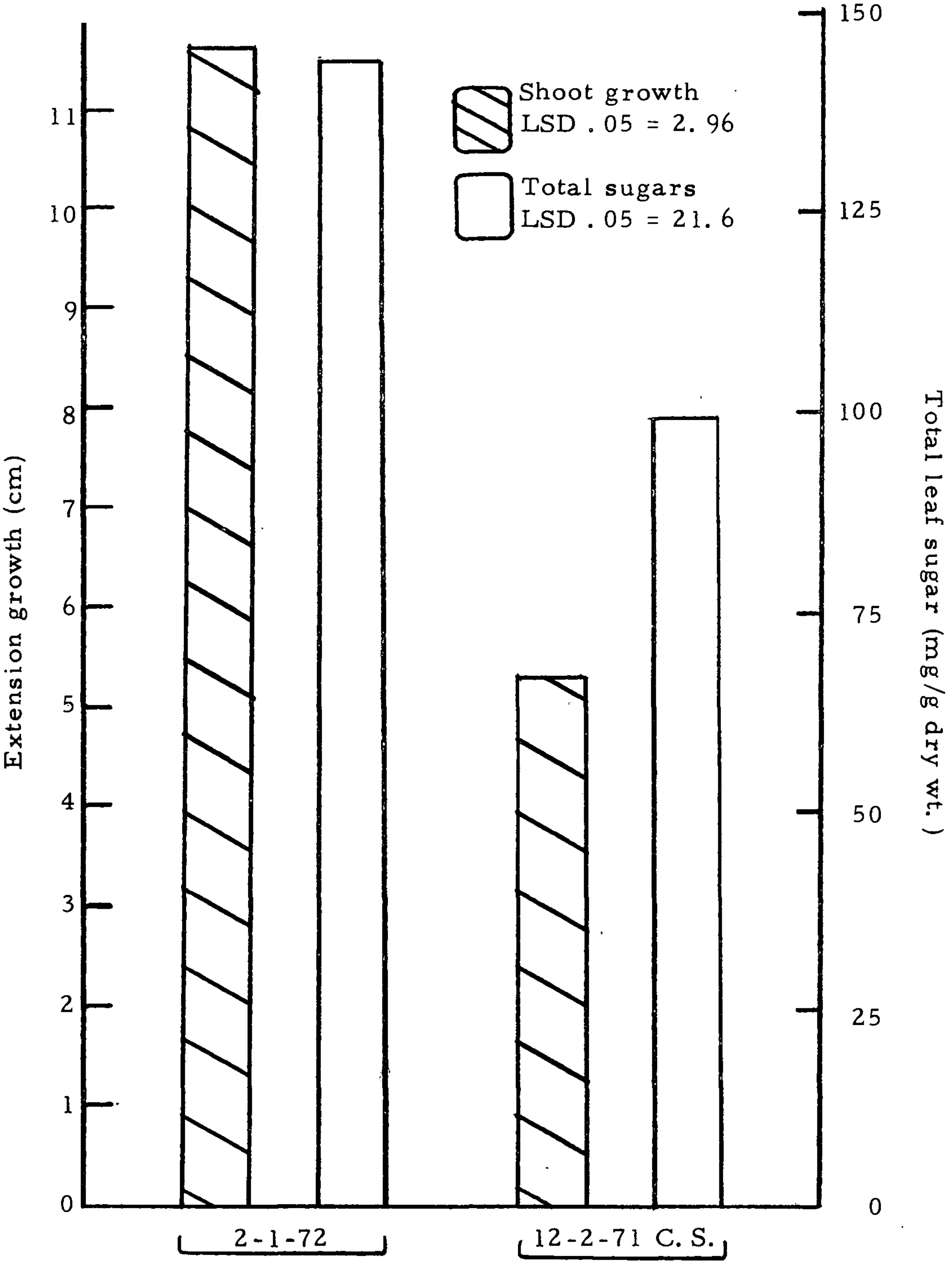

Figure 3. Relationship of needle sugar levels in Douglas-fir cuttings to subsequent extension growth. Dates are harvest dates of cuttings. C. S. = cold stored cuttings.

Prepared for Submission to Forest Science

THE PROBLEM OF PLAGIOTROPIC GROWTH IN ROOTED DOUGLAS-FIR STEM CUTTINGS

ABSTRACT. Branch cuttings of Douglas-fir display a plagiotropic growth habit for some time after rooting. The natural transition of these cutting ramets to the orthotropic growth habit occurs slowly and was not influenced by staking, pruning, re-potting, or unidirectional light. The transition of some young clones occurs in three years, while others may take as long as seven or eight. Physiologically old clones remain plagiotropic for a longer period of time. The significance of preliminary findings in solving the problem of plagiotropism by cutting selection is discussed. Cuttings from young clones, terminal branch positions, top crown sections, and upright shoots of sheared trees appear to become orthotropic more rapidly than from other sources.

ADDITIONAL KEY WORDS. Pseudotsuga menziesii (Mirb.) Franco, orthotropic growth, cutting ramet.

RESEARCH on the vegetative propagation of Douglas-fir from cuttings has been underway at Oregon State University for six years. During this period, it has been established that cuttings from both juvenile and mature trees can be rooted in fairly large numbers, and that there are easy- and difficult-to-root genotypes in both categories (7, 119, 120). A problem as difficult, if not more difficult, as root initiation on the cutting is the establishment of orthotropic growth in the rooted cuttings. Cuttings from both young and mature material take several years to grow out of their plagiotropic habit. The nature and extent of the problem and its solution need priority attention. Few references to this problem can be found in the literature. However, experienced propagators, both nurserymen and researchers, who have worked with genera such as Pseudotsuga, Abies, Picea, Araucaria, and similar conifers have recognized the problem. McCulloch (95), reporting on three-year-old Douglas-fir cutting ramets in the field, noted they had failed to develop well-defined leaders. Lateral branches were well developed, and he concluded that if growth continued in this manner the trees would develop as weepers. Similar observations have been made by researchers working with other conifers (16, 22, 44, 59, 73, 74, 97, 134, 146).

