ROOT INITIATION AND DEVELOPMENT IN AIR-LAYERED PINE AND SPRUCE

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James Roger Feucht

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ROOT INITIATION AND DEVELOPMENT IN AIR-LAYERED PINE AND SPRUCE

By

JAMES ROGER FEUCHT

AN ABSTRACT

Submitted to the School for Advanced Graduate Studies of Michigan
State University of Agriculture and Applied Science
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DOCTOR OF PHILOSOPHY

Department of Horticulture

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Approved: Donald P. Watson

JAMES ROGER FEUCHT ABSTRACT

Exploratory experiments were conducted in the 1958 growing season to establish the value of nine different growth-regulators for the induction of root initials in air-layered branches of seven to 12-year-old trees of Picea glauca and Pinus sylvestris.

From the results of the exploratory experiments growth-regulators were selected for use in further investigations conducted in the 1959 growing season. Air-layers were prepared on the lower, middle and top whorls of 15 trees of each species at the beginning of April, May, June, July and August, 1959. The air-layers at each of the three whorls on Picea glauca were injected with aqueous solutions of one of the following treatments: 0.5 ppm 2,4,5-Trichlorophenoxyacetic acid, 100 ppm naphthaleneacetic acid, 100 ppm 4-thianaphtheneacetic acid, 1,000 ppm indolebutyric acid and distilled water, and Pinus sylvestris air-layers were injected with 1 ppm 2,4-dichlorophenoxyacetic acid, 100 ppm naphthaleneacetic acid, indolebutyric acid, and 4-thianaphthaleneacetic acid, 100 ppm indoleproprionic acid and distilled water.

Results 100 days after treatment showed that there were no significant differences in rooting between treatments, or between positions of the air-layers on the trees. A significantly greater number of Picea glauca stems rooted, however, when air-layers were applied at the beginning of May, little rooting occurred when applied in July.

Mean root length was significantly greater at the end of the June treatments when compared with April, July and August.

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The number of rooted stems and the mean root length were correlative with the mean minimum and maximum air temperatures which might suggest that temperatures above 80° F occurring at the beginning of a treatment hindered root initiation and increased the incidence of stem death, but that root length was increased by gradually rising daily mean temperatures and hindered by temperatures which prevailed below 60° F.

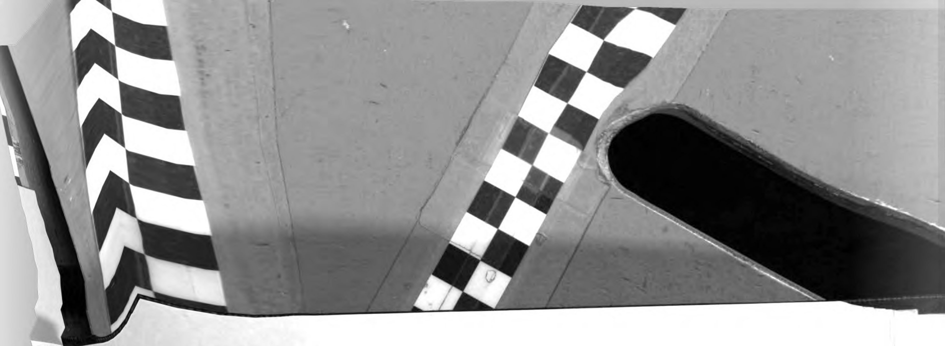
Anatomical descriptions of typical stem pieces were included.

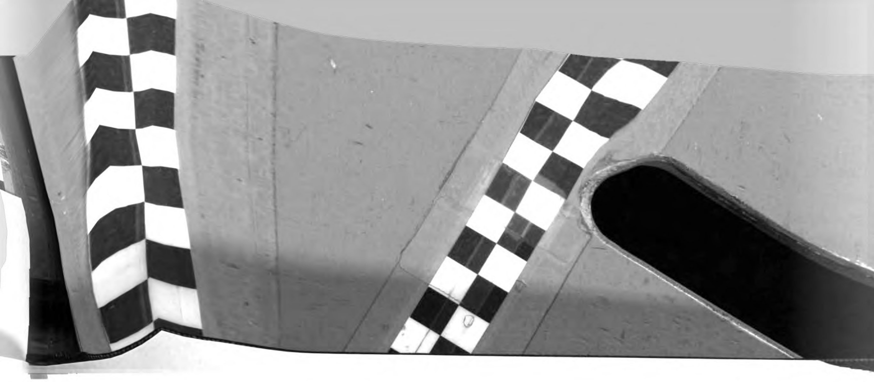
Examinations of sections cut through the wounded area of Picea glauca and Pinus sylvestris stems showed that root initials arose from secondary phleom rays of tissue proliferations produced by the vascular cambium. Root initials in stems of Pinus sylvestris invariably occurred at the apex of a knob-shaped tissue proliferation.

Anatomical examinations of dead stems of Picea glauca showed that no meristematic activity had occurred at or near the wounded area and it was suggested that high temperatures at the beginning of the treatment period may have prevented the formation of a protective periderm over the cut surface, causing stem death.

Survival of rooted air-layers of Picea glauca planted in September was 100 percent after 70 days in contrast with 52 percent in July when observed after 130 days.

Eleven tables and nine figures were included.





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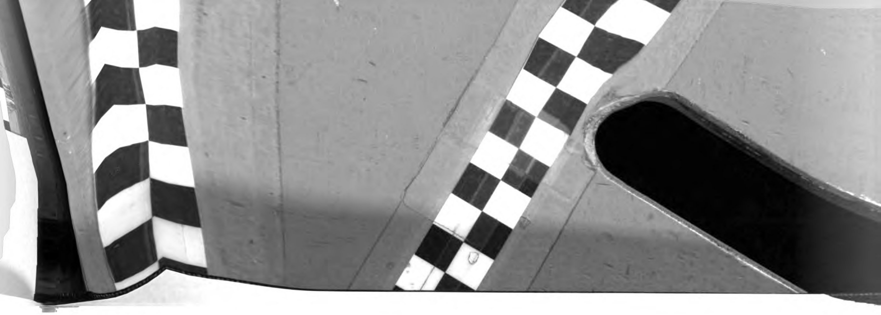

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INTRODUCTION

Vegetative propagation of woody and herbaceous plants is used primarily for the perpetuation of individuals selected for desirable characteristics. Propagation by vegetative means is used most commonly by nurserymen and foresters and is often the sole means of producing plants with some of the most useful, aesthetic, and economic features.

Many species of plants are propagated vegetatively by cuttage and graftage; others are reproduced but with great difficulty by either method. The lack of clonal material among many genera can be attributed (to a great extent) to the absence of a suitable means of vegetative propagation.

As a result of these difficulties clonal populations which do exist in these genera are few in number and consist of selections from only a small representation of the species. Rooting of cuttings and successful graft unions are rare in numerous species of <u>Pinus</u> and <u>Picea</u>.

Interest in the process of air-layering has stimulated investigation into the feasibility of this propagation method of plants which do not produce roots easily. The present investigation was conducted to determine the feasibility of air-layering as a method of reproducing two species of conifers, to screen the effects of growth regulators on root initiation and development, to study inherent and environmental factors affecting rooting and to examine the anatomical abberations exhibited in stems that were air-layered.

REVIEW OF LITERATURE

Natural Layering in Conifers and the History of Air-layering

Few species of coniferous plants reproduce naturally by vegetative means, but drooping branches of *Thuja occidentalis* growing on the limestone cliffs and shallow-soiled uplands of Mackinac Island, Michigan, have been observed to root when covered with soil and humus (Potzger, 1937). Successful layering of branches has also been reported in *Abies balsamea*, *Abies lasiocarpa*, *Cryptomeria japonica*, *Juniperus pachyphloea*, *Picea canadensis*, *Picea excelsa*, *Picea mariana*, *Picea sitchensis*, *Pinus chihuahuana*, *Pinus echinata*, *Pinus montana*, *Pinus rigida* and *Pinus sylvestris* (Potzger, 1937). Potzger has also noted that natural rooting by layering may occur in *Pseudotsuga*, *Sequoia*, *Tsuga* and *Tumion*, but with the exception of *Thuja occidentalis* the occurrence of natural layering in these species is not common.

Cooper (1911) found that natural layering of conifers is more common in regions of higher altitude and latitude. He cited *Abies balsamea* and *Picea sitchensis* as the most commonly layered conifers in these regions and described the layering habit of *Picea sitchensis*, *Tsuga mertensiana* and *Tsuga heterophylla* (Cooper, 1931).

Natural branch layering of *Picea mariana* in sites where the soil is shallow has been described by Fuller (1913) who also observed that a single parent tree might give rise to a circular colony of young vegetatively propagated trees when its lower branches were gradually covered by soil.

Lemberg (1933) reported that in many instances Pinus sylvestris partially buried by sand dunes on the coast of Finland will layer and form circular "colonies" of rooted branches.

A method of inducing roots known as "air-layering", "Chinese layering", "marcottage", "mossing-off", or "propagation from gootes", has been used by the Chinese for over 2000 years (Thakurta, 1940; Mergen, 1955). The manufacture of plastic films has increased the use of the air-layering method within the last 12 years because of its properties of high gaseous exchange and low water permeability.

The first to use plastic films for air-layering was Colonel William Grove of Laurel, Florida in 1947 (Grove, 1947). Grove experimented with two types of plastic films, "Pliofilm" and "Vitafilm"[1/] on the Lychee, Litchi chinensis. Pliofilm disintegrated before roots could form, but the heavier Vitafilm was found to be suitable. Both films have similar properties in that they prevent water evaporation, but permit the passage of gases through the film (Grove, 1947). The film currently of widespread use in air-layering is called polyethylene[2/], and sold under various trade names. All polyethylene has the properties of very low transmission of water vapor and high gas permeability (Wyman, 1952).

[1/] Manufactured by Goodyear Tire and Rubber Company, Inc., Akron, Ohio.

[2/] Polyethylene is a high polymer of ethylene developed by the Imperial Chemical Industries Ltd. of England and licensed for manufacture in the United States in 1943 by E. I. DuPont de Neumours and Company.

Creech (1950) successfully rooted the Rhododendron hybrid "America" with the use of polyethylene-wrapped air-layers. Of 18 air-layers made, 11 rooted and grew after being severed from the source plant and placed in soil.

Rooting of Magnolia grandiflora, Acer palmatum and selected hybrid tea roses was obtained through air-layering by Hanger et al. (1954). In nearly all of the stems which were rooted, however, considerably injury to the current growth was observed.

A study of air-layered Queen Elizabeth roses in which black and white colored polyethylene films were used to prevent moisture loss showed that this rose variety produced numerous roots six weeks after the initial air-layering, but no differences in rooting occurred between black and white polyethylene wrapped stems (Ching et al., 1956).

Abraham (1956) experimented with the air-layering process on cashew, Anacandium occidentale at the Central Cashewnut Research Station, Madras, India and reported that an average of 75 percent rooting can be obtained with this species throughout the year.

Certain species of Artocarpus were rooted by marcottage by Fabello (1934) and this method of propagation is widely used on many tropical and sub-tropical fruits, principally the mango, Mangifera indica L., and the avocado, Persea gratissima Gaertn., (Fielden, 1936; Singh, 1953; San Pedro, 1935).

Limbs of the guava, *Psidium guajava* Linn., more than 1/2 inch in diameter were rooted in 3 to 5 weeks, using the air-layering methods Ruehle, 1948).

Rao *et al.* (1952) attempted air-layering on *Diospyros kaki* Linn., no rooting occurred.

Wyman (1952) attempted air-layering on 250 plant species representing 58 genera of "hard-to-root" woody plants, including some gymnosperms, and successfully rooted 140 species. Species of *Pinus* and *Picea* were not included.

In 1952, Mergen air-layered branches of *Pinus elliottii* Engelm. prepared by completely removing a ring bark and cambium 1/4 inch in width treating with Hormodin No. 3[1/] and wrapping with moist sphagnum enclosed in a tight wrap of polyethylene film. Although no rooting was recorded 5 1/2 months after treatment, his later studies showed that rooting of *Pinus elliottii* by air-layering was more successful than by cuttings (Mergen, 1955).

Hoekstra (1957) conducted additional studies of this same species and reported rooting as high as 93.6 percent using six-year-old trees. Extensive studies conducted by Mergen and Cutting (1958) using air-layered *Picea abies* and *Picea pungens* resulted in 15 and 3 percent rooting, respectively. Less extensive studies of air-layered stems of *Pinus palustris* were performed by Johansen and Kraus (1958) in which 9 stems of the 15 air-layered stems produced roots.