Important questions still unanswered are (1) will natural transition of plagiotropic plants occur, and (2) can the transition to orthotropic growth be sped up or plagiotropism overcome by proper selection of cutting material or manipulation of the ramet plant? The objectives of these studies were to (1) study the effects of pruning, staking, re-potting, and exposure to unidirectional light on the plagiotropic growth habit of the cutting ramet (Figure 1), and (2)

Figure 1. Rooted cuttings of two clones still showing typical plagiotropic growth habit after three years growth. Clone 5 (top) and Clone 7 (bottom).

determine the effects of selecting cuttings from different branch positions, crown levels, growth angles, and tree ages on their subsequent growth habit.

MATERIALS AND METHODS

Staking and Re-potting: Effects on Growth of Cutting Ramets

Nurserymen commonly stake plants in an upright position to obtain orthotropic growth. Utilizing this practice and attempting to determine the nature and persistence of plagiotropic growth in cutting ramets, two age classes of two- and three-year-old rooted cuttings were randomly selected from two clones and treated as follows: (a) Control, (b) plant potted upside-down and suspended above the greenhouse bench, (c) plant staked into an upright position, and (d) plant tied into horizontal position (Figure 2). Five plants from each of the two age classes in the two clones were given each of the treatments and their growth habit observed for two years. The two clones selected for study differed in growth habit, clone 12 having an extreme and persistent plagiotropic habit and clone 7 more orthotropic even after rooting, and proceeding rapidly in its transition to a complete upright habit.

Figure 2. Leader staked upright having returned to the plagiotropic growth habit (top). Plant potted upside down having returned to the plagiotropic growth habit of the control (bottom). This is clone 12. Note the similar growth angle displayed by all these plants.

Pruning: Effects on Growth of Cutting Ramets

A reduction in the shoot/root ratio in many plants will force a more vigorous upright growth in the remaining buds, particularly the leader. This technique might be used successfully in hastening the transition from plagiotropic to orthotropic growth habit in Douglas-fir. In order to test this assumption, one-, two-, and three-year-old ramets with plagiotropic habit were selected randomly and treated as follows: (a) control, (b) pruned to a main leader and staked in upright position, (c) heavily pruned to one basal shoot or bud. Ten plants of nine clones in each of three age classes were given each treatment. These potted plants were selected for treatment in the autumn of 1970, and lined out in the field. Since there was heavy winter kill in treatment (c), the experiment was repeated on a smaller scale the following spring in the greenhouse.

Phototropic Studies

Roussel (132) showed Douglas-fir to be phototropic as seedlings but they lost this response mechanism in their third or fourth year. Cutting propagation has been shown to have a rejuvenating effect on this species (7). In this study the objectives were to determine the effects of reduced light intensity and unidirectional light on the growth behavior of randomly selected cutting ramets. Three plants/three age classes (1, 2, and 3 years) /three clones, showing plagiotropic growth habit, were included in the study. The cutting ramets were given the following treatments: (a) control, (b) plant grown in four-inch diameter tube open only at top (plant was secured upright by staking), (c) same as b with the top of the tube closed but open on the side with four-inch diameter hole, (d) tube used as in b with plant pruned to one lateral shoot, (e) tube as in c with plant pruned as in d.

The experiment was begun February 1, 1971, under greenhouse conditions with naturally chilled plants. The plants were observed for one year.

Rate of Natural Transition of Cutting Ramets

Cutting ramets from physiologically old ortets were observed to make a natural transition from plagiotropic to orthotropic growth habit. Plants observed include those propagated by McCulloch (95), which are located in McDonald Forest near Corvallis, those in our own nursery, and those in the David T. Mason seed orchard near Sweet Home, Oregon, propagated by Jack Barringer. Several characteristics distinguish the plagiotropic from the orthotropic plant. Obvious morphological differences include needle orientation and bud and branch arrangement, which are bilateral in plagiotropic shoots, but radially summetrical in orthotropic ones. The most noticeable and easily measured difference, however, is the growth angle of the main stem. In order to determine the rate of transition to the orthotropic habit, the growth angle of the main stem was measured on ten plants of each of four clones of cutting ramet trees in four age classes (1, 2, 3, and 4 years). The measurements were made with the Suunto PM-5 Clinometer, and recorded in degrees from vertical.

The trees used as a source of cutting wood for the ramet plants used in each of the above studies were understocks of grafted trees in the David T. Mason seed orchard near Sweet Home, Oregon.

Source of the Cutting Material: Effects on Growth of Cutting Ramets

Cutting origin has been shown to have an important effect on the growth habit of ramets in other conifers (44, 59, 73, 74, 97, 146). These findings have been recently reviewed (7). Studies on the effects of cutting origin on the rooting of Douglas-fir shoots have also been reported (7). These studies have included the effects of ortet age, crown position, branch order position, shearing--including its rejuvenating effects, different growth angles of shoots within the crown, and successive propagation--including grafted and cutting ramets on rooting and subsequent growth of cuttings. Rooted cuttings from these experiments are growing at the Lewis Brown Horticultural Farm near Corvallis. The growth habit of these cutting ramets will be reported in detail at some later date.