[1/] A commercial preparation of Merck and Company, Rahway, New Jersey.

Hitt (1955) attempted air-layering on Pinus resinosa, Pinus banksiana, Pinus strobus and Pinus sylvestris. No rooting results were given.

Pinus echinata and Pinus taeda were rooted by Zak (1959) using a unique method of air-layering. Two and one-half-year-old plants growing in pots were placed horizontally so that the stems were resting in notches of a wooden frame filled with moist sphagnum. An aluminum cover was then placed over the frame for 53 days. Eight stems of Pinus echinata and six of P. taeda out of ten stems each rooted using this method. Zak also used DuPont synthetic sponges in the place of sphagnum with some rooting response. The roots were not able to penetrate the sponge material and, therefore, Zak suggested using cup-shaped sponges to prevent the retarding of root growth.

In Sumatra, Lasschuitt (1950) air-layered Pinus merkusii and reported that eight months after applying the air-layers, seven of the 75 treated branches had produced roots.

Frolich (1957) air-layered Picea abies, P. abies pygmaea, P. abies nidiformis, P. pungens glauca, Larix decidua, L. leptoleptis, Abies concolor Chamaecyparis Lawsoniana and Pinus sylvestris in addition to ten Angiosperms including the genera Tilia, Populus, Quercus, Acer, Ulmus, Rhamnus, Pirus and Carpinus. All of the Angiosperms rooted well, usually higher than 60 percent, and many 100 percent. The coniferous species rooted from 20 to 80 percent, with the exception of Pinus sylvestris which did not root at all.

Extensive literature reviews concerning factors affecting rooting of cuttings have been compiled by Priestley and Swingle (1929), Swingle (1940) and Nienstaedt (1958) which indicated that these studies have been more frequent than those with air-layering, yet factors having influence on the rooting of cuttings have also been found to play a role in the rooting of air-layers. For this reason some of the findings must not be overlooked.

Influence of Physiological Age on Rooting

The term "physiological aging" has been used to refer to an inherent change of an organism which brings it closer to senescence (Robbins, 1957). According to Schaffalitsky de Muckadell (1959) this change is associated with meristematic aging. She states that "Generally speaking the annual shoot is rightly considered the youngest part of a tree. It is important to notice that in studies of ontogenetic development, the situation is quite the contrary. The annual shoot should be looked upon as the latest developed, or even the 'oldest' part of the plant."

Physiological aging is apparent in most plants, but is particularly pronounced in plants which exhibit a wide variety of leaf-shapes. Difficulties arise, however, in determining whether the change in leaf shape is caused by "meristematic aging" or environmental influences. Molish (1915) originated the term "topophysis", which he describes as a condition in which shoots exhibit individuality as related to position on the tree. This effect has been exhibited on cuttings of Araucaria excelsa where cuttings from first order terminals develop into upright plants and those from second order branches develop into single "thread-like" horizontal shoots (Vöchting, 1904).

Seeliger (1924) has referred to the effect of aging on plants as "zyklophysis" (cyclophysis) and considered cyclophysis to be identical with topophysis. Schaffalitsky de Muckadell (1959), however, rejected Seeliger's view and differentiated between topophysis and cyclophysis in that cyclophysis was a definite effect of age of the meristems and topophysis, an effect of shoot individuality as proposed earlier by Molish (1915).

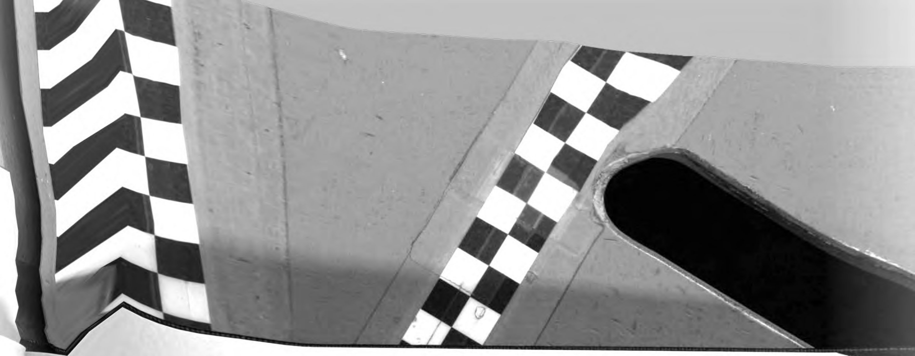
A condition in a tree or its ramets which was caused by earlier effects of the environment has been termed "periphysis" by Büsgen and Münch (1957). The adult form of Hedera helix is conditioned by cyclophysis and periphysis because the condition arises only in old age and everywhere on the old plant where it is exposed to full light eliminating the change in leaf form being due to topophysis (Büsgen and Münch, 1957).

Rooting ability in plants also appears to depend upon the cyclophysis and periphysis phenomena. Delisle (1942) using lateral and terminal cuttings of the current season's wood taken from two-, four- and over four-year-old trees of Pinus strobus, found that the cuttings from two-year-old trees rooted only with the aid of an auxin and those from trees over four years old did not root at all. Further studies using the brachyblast

(fascicles or short-shoots) showed only three to five percent rooting when the short-shoots were taken from older trees (over four-year-old) and up to 74 percent rooting when taken from younger trees. There was no reported difference in rooting between terminal and lateral shoots. Doran and Holdsworth (1940), however, obtained 70 percent rooting of Pinus strobus cuttings from a 33-year-old tree when the cuttings were taken from lower branches and treated with an aqueous solution of 200 mg per liter indolebutyric acid. No rooting was observed from cuttings taken near the top of the 33-year-old tree.

The effect of the position of the cutting on the plant was also observed in a remarkable experiment by Toole (1948) in which stem cuttings of Albizzia julibrissin Durazz. originating directly from roots, rooted 100 percent, while no rooting occurred when the cuttings were obtained from stems originating distal to the roots. Toole suggested that the ease of rooting increases as cuttings are taken from nearer the root system due to a greater concentration of root-promoting substances in the root than in the aerial parts of a plant.