RESULTS AND DISCUSSION

Staking and Re-potting: Effects on Growth of Cutting Ramets

The plagiotropic growth habit displayed by rooted cuttings from young understocks was consistent within clones, but varied greatly between clones as far as growth angle was concerned (Table 1). Regardless of the treatment used, the shoots always re-oriented themselves to their original angle of growth (Figure 2). The transition was not rapid and, surprisingly, occurred as a result of the stem bending in the older, more lignified wood (in some cases three-year-old wood). This adjustment was completed within a year with the leading shoots assuming the same growth angle as the untreated controls. Some young needles re-oriented themselves to a right-side-up position within the first month, but most of the older ones did not right themselves. The new needles emerging at bud burst were properly oriented.

Pruning: Effects on Growth of Cutting Ramets

Decreasing the shoot/root ratio by pruning did not hasten the transition to orthotropic growth habit. The three-year-old control plants of clones 4, 5, 7, 8, and 11 were more orthotropic than those that had been pruned or staked (Table 1). Heavy pruning increased

Table 1. Length and angle of main stem growth on four-year-old cutting ramet trees. Note the variability between clones and treatments. The angle of shoot growth is in degrees from vertical. Height is in cm.

Clone	Control		Staked*		Pruned*	
	angle	length	angle	length	angle	length
	deg.	cm	deg.	cm	deg.	cm
1	8.5	64.8				
2	15.0	60.1				
3	19.0	67.9				
4	24.3	65.8	25.3	64.3	33.2	32.8
5	25.1	69.3	28.9	71.9	26.7	33.8
6	23.1	65.8	30.6	61.7	dead	
7	10.9	61.0	16.5	64.0	18.7	22.1
8	42.9	71.9	47.2	83.3	45.0	31.8
9	25.0	76.2	29.0	74.4	dead	
10	8.3	73.5				
11	42.2	62.2	54.2	68.6	56.7	27.9
12	35.6	75.2	33.5	76.2	40.0	27.9
13	45.0	66.0	50.0	63.5	dead	

*See Experiment 2.

the growth of remaining shoots, but the angle of new growth was the same as that of the controls (Figure 3). Staking was difficult to maintain since the reaction wood formed in the old portions of the stem created considerable pressure on the stakes. The bamboo stakes were bent with such force that some were broken. When bending was prevented, the extension growth was at the same angle as before staking (Figure 2). These and other observations indicate that pruning and staking treatments used were not effective in hastening the transition of Douglas-fir from the plagiotropic to orthotropic growth habit.

Phototropic Studies

There was no evidence of a phototropic response in any of the plant treatments. With bud break (mid-February for clone 12, and early March for clones 2 and 7), and shoot elongation, the shoots tended to curve around the tube as they came in contact with it. Only those shoots already oriented toward the tube opening grew in that direction. The central leader of the staked and pruned plants grew plagiotropically in the tube and continued this habit after the tube was removed. Since these cutting ramets did not respond phototropically, they were apparently physiologically older than those found to respond in Roussel's studies.

Figure 3. Plagiotropic growth habit not changed by heavy pruning. Angle of shoot growth the same for plant pruned back to one basal shoot (right) as control (left).

Rate of Natural Transition of Cutting Ramets

Clonal differences in plagiotropic growth habit of newly rooted Douglas-fir cuttings are evident almost as soon as bud break occurs. Some clones display an almost horizontal growth habit. The rate of transition from the plagiotropic to the orthotropic habit is also different between clones (Figure 4). The transition occurs gradually and at a somewhat predictable rate over several years. During the transition, the stem becomes more upright and the arrangement of the needles, buds, and branches becomes more radially symmetrical. This study, and observations on another 20 clones. indicated that orthotropic plants from cuttings can be expected after three years with some clones, but requires longer, even up to six to ten years with others. There were wide clonal differences in the growth habit of four-year-old cutting ramets, even when the ortet source plants for these ramets were near the same age chronologically (Table 1).

McCullock (95) reported that cutting ramet plants were still plagiotropic after three years in the field. Thirty years later the trees that McCullock reported as plagiotropic are now symmetrical, orthotropic, uniform trees, which would compare favorably with seedling trees of the same age. The ortet from which these ramets were propagated was an old tree. It thus appears that given enough time, cutting ramets from even old clones will become orthotropic.