Gardner (1929) studied the relationship between tree age and the rooting of cuttings from one- to twelve-year-old trees of Pyrus malus, P. communis, Prunus avium, P. mahaleb, P. cerasifera, P. persica, Ulmus americana, U. pumila, Acer saccharum, A. dasycarpum, Ilex opaca, Robinia pseudoacacia, Catalpa speciosa, Amorpha fruticosa, Pinus strobus, P. resinosa, P. taeda,



P. sylvestris, *Picea abies*, *Thuja occidentalis* and *Taxodium distichum*. Nearly all of the species rooted faster and in greater abundance when one-year-old trees were used as a source of cutting material. Less rooting occurred the older the parent tree. Outstanding examples of the age phenomenon as observed in Gardner's experiment are listed as percent rooting for one-, two-, and three-year-old trees, respectively: *Pinus sylvestris*, 77, 8 and 0 percent; *Pinus strobus*, 98, 51 and 12 percent; and *Ilex opaca*, 100, 64 and 47 percent.

Rooting investigations of hard-to-root plants by Thimann and Delisle (1939) led the investigators to conclude that the ".... most important factor..." in rooting plants is the age of the ortet.

Bodman *et al.* (1952) also found that the age of the ortet is an important factor in rooting *Pseudotsuga taxifolia glauca* cuttings and even affects the survival of out-planted rooted cuttings. Rooting of cuttings from five-, 12-, 17- and 42-year-old trees was 16, 25, 30 and 0 percent respectively, while survival was 43, 29 and 43 percent, respectively.

Hoekstra (1957), after applying air-layers to the distal tips of the branches in the upper one-third of crown in six- and 23-year-old *Pinus elliottii*, found that rooting occurred in 12 weeks in both age classes. Six-year-old trees produced rooting up to 93.6 percent and 23-year-old trees rooted 80 percent.

McCullock (1943) made cuttings using one-, two- and three-year-old wood from a 50-year-old Pseudotsuga menziesii glauca and observed rooting from one-year-old wood only despite chemical treatments.

Johansen and Kraus (1958) reported that nine out of the fifteen air-layers applied to a 52-year-old Pinus paulustris rooted. All fifteen air-layers were applied to the crown region of the tree.

Deuber (1940) experimented with cuttings of Picea abies, Pinus strobus, P. resinosa, P. bungeana Zucc., P. densiflora Sieb. and Zucc. and Tsugo canadensis L. Carriere. Five-year-old Picea abies cuttings rooted more readily, in general, than cuttings from 26- and 40-year-old trees, however, the season of the year in which the cuttings were made influenced the effect of age. Cuttings of Pinus strobus from juvenile ages, especially from seedlings two to four years old, rooted to a greater extent and more consistently than cuttings from older trees. Some rooting did occur, however, from trees 5, 7, 15, 18, 25, 30, 40 and 60 years old. Lateral cuttings rooted "more abundantly" than terminal cuttings. No rooting occurred in the limited trials of Pinus resinosa. Ten-year-old Pinus bungeana cuttings rooted 52.5 percent, 15-year-old Pinus densiflora, 25.7 percent and four-year-old Tsuga canadensis, up to 65 percent.

Grace (1939) investigated the difference in rooting response of cuttings taken from upper and lower regions of 18-year-old Picea abies and observed that 75 percent of the cuttings rooted when taken from the lower branches and

43 percent rooted from branches taken near the top of the tree. Position, however, had no significant effect on the number of roots produced per cutting. Tsunahide and Ogasawara (1957), on the other hand, found that cuttings of Metasequoia glyptostrobeides from lower branches formed a larger number of roots and longer roots per cutting.

Effects of position of a given whorl on rooting were investigated by Farrar and Grace (1942) using cuttings from lower branches of Picea abies. Cuttings were classified into six types, as follows: Type 1, first order terminals which averaged 127 mm in length; Type 2, second order terminals averaging 81 mm in length; Type 3, second order large laterals averaging 93 mm in length; Type 4, second order small laterals averaging 71 mm in length; Type 5, third order laterals averaging 59 mm in length; and Type 6, the distal part of Type 1, averaging 79 mm in length. They concluded that the position of the cutting on the branch had "little" effect if the cuttings were made six to nine cm long. Sixty-seven percent of the first order terminals rooted but when made shorter than six cm, only 32 percent rooted.

When a "heel" of older wood was left on cuttings of Picea abies, rooting was inhibited (Deuber and Farrar, 1940). Farrar and Grace (1942), however, found in later experiments that heel cuttings appeared to favor root length when placed in a peat medium and survival and rooting was higher with heel cuttings than with simple cuttings when used in sand. No explanation was given for this effect of age of wood and media type.

Effect of Season of Year on Rooting

The importance of the month of collection of cuttings has long been recognized by plant propagators whether working with gymnosperms or with angiosperms.

Farrar and Grace (1941) collected cuttings of Picea abies at 24 intervals throughout a year making seven collections when new growth was forming, six at semi-monthly intervals from July to September, four during October and seven at monthly intervals during the period of winter to April. Rooting success gradually decreased during the summer months, and increased to a high of 80 percent in September and October. Cuttings stored over winter or taken in April and May did not respond well. Results also indicated that cuttings taken just as the buds are opening rooted more readily than cuttings taken before and immediately after bud expansion. Deuber and Farrar (1940), using a total of 3200 cuttings of Picea abies, collected at monthly intervals, reported that rooting increased from October to a high in December and decreased rooting occurred in January. In contrast, a study of the seasonal variation in the natural rooting capacity of Picea abies and P. sitchensis (Larsen, 1955) showed that rooting was highest in June and July, lowest in September, with a gradual increase in rooting occurring on cuttings made during the winter months. Differences in results between Deuber and Farrar and the work of Larsen might be attributed to the climatic differences between Ottawa, Canada

and Denmark, and to the climatic differences in the years during which the work was carried out.

Doran et al. (1940) found that Pinus strobus cuttings yielded 70 percent rooting when taken in March.

Systematic studies of cuttings from Picea abies and Pinus strobus made by Deuber (1940) clearly showed that dormant stems collected in mid-winter season root more readily than at any other season. Deuber termed the period of best root production in Picea abies from October to December as the "grand period of root generation", while Pinus strobus cuttings rooted most abundantly when collected in December, January and February.

Cuttings of Pseudotsuga menziesii glauca and Picea sitchensis were found to root best when taken in December through February (Griffith, 1940).