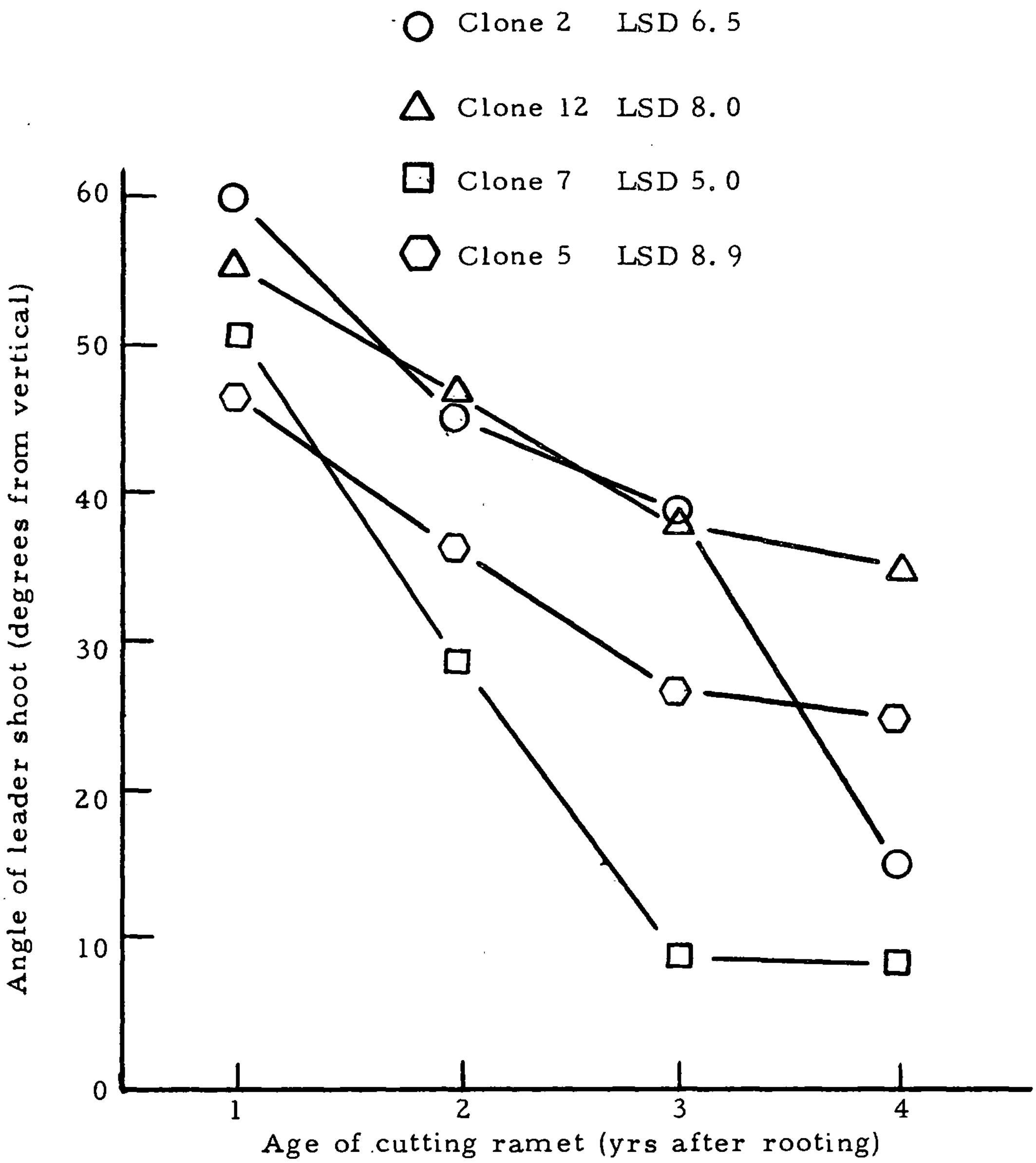

Figure 4. A comparison of the rate of transition from plagiotropic to orthotropic habit in cutting ramets from four Douglas-fir clones. All cuttings were from similar understock parents.

Source of Cutting Material: Effects on Growth of Cutting Ramets

The angle and length of shoot growth made by rooted cuttings from trees of different age, crown levels and branch positions were determined in the spring of 1972, after two growth flushes (Table 2a). To a greater or lesser degree, these plants all displayed a plagiotropic growth hahit, and the differences that now exist will likely become more pronounced with time. The only apparent difference at present in growth of cuttings from non-sheared trees is that terminal shoots from the top crown section of young trees are more orthotropic than those from other sources. Cutting ramets from sheared trees originating from shoots representing different degrees of plagiotropism, showed that the more orthotropic the shoots were growing when the cuttings were taken from them, the more orthotropic the ramets were two years after rooting (Table 2b). Observations of cutting ramets from old clones, not included in this study, suggest that with increasing age of the ortet, the more persistent the plagiotropic growth habit of the cutting ramet will be.

GENERAL DISCUSSION AND CONCLUSIONS

The results of these experiments suggest that the plagiotropic growth habit which develops in Douglas-fir branches with advanced differentiation cannot be easily altered. The growth angle of the shoot at the

Table 2a. Growth angle of cuttings from different tree age classes, crown levels, and branch positions.

Crown level	Branch position	Age of the parent (ortet) plant (yrs)							
		4		8		14		24	
		No. of plants	Growth angle	No. of plants	Growth angle	No. of plants	Growth angle	No. of plants	Growth angle
Top 1/4	Terminal	8	54	10	22				
	Lateral	14	29	10	15				
Second 1/4	Terminal	15	24	10	10	1	20		
	Lateral	11	15	20	22	9	6	10	8
Third 1/4	Terminal	15	37	11	26	4	10		
	Lateral	10	22	19	21	12	12		
Bottom 1/4	Terminal	12	29	20	18	2	5		
	Lateral	13	18	20	21	13	7		

Table 2b. Growth angle of cuttings from shoots oriented at different angles within the crown of sheared trees. Means are based on the average of ten cuttings from each source.

Ortet shoot angle	Age class	Growth angle (degrees from horizontal)			
		60-90° (↑)	30-59° (↗)	0-29° (→)	<0° (↘)
Ramet shoot angle	24-28	53	34	31	20
	35-42	58	43	36	20
Mean		56	39	34	20

LSD .05 = 14

time it is severed from the tree is carried over into the ramet. The ramet from a branch cutting displays for some time a plagiotropic growth habit in varying degrees regardless of the source. The time required for transition to the orthotropic form varies as a function of the crown level, branch position, age, and orientation of the shoot within the crown. It seems that the ramets in this species will eventually become orthotropic regardless of their source.