Childers and Snyder (1957), using 16-year-old pistillate trees of Ilex opaca 'Arden' and 'Cumberland' and 40-year-old staminate trees 'Old Hale and Hearty', collected cuttings on the first and fifteenth day of each month from August 1 to November 15 with variable results. 'Arden' was found to be the easiest to root and no significant differences in seasonal effects on rooting were observed. 'Old Hale and Hearty' rooted highest when cuttings were collected in September and November. 'Cumberland', the most difficult to root, rooted highest when cuttings were obtained on September 1 with very low rooting occurring when collected on November 15. It was interesting to observe that cuttings Collected from the oldest parent, 'Old Hale and Hearty', exhibited a higher rooting capacity than the cuttings from the younger cultivar, 'Cumberland'.

Recent interest in the practice of air-layering of conifers stimulated Mergen (1955) and Hoekstra (1957) to investigate the seasonal variation in rooting of air-layered Pinus elliottii. Mergen, in a preliminary experiment, showed that rooting would occur when air-layers were applied to 1/4-inch girdled stems in October, May and August. No significant differences were obtained in the number of branches rooted, but August treatments yielded more roots per marcotte and in a much shorter time than the October and May treatments.

Hoekstra (1957) concluded that July was a better month than September to apply air-layers on Pinus elliottii growing in Lake City, Florida. He considered the gradual decrease in daily mean temperatures to be an important factor in the reduction of rooting capacity in the September treatments.

Recent air-layering studies conducted by Mergen and Cutting (1958) using Picea abies and P. pungens growing in New York, demonstrated that rooting was highest when the marcotte was applied in April before active shoot elongation had taken place.

Johansen and Kraus (1958) were successful in rooting air-layered Pinus paulustris stems which were applied in October. Only 15 air-layers were attempted, however, and no seasonal variation study was conducted.

Rooting Responses as Influenced by Growth-regulator Applications

Within the past two decades plant propagators have become keenly interested in the use of growth-promoting substances for root induction and growth in cuttings. As a result, there are large quantities of literature concerning the effects of chemical substances on the formation of adventitious roots under greatly varied conditions and on many plant species and varieties.

Experiments conducted with coniferous species concern, for the most part, attempts to determine the value of various growth regulators in the rooting of cuttings. Some workers in this field have concluded that for the particular species they are studying, growth-regulators are of no significant value, yet others using the same species, will conclude that growth-regulators are of great value in stimulating adventitious roots.

Deuber (1940), using indoleacetic acid at four mg per gram of talc, Hormodin No. 1 and No. 3, and indolebutyric acid at one, two and four mgs per gram of talc, concluded that on *Pinus strobus* cuttings "...there were relatively few instances in which the chemical treatments materially increased the rooting response." Delisle (1942), on the other hand, reported the *Pinus strobus* cuttings root "very little" without chemical treatments. Although Delisle used indolebutyric acid for treatments, as did Deuber, Delisle increased the concentration three-fold, which may explain the differences in conclusions between the two authors. Another rooting study of *Pinus strobus* by Doran (1940)

showed that 70 percent rooting was obtained from a 33-year-old tree using wood from lower branches and treated with indolebutyric acid at 200 mg per liter for five hours. Treatment in the aqueous solution for 24 hours prevented root formation.

Grace (1940) treated cuttings of Picea abies with talc preparations containing indolylacetic acid and napthylacetic acid each at 0, 1000 and 5000 ppm, and combined with 0 and 10 percent cane sugar and ethyl mercuric phosphate at 0 and 50 ppm. Indolylacetic acid at 1000 ppm increased rooting from 10 to 42.5 percent, but at 5000 ppm reduced rooting significantly. Naphthylacetic acid reduced rooting at both concentrations when compared with control cuttings. Mean root length was increased only by indolylacetic acid at 1,000 ppm.

Farrar and Grace (1940) found that Pinus strobus and Picea glauca cuttings received no benefit from applications of indoleacetic acid.

Kirkpatrick (1940) lists 18 species of broad-leafed evergreens and 61 species of conifers which root more readily when treated with indolebutyric acid at concentrations ranging from 10 to 80 mg per liter of water. Kirkpatrick concluded that "... there is no question but that indolebutyric acid is helpful and beneficial in inducing roots."

Two hundred mg per liter of water of indolebutyric acid was found to be very beneficial in rooting Pinus strobus by Doran (1946). Doran pointed out that the benefit may be influenced by individuality of trees in their rooting capacity even though all of the trees used were of the same age.

In addition to *Pinus strobus* the rooting ability of cuttings from other five-needled pines was studied by Deuber (1942), including *Pinus monticola*, *P. parviflora*, *P. flexilis*, *P. koraiensis*, *P. peuce*, *P. cembra* and *P. lambertiana*. Indolebutyric acid at 2 to 200 mg per gram of talc increased rooting in *P. strobus*, *P. monticola* and *P. cembra*, but no rooting occurred in the other species. *Pinus strobus* rooted as high as 60 percent on 15-year-old trees with 2 mg per gram of talc. Only 5.5 percent rooting was obtained with the cuttings of *Pinus monticola* and 30 percent with *P. cembra*. The low rooting response obtained with *Pinus monticola* was attributed to using trees 56 years old.

The effects of indolebutyric acid, indoleacetic acid and alpha naphthoxyacetic acid on the rooting of *Pseudotsuga menziesii glauca* and *Picea sitchensis* were investigated by Griffith (1940) by soaking the basal ends of the cuttings in aqueous solutions of the growth-regulators for 24, 48 and 72 hours. The solution concentrations were 0, 6.35, 12.5, 25.0, 37.5, 50.0, 75.0, 100.0, 125.0, 150.0 and 200.0 ppm. Results obtained 160 days after treatment showed that indolebutyric acid at 25.0 ppm gave 100 percent rooting on *Picea sitchensis* and 80 percent rooting on *Psuedotsuga menziesii glauca* when collected in February. Bodman (1952), on the other hand, found that the best rooting on *Pseudotsuga menziesii glauca* was obtained with 50 ppm indolebutyric acid.

Komissarov (1938) treated cuttings of *Pinus sylvestris* with beta-indolylacetic acid at a concentration of 100 ppm. No rooting occurred in the first

attempts but a repeated experiment yielded 70 percent rooting with cuttings obtained from three-year-old trees treated in the same manner as in the previous experiment. Komissarov concluded that chemical treatment is of great importance in rooting this species.