The plagiotropic growth habit is apparently controlled by gravity. It is as though the cells in the ramet plant are programmed to a certain growth habit and react as they would on the parent plant.

In Christmas tree plantations of this species the top several feet of the tree is often removed. If the cut is not made too close to the ground, the lateral branches in the upper whorl begin to grow more orthotropic. Ultimately, one or sometimes several of these become predominant with the others resuming the plagiotropic growth angle specific to that genotype. This built-in program suggests apical dominance with a message coming from the leader keeping the side branches suppressed. It is difficult, however, to see how apical dominance is operating to control plagiotropism in cutting ramets when branch cuttings maintain their plagiotropic growth habit removed from the influence of the parent plant and established on their own roots. Cutting ramets from lateral branches of some species, such as Araucaria excelsa, maintain their plagiotropic habit indefinitely--for at least 60 years (152). It appears that the plagiotropic program becomes more fixed the farther removed the shoots are from the apical meristem, and the older this meristem becomes physiologically. What causes the already plagiotropically programmed shoot in Douglas-fir to eventually become orthotropic is not clear. It may be that it takes a certain length of time for the biochemical control program to change and dedifferentiation to occur, being longer in more highly differentiated shoots, and occurring at a faster rate the larger the root and shoot systems become.

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APPENDIX

Appendix Table 1. The effects of age on the response of cuttings from different age classes of non-sheared and sheared trees.

Tree age (yrs)	Dec. 1, 1970 C. S. [a]	Aug. 1, 1971 N. S. [a]	Dec. 1, 1971 N. S.	Dec. 1, 1971 C. S.
		Percent rooting		
Non-sheared				
4	55. 2	6. 3	57. 0	67. 5
8-9	62. 7	23. 0	35. 3	45. 5
14-15	21. 5	2. 5	9. 5	19. 8
24-25	4. 7	1. 9	0. 8	0. 8
Sheared				
24-28	45. 2	2. 0	11. 2	26. 0
35-42	15. 3	1. 0	14. 0	6. 0
LSD . 05	5. 8	4. 3	3. 4	3. 15
		Percent of rooted cuttings good quality		
Non-sheared				
4	12. 0	14. 0	49. 0	61. 0
8-9	57. 0	24. 0	42. 0	27. 1
14-15	15. 0	0. 0	16. 0	11. 4
24-25	0. 0	0. 0	0. 0	0. 0
Sheared				
24-28	18. 0	25. 0	15. 0	6. 1
35-42	18. 0	0. 0	11. 0	6. 7
		Percent bud break in bench		
Non-sheared				
4	61. 5	0. 0	55. 0	98. 9
8-9	59. 4	0. 0	5. 8	49. 0
14-15	43. 0	0. 0	13. 3	99. 0
24-25	8. 0	0. 0	0. 0	38. 8
Sheared				
24-28	67. 0	0. 0	8. 0	70. 4
35-42	45. 0	0. 0	26. 0	84. 8
LSD . 05	6. 4	0. 0	11. 0	1. 97

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Appendix Table 1. (Continued)

Tree age (yrs)	Dec. 1, 1970 C.S.[a]	Aug. 1, 1971 N.S.[a]	Dec. 1, 1971 N.S.	Dec. 1, 1971 C.S.
	<u>Dead cuttings in the rooting bench</u>			
<u>Non-sheared</u>				
4	0.0	24.3	3.8	0.0
8-9	7.5	12.0	11.8	5.5
14-15	16.8	12.0	21.8	1.0
24-25	27.0	17.8	1.8	0.5
<u>Sheared</u>				
24-28	19.3	48.5	11.6	2.8
35-42	30.7	33.5	1.2	13.2
LSD .05	7.5	8.1	9.3	2.0
	<u>Alive callused unrooted cuttings</u>			
<u>Non-sheared</u>				
4	21.5	16.8	34.3	6.3
8-9	15.7	40.0	46.5	46.5
14-15	39.9	59.0	66.3	75.0
24-25	50.1	54.5	96.0	98.5
<u>Sheared</u>				
24-28	33.0	26.0	67.2	71.0
35-42	53.0	57.5	74.4	78.0
LSD .05	10.8	11.9	15.1	6.0
	<u>Percent mortality of rooted cuttings</u>			
<u>Non-sheared</u>				
4	7.0			
8-9	11.6			
14-15	16.8			
24-25	44.0			
<u>Sheared</u>				
24-28	14.0			
35-42	31.7			
LSD .05	17.3			

[a] N.S. = non-stored cuttings
C.S. = cold stored cuttings

Appendix Figure 1. The rejuvenation of rooting potential as a result of shearing. Cuttings from the non-sheared and sheared portions of the same old tree.

Appendix Table 2. A comparison of the rooting potential of cuttings from the sheared and non-sheared portions of the same old trees. Values in percent rooting.

Clone	Age (yrs)	Aug. 1, 1971		Dec. 1, 1971		Dec. 1, 1971 (C. S.)[a]	
		sheared	non-sheared	sheared	non-sheared	sheared	non-sheared
		%	%	%	%	%	%
70	61	0.0	0.0	25.0	5.0	25.0	20.0
71	56	0.0	0.0	10.0	0.0	25.0	5.0
72	28	7.0	0.0	0.0	0.0	10.0	0.0
73	25	3.3	0.0	0.0	0.0	0.0	5.0
74	40	3.3	0.0	5.0	0.0	0.0	0.0
75	32	0.0	0.0	0.0	0.0	10.0	0.0
76	40	50.0	10.0	30.0	25.0	75.0	40.0
77	38	33.0	0.0	10.0	0.0	15.0	5.0
Mean		9.3	1.2	10.0	3.8	20.0	9.4
LSD .05		7.0		6.09		11.22	

[a] C. S. = cold stored cuttings

Appendix Figure 2. Rooting of cuttings from different origins of mature clones. Ortet (top), grafted ramet (middle), cutting ramet (bottom).

Appendix Table 3. A comparison of the rooting potential of cuttings originating from the ortet, grafted ramet, and cutting ramet. Cold stored cuttings are separated from non-stored cuttings for comparison. The values are in percent rooting.

Clone	Age	Ortet (parent)				Grafted ramet				Cutting ramet			
		poor	fair	good	total	poor	fair	good	total	poor	fair	good	total
						December 1, 1971 non-stored cuttings							
368	84	0	0	0	0	0	0	0	0	0	5	5	10
171	25	0	5	0	5	0	0	0	0	45	10	5	60
173	34	0	0	0	0	5	5	0	10	15	20	5	40
175	27	0	0	0	0	15	10	0	25	0	0	100	100
176	25	0	0	0	0	10	0	0	10	15	5	5	25
Mean - LSD .05 = 10.3					1.0				9.0				45.0
						December 1, 1971 cold stored cuttings							
368		0	0	0	0	0	0	0	0	10	0	0	10
171		0	5	0	5	0	0	0	0	65	20	0	85
173		0	0	0	0	5	0	0	5	20	10	0	30
175		30	0	0	30	10	10	5	25	15	15	70	100
176		0	0	0	0	0	0	0	0	25	0	0	25
Mean - LSD .05 = 12.0					7.0				6.0				48.0
						February 1, 1972 non-stored cuttings							
368		0	0	0	0	0	0	0	0	10	0	0	10
171		0	0	0	0	0	0	0	0	15	15	0	30
173		0	0	0	0	5	5	0	10	5	10	5	20
175		0	0	0	0	0	0	0	0	15	5	0	20
176		0	0	0	0	0	0	0	0	15	15	20	50
Mean - LSD .05 = 4.1					1.0				2.0				26.0

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Appendix Table 3. (Continued). A comparison of the percent rooting of cuttings originating from parent understock trees (ortet) and 3-5 year old cutting ramets.

Clone	Ortet (parent)				Cutting ramet			
	poor	fair	good	total	poor	fair	good	total
				December 1, 1971 non-stored				
1	20	5	0	25	25	0	5	30
2	0	5	0	5	15	5	0	20
3	0	0	0	0	10	10	0	20
4	10	5	0	15	20	40	15	75
5	0	5	0	5	20	35	30	85
6	0	0	0	0	10	0	0	10
7	5	0	0	5	15	15	20	50
8	25	5	5	35	15	5	20	40
10	10	0	0	10	10	5	15	30
12	10	5	10	25	10	5	20	35
13	10	5	0	15	35	0	5	40
LSD .05 = 8.3	Mean			12.7				39.5
				December 1, 1971 cold stored				
1	10	15	5	30	10	15	10	35
2	20	0	0	20	10	15	25	50
3	0	0	0	0	10	5	10	25
4	25	5	15	45	20	25	25	70
5	30	5	0	35	5	5	15	25
6	0	0	0	0	0	5	5	10
7	10	0	0	10	15	10	5	30
8	0	0	0	0	10	5	10	25
10	5	0	0	5	15	20	5	40
12	10	35	30	75	15	15	55	85
13	15	0	0	15	15	20	10	45
LSD .05 = 17.8	Mean			9.0				36.0

Appendix Table 4. Rooting, mortality and growth of Douglas-fir stem cuttings from different crown positions. Four replications of ten cuttings X 2 branch order positions X 4 age classes X 5 clones per age are included for comparison. Differences due to crown positions are not significant at .05 level. Values are in percent rooting.

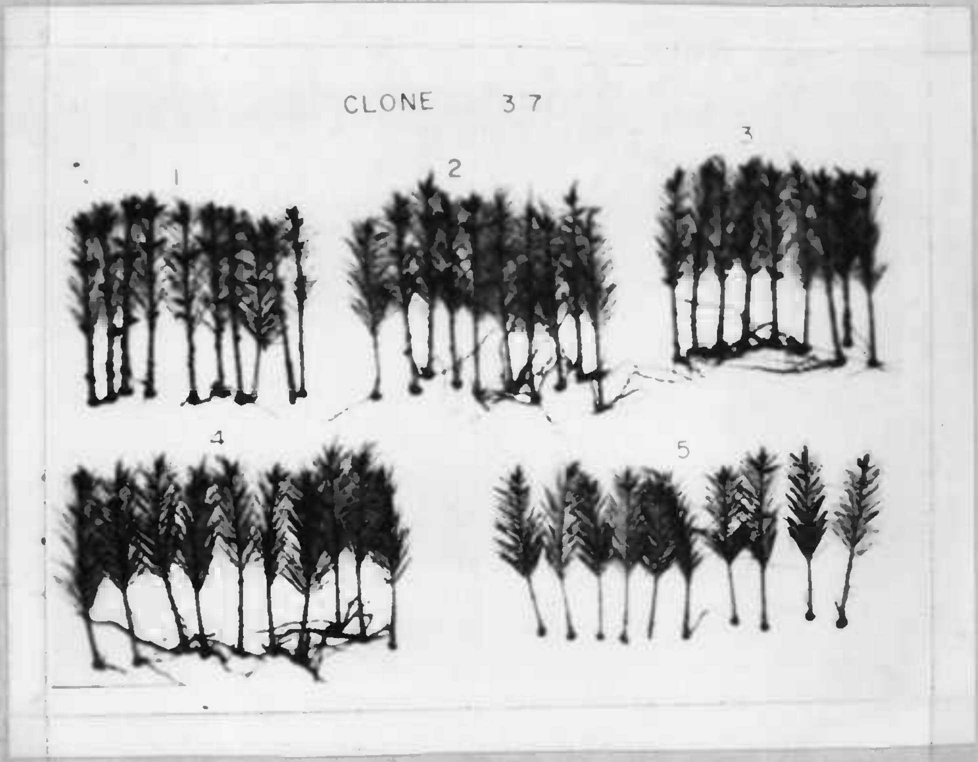
	Dec. 1, 1970 cold stored				Aug, 1, 1971 non-stored				Dec. 1, 1971 non-stored				Dec. 1, 1971 cold stored			
Crown level[a]-	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Total rooting	27	24	30	33	12	10	9	9	21	27	26	29	32	30	31	41
Callused unrooted	26	30	40	32	40	38	32	29	58	58	66	62	57	69	68	57
Dead in bench	21	14	6	10	13	16	14	23	14	10	5	5	5	1	0	1
Bud break	49	41	43	41	0	0	0	0	12	18	26	20	67	75	71	71
Good rooting													47	33	55	40