The comparative activity of the stimulation of roots in cuttings was investigated by Hitchcock and Zimmerman (1939). Aqueous solutions of 1 to 80 mg per liter of water produced essentially the same effects as talc preparations at the concentration range of 0.5 to 50 mg per gram of talc. Potassium salts of indoleacetic, indolebutyric and naphthaleneacetic acids were more effective than the acids; of these, the salt of indolebutyric acid was more effective than indoleacetic acid and naphthaleneacetic acid on most of the plants tested. A later study by Hitchcock and Zimmerman (1940) indicated that mixtures of indolebutyric acid and naphthaleneacetic acid were more effective than individual substances as characterized by more uniform rooting, a greater number of roots and a greater rooting percentage.

Stoutemyer and O'Rourke (1945) obtained significant increases in rooting percentage by spraying 2, 4, 5-trichlorophenoxyacetic acid and its sodium salt on cuttings of Buxus sempervirens handsworthii, Ilex crenata convexa, I. vomitoria, Ligustrum amurense and L. ibolium. Effective concentration depending upon the species.

In a later paper Stoutemyer (1954) found that 2, 4-Dichlorophenoxyacetic acid and 2, 4, 5-T were very effective in root promotion at a concentration as

low as 0.5 ppm. Bud inhibition, however, may result with the use of 2, 4-D. No bud inhibition was observed with application of 2, 4, 5-T to the cut ends of numerous species of cuttings.

The Effect of Genetic Variation on Rooting Capacity

Genetic variation between individual plants of a given species or between clones has been found to have a marked effect on the rooting capacity of some plants.

Deuber (1940) observed highly significant variations in rooting ability in one collection of cuttings from *Picea abies* in which rooting of cuttings taken from various individual trees ranged from 0 to 100 percent. Similar variation of rooting was observed in hybrid seedlings of *Pinus attenuradiata* in an experiment conducted by Duffield and Liddicoet (1949). The variation of rooting was attributed solely to genetic differences between seedlings.

A range of rooting of cuttings from 3 to 55 percent between 15 clones of *Populus deltoides* were recorded by Cunningham (1953). Hybrid seedlings of *P. deltoides* showed rooting up to 97.5 percent.

Snow (1941) tested the rooting responses of 24 clones of *Acer rubrum* and reported a rooting range of 17.5 to 97.5 percent between the clones.

Gregory and Van Overbeek (1945) found that the red-flowered *Hibiscus rosa-sinensis* rooted readily, yet a white-flowered selection of this variety rooted with difficulty.

In 1942, O'Rourke found marked differences in rooting capacity and survival of cuttings taken from 13 clones of Robinia pseudoacacia. Scions were grafted onto seedling rootstocks and after growing in the field for one year cuttings were collected and their bases placed in a sandy loam soil. After eight months, the number of survivors of each clone was recorded. A range of 0 percent to 50 percent survival was obtained. The average length of growth was also recorded in this experiment and the data indicated that the percent survival paralleled the average vigor. Those clones which resulted in a higher survival also attained a greater length of growth.

The physiological significance of inherent rooting capacities is not understood at the present time, but Mirov (1941), in investigations which did not involve rooting, has shown that the concentration of naturally occurring growth regulators in the stems of Pinus ponderosa and Pinus torreyana parallels the vigor of the plant and is a hereditary trait.

The Formation and Development of Adventitious Roots from the Standpoint of Anatomy and Histochemistry

Adventitious root formation may be induced in many types of plants as evidenced by the preceding review of the factors that affect rooting. The effect of these factors differ with the species of plant tested. The type of tissues involved in the initiation and subsequent development of adventitious roots vary with the species and its anatomical characteristics.

Connard and Zimmerman (1931) studied the origin of adventive roots in cuttings of Portulaca oleracea L. and found that the pericycle, cambium and ray cells all take part in root formation, but that the root initials arise only from "ray" cells adjacent to primary vascular bundles. Roots were well differentiated before emerging from the stem and appeared to dissolve interfering cells of the cortex and epidermis.

Adventitious roots of Vaccinium corymbosum were shown by Mahlstede and Watson (1952) to originate from the phloem region and usually immediately adjacent to a xylem ray. Roots were found most commonly in association with a bud gap. A mechanical inhibition of root emergence from the stem caused the developing roots to bend in the cortex before emerging through the epidermis. Mahlstede and Watson suggested that the repression of root development was caused by the presence of a dense ring of pericyclic fibers or was the result of a thick cuticle which was equal in thickness to the subtending epidermal layer.

Jiminez (1937) reported that Ceiba pentandra and Sandoricum koetjape cuttings formed roots arising from newly formed phellogen cells and Bixa orrelis cuttings developed roots which originated from "callus" or from preformed meristematic areas.

Studies by Carlson (1929, 1933) using stem cuttings of Dorothy Perkins and American Pillar roses showed that roots originate from parenchymatous cells of the secondary phloem, but may occasionally arise from the cambial area, pericycle, epidermis and cortex, and bud-gap parenchyma.

Anatomical studies of the origin of roots in stem cuttings of red and black raspberries by Sudds (1935) showed that most of the adventitious roots arose from near the protoxylem points at the periphery of the pith.

Stangler (1949) found that roots invariably originated in the pericycle of *Chrysanthemum morifolium* Bailey, *Dianthus caryophyllus* L. and *Rosa dilecta* Rehd. In *Dianthus* there was a band of thick-walled fibers external to the point of root origin which prevented roots from developing vertically through the cortical region and therefore curved downward, emerging at the base of the cuttings. No mechanical hinderance of root development was observed in *Chrysanthemum* and *Rosa*.

Adventitious roots were found to be initiated in woody stems of *Acanthus montanus* T. Anders, (Taylor, 1926), *Forsythia suspensa* (Swartley, 1943) and *Salis* sp., *Vitis* sp. and *Ribes* sp. (Corbett, 1897). In addition to roots arising from the cambium, Sandison (1934) observed pre-existing root initials in an area between the cambium and pericycle of *Lonicera japonica* stems. Sandison reported that adventitious roots will arise from the cambium in this species only when the tissues surrounding the cambium are wounded.

Van Tieghem and Douliot (1888), after extensive studies of the origin of roots in many vascular plants, concluded that adventitious roots which occur naturally in young stems are initiated only in the pericycle. Older stems may contain adventive roots which arise in the phloem parenchyma and later in the cambium.

Stages in the initiation and subsequent development of adventitious roots from cuttings of *Taxus cuspidata* were observed by Hiller (1951). Roots which emerge from near the base of the cuttings were initiated from the rays in the secondary phloem and roots which emerged more distal to the basal end of the cuttings were initiated in secondary phloem ray cells and the surrounding parenchyma.

Priestley and Swingle (1920) emphasized that young stems in general form roots from the pericycle and in older stems from cambial cells, but in either case the roots are intimately associated with the rays and involve more than one layer of cells. This concept was supported in a later study by Datta and Majundar (1943) of several dicotyledonous plants.

Ray-cell association with adventitious roots was found by Bannan (1941) in an anatomical study of *Thuja occidentalis* L. stems which had rooted by natural layering. Roots originated adventitiously from "outer tissues" and invariably were continuous with xylem rays composed of parenchyma cells only.

The association of adventitious root formation with rays was also noted in species of *Populus* and *Salix* by Trecul (1846) and van der Lek (1924) in *Begonia maculata* and *B. semperflorens* (Smith, 1936) and in *Tropaeolum majus* L. (Smith, 1942). Adventitious roots in *Populus* and *Salix* species originated from near the cambium and at the extremity of convergent rays, or in some instances, from a single ray (Trecul, 1846). In the more succulent species, *Begonia* and

Tropaeolum, roots arose from the interfascicular cambium and occasionally in the fascicular cambium at the edge of a vascular bundle (Smith, 1936, 1942).

Delisle (1942) described the sequence in the initiation and development of roots arising from stem cuttings of Pinus strobus L. In tissues from both "young" and "old" trees, the first apparent change in cellular activity near the exposed end of the cutting was the formation of a periderm from subepidermal cells and "extensive activation" of the cortical and pericyclic cells. Cortical cells generally acquired a binuclear condition after periderm formation, while cambial cells became active and produced considerable parenchyma as well as xylem and phloem. Cambial activity did not proceed equally in a radial direction throughout the circumference of the cuttings. More active areas produced "cushions" of cells which were located at the ends of a congerie of rays and adjacent to leaf traces. These "cushions" were shown to eventually develop into adventitious roots. Delisle also observed roots arising from the parenchyma of leaf traces. Auxin treatment caused tracheal cells of the proliferated xylem to form smaller but numerous bordered pits on both the tangential and radial walls.

Adventitious roots of Cotoneaster dammeri arise only from one of the two groups of parenchymatous cells in the divided bud gap and is an apparent resumption of the activity of the parenchyma cells (Wolfe, 1934). Wolfe also found in this same species that vascular elements of young roots differentiate very early in their development and that the roots are fully differentiated into a cortex and central vascular cylinder by the time that emergency from the stem takes place.

Esau (1953) concluded that, in general, if the organ is young the adventitious primordium is initiated by a group of cells near the periphery of the vascular tissues, and if the organ is older, the origin of adventitious root primordia are located more closely to the vascular cambium. Younger stems form roots more commonly from interfascicular parenchyma and older stems from vascular rays.

Callus development in relation to root initiation and development was studied by Swingle (1929) with Malus sp. and Salis sp. cuttings. Swingle concluded that rooting and callusing are two distinct processes and that no relationship exists between callus formation and root initiation and development. Taylor (1926) also concluded that callus tissues have no part in the formation of roots, in a study of Acanthus montanus cuttings. Snyder (1954) on the other hand, reported that "... in some instances a cambium develops within the callus tissue..." from which root initials may arise.

Some confusion of the term "callus" is apparent in the literature. Swingle (1929) defines callus as a form of "tissue hypertrophy", which sometimes results in large masses of parenchyma. Sledge (1930) referred to wood proliferations as "callus:, and Hiller (1951) called proliferations of the xylem, phloem and cortex, a "callus" structure.

Bloch (1941) characterized the tissues which proliferate during the healing

of wounds as "wound callus", and parenchyma proliferations which are not organized into a definite tissue as "cell proliferations".

After extensive studies of a large number of pteridophytes and representatives of both monocotyledonous and dicotyledonous plants, La Rue (1937) concluded that pteridophytes do not develop callus on wounds, only a few monocotyledonous plants form callus; sclerophyllous and succulent species, in general, tend to form periderm only, and most dicots form callus upon wounding unless they possess specialized structures for vegetative reproduction.

The histological responses of stem cuttings to applications of growth-regulators were examined by Beal (1951) using Phaseolus vulgaris L. A three percent concentration of indoleacetic acid in lanolin paste applied to the base of bean cuttings caused tumor-like outgrowths and the formation of adventitious roots 110 to 120 hours after treatment. Cells of the epidermis and pericycle responded less actively than other tissues. Parenchyma of the cortex enlarged many diameters when compared to non-treated stems and cells near the endodermis became meristematic, differentiating into phloem and xylem elements and large parenchyma cells. Some cells remained meristematic and formed root histogens. Near the surface of application the cells of the rays adjacent to the xylem proliferated and differentiated into a confused mass of tracheids. Rays of proliferated phloem often gave rise to root histogens.

Struckmeyer (1951) conducted experiments on the effects of one percent 2, 4-dichlorophenoxyacetic acid (2, 4-D) on the stem anatomy of Lycopersicon

esculentum, Coleus blumatus and a species of Xanthium, Dracena and Philodendron. Struckmeyer found that 2, 4-D stimulated rooting activity in all of the species except the Philodendron sp. and rooting was usually preceded by heavier tissue proliferations than in untreated cuttings. Rooting occurred rapidly in Lycopersicon, Xanthium and Coleus, but in Dracena, rooting did not occur until a large mass of proliferated vascular tissue had formed.

The influence of the carbohydrate and nitrogen content on the rooting of cuttings of Lycopersicon esculentum and Tradescantia virginiana was studied by Starring (1923) employing the iodine-potassium iodide (IKI) test for starch, Benedict's solution for a test of sugars, and diphenylamine-sulfuric acid test for nitrates on stem tissues of each species. The amount of carbohydrates and nitrogen in the plants was superficially controlled before making the cuttings by increasing or decreasing the nitrogen in a fertilizer and increasing or decreasing the duration of natural light. Very little rooting occurred in stems which were observed to have a low carbohydrate and high nitrogen content. The greatest amount of roots were produced when stems contained a high nitrogen and high carbohydrate level; however, longer and slightly fewer roots were produced with a high carbohydrate and low nitrogen content.