[a] Level 1 - top 1/4 of crown
Level 2 - second 1/4 of crown
Level 3 - third 1/4 of crown
Level 4 - bottom 1/4 of crown

Appendix Table 5. Rooting, mortality and growth of Douglas-fir stem cuttings from terminal and lateral branch positions. Four samples of 10 cuttings X 4 levels X 4 age classes X 5 clones per age.

	Dec. 1, 1970 cold stored		Aug. 1, 1971 non-stored		Dec. 1, 1971 non-stored		Dec. 1, 1971 cold stored	
	terminal	lateral	terminal	lateral	terminal	lateral	terminal	lateral
Total rooting (%)	30.2	40.6[a]	8.4	6.6	24.5	26.7	39.9	26.8
Percent of rooted cuttings good quality	29.2	40.6[a]	23.0	0.0[a]	37.5	43.0	50.0	29.5
Percent of cuttings callused unrooted	26.0	44.5[a]	42.7	43.1	55.8	65.6[a]	55.5	70.1
Percent of cuttings dead in rooting bench	18.2	8.8[a]	16.4	18.2	19.8	3.7[a]	3.0	0.5
Percent cutting mortality six months after rooting	30.0	14.0[a]						
Shoot growth the summer following rooting (cm)	9.3	7.2[a]						

[a] Differences due to branch order position significant at .05 level.



Appendix Figure 3. The rooting of cuttings from five branch order positions of a nine-year-old tree. (1) first order terminal, (2) second order terminal, (3) first order lateral (large), (4) first order lateral (small), (5) second order lateral.

Appendix Table 6. The percent rooting of Douglas-fir stem cuttings from four different crown positions and two branch positions of four different age classes. Differences due to crown positions are not significant at the .05 level.

Level[a]	Harvest date		Age classes				Branch positions	
			4	8-9	14-15	24-25	terminal	lateral
1	December 1, 1970	C.S.[b]	55.0	58.0	17.5	5.5	24.5	43.3
	December 1, 1971	N.S.[b]	35.0	39.0	7.0	1.0	19.4	29.0
	December 1, 1971	C.S.	75.0	35.0	19.0	0.0	35.1	29.0
2	December 1, 1970	C.S.	60.5	57.3	19.5	8.5	30.3	42.3
	December 1, 1971	N.S.	61.0	41.0	5.0	1.0	24.7	34.0
	December 1, 1971	C.S.	50.0	58.0	12.0	0.0	36.0	24.0
3	December 1, 1970	C.S.	60.0	63.0	24.5	2.0	36.2	38.7
	December 1, 1971	N.S.	66.0	25.0	13.0	1.0	28.5	23.9
	December 1, 1971	C.S.	70.0	38.0	12.0	2.0	40.0	21.0
4	December 1, 1970	C.S.	46.0	72.0	24.5	3.0	34.9	37.6
	December 1, 1971	N.S.	66.0	36.0	13.0	0.0	30.0	27.5
	December 1, 1971	C.S.	75.0	51.0	36.0	1.0	48.0	33.5

[a] Level 1 - top 1/4 of crown
Level 2 - second 1/4 of crown
Level 3 - third 1/4 of crown
Level 4 - bottom 1/4 of crown

[b] C.S. - cold stored cuttings
N.S. - non-stored cuttings

Sugar Determination

A 300 mg sample of dried, ground (40 mesh) leaf tissue was placed in a 50 ml Erlenmeyer flask containing 30 ml of 0. 2 percent benzoic acid. The flasks were then capped and placed on a shaker for 30 minutes for dissolution, at which time the contents were filtered through Whatman No. 1 paper. An aliquot of the filtrate was then analyzed for sugar content. By experimentation, this sample size was found to be satisfactory for the sugar content in Douglas-fir leaves.

The recorder was adjusted to 100 percent transmittance with water only flowing through the lines (Appendix Figure 4). If when ferricyanide was added to the flow, transmittance was above 16 percent, small amounts of five percent ferricyanide were added to the colormetric solution until the value fell within the acceptable range (13 ± 3 percent T.). The sensitivity of the procedure was adjusted experimentally by altering the size of the diluent tube and sample tube.

Reagents (1) Alkaline ferricyanide

sodium chloride	162. 0 g
potassium ferricyanide	4. 5 g
sodium carbonate	360. 0 g
water to	18, 000. 0 ml

Each of the chemicals was dissolved in about one liter of water and then combined in a five gallon bottle and diluted to volume. Briz. , a detergent, was added just prior to use at the rate of 0.5 ml/1000 ml.

(2) Stock glucose standard, 10 mg/ml

dextrose	20.0 g
benzoic acid	4.0 g
water to	2,000 ml

The dextrose and benzoic acid were dissolved in about one liter of water and transferred to a two liter volumetric flask and diluted to volume.

(3) Dilute glucose standards

Volume of stock	Dilute to	mg/100 ml	Percent	Percent in sample based on 300 mg diluted to 30 ml (100 x dilution)
25 ml	1000 ml	25	0.025	2.5
50	"	50	0.050	5.0
75	"	75	0.075	7.5
100	"	100	0.100	10.0
125	"	125	0.125	12.5
150	"	150	0.150	15.0
200	"	200	0.200	20.0

Each of the stock solutions was diluted to 1000 ml with 0.2 percent benzoic acid.

(4) Stock sulfuric acid solution, 0.25 N

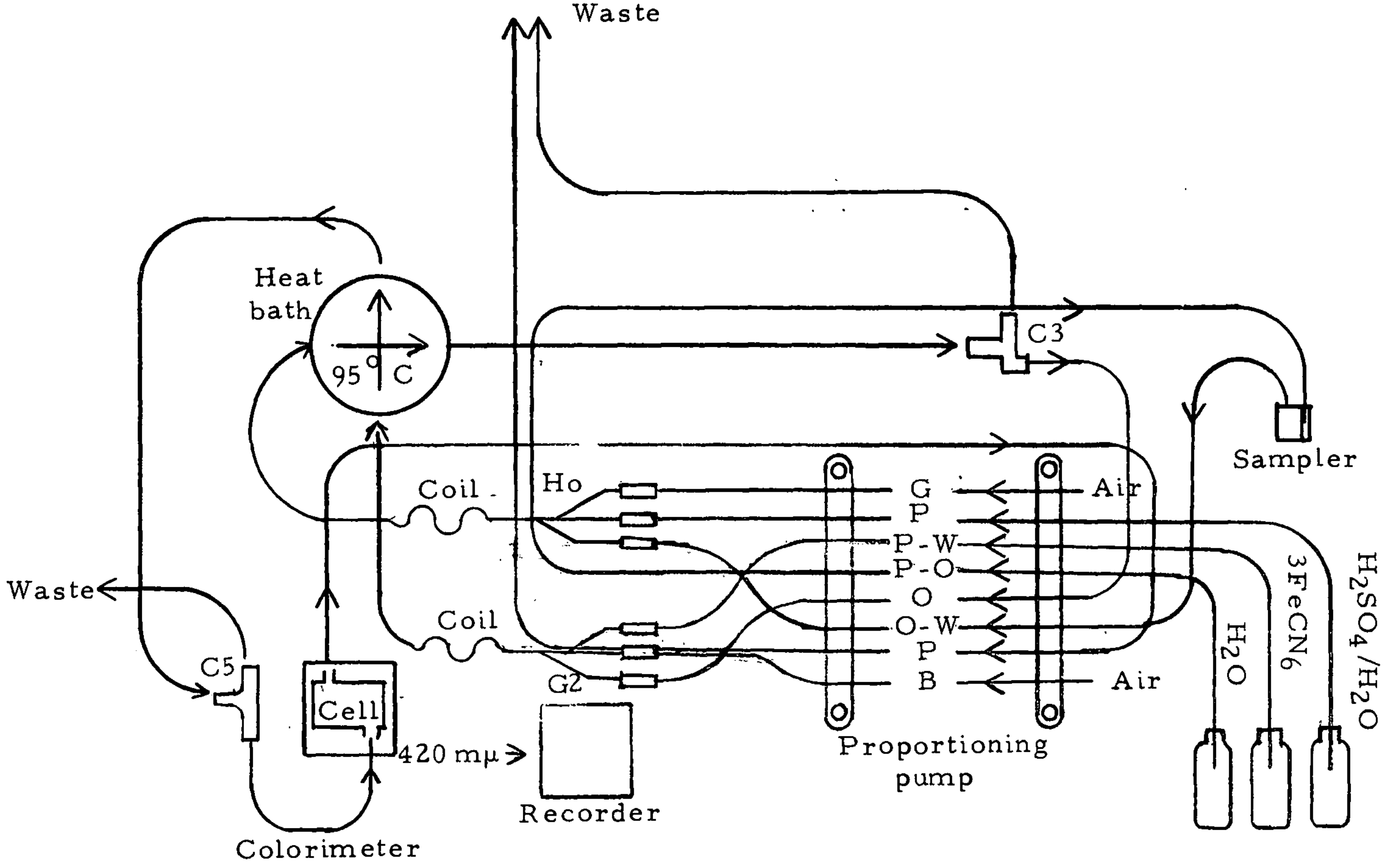
sulfuric acid, conc.	125 ml
water to	18,000 ml

(5) Working sulfuric acid solution, 0.05 N

stock solution	400 ml
water to	2,000 ml

(6) Benzoic acid solution, 0.2 percent

benzoic acid	36.0 g
water to	18,000.0 ml



Appendix Figure 4. Flow diagram for reducing and non-reducing sugars using the Technicon Auto Analyzer. Total sugars would result as shown. For reducing sugars the H_2SO_4 tube is placed in H_2O. The tube sizes are letter coded--the letter represents color.