Carlson (1929) conducted microchemical tests on rooted and non-rooted cuttings of Dorothy Perkins and American pillar roses. The Dorothy Perkins rose rooted more readily than the American pillar rose and results of iodine-potassium iodide tests for starch showed that the former variety contained a greater quantity of reserve starch in the stem tissues. Brandon (1939), on the other hand, showed that the content of starch in 42 rose varieties including those used by Carlson (1929), did not parallel the ease of rooting. Brandon conducted the study from November to July, inclusive, and Carlson in March and April.

EXPERIMENTAL METHODS AND MATERIALS

Exploratory

Exploratory experiments to determine the effects of nine growth-regulators on the rooting of air-layered *Picea glauca* and *Pinus sylvestris* were conducted between February 23, 1958 and June 10, 1959.

Five six-year-old plants of *Picea glauca* were planted in 12-inch clay pots and grown in a 60° F greenhouse. When the buds were beginning to expand, treatments were prepared and applied by the following methods: Twenty-five 50-gram lots of air-dried, sphagnum were placed into individual polyethylene bags and to each bag 1, 000 grams of water was added. The bag was sealed and allowed to stand at room temperature for 24 hours to ensure complete saturation of the sphagnum by the water. At the end of this period 600 grams of water was extracted by squeezing from each lot yielding a moist, but not saturated, rooting medium. After water extraction five grams of 100, 1, 000 and 5, 000 ppm each of one of the following growth-regulators prepared in talc by standard alcohol evaporation was added to the sphagnum and mixed thoroughly: 2, 4-dichlorophenoxyacetic acid (2, 4-D), 2, 4, 5-Trichlorophenoxyacetic acid (2, 4, 5-T), alpha-naphthaleneacetic acid (NAA), indolebutyric acid (IBA), gibberellic acid (GA), beta-naphthoxyacetic acid (BNA), indole-3-proprionic acid (IPA) and indole-3-acetic acid (IAA)[1/], making a total of 25 different treatments.

[1/] For convenience, abbreviations of the growth-regulators will be used hereafter.

Air-layers were prepared by selecting first order terminals of lateral branches, removing the leaves from five inches of the one-year-old stems beginning leaf removal three to four inches distal to the terminal bud and removing a ring of bark two mm wide from around the stem near the center of the leaf-free area using a tool designed for this purpose (Figure 1). Fifteen grams of the treated sphagnum were immediately applied around the wound and wrapped tightly with a 4" x 5" x 0.0015" sheet of polyethylene film[1/] secured at each end with plastic covered wire. The polyethylene was overlapped at least one-third of the circumference of the sphagnum mass to reduce the amount of evaporation of water from within the air-layers.

Ten air-layers per treatment were applied to the trees at random, precautions being made that each tree contained at least one air-layer of each treatment. Superficial observations of survival of the air-layers were made at periodic intervals and 70 days after treatment the branches containing the air-layers were cut from the trees by severing the stem immediately proximal to the location of the air-layer. By removing the polyethylene film and carefully removing the sphagnum, rooting, wound, proliferation and injuries were observed.

To determine the seasonal effect on the rooting response of Picea glauca and Pinus sylvestris, studies were conducted at the Max Gruner Tree Farm located in Shiawassee county near Perry, Michigan. Air-layers were applied

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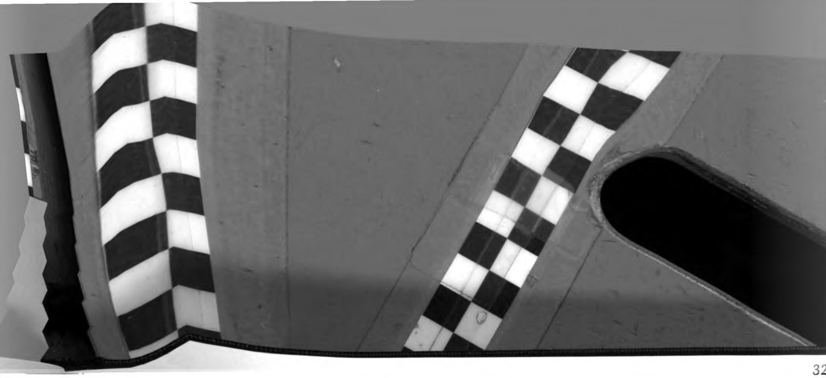
[1/] Cut from polyethylene film obtained from the Bakelite Plastics Company, Boundbrook, New Jersey.



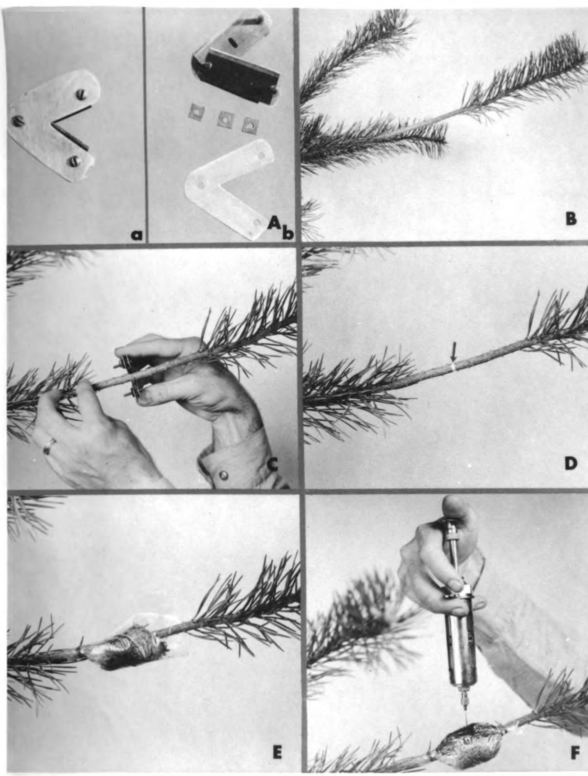


Figure 1. Preparation and treatment of air-layers. A_a - assembled girdling tool; A_b - aluminum cover removed to show arrangement of one pair of the razor blades; B - leaves removed from stem; C - cutting bark with tool; D - ring of bark removed (arrow); E - application of sphagnum and plastic; F - treatment by injection with a hypodermic syringe.

on April 30, 1958 to nine-year-old *Pinus sylvestris* trees and to nine- to twelve-year-old *Picea glauca* trees on May 7, 1958. Two hundred and thirty air-layers were prepared for each species, 115 per species on one-year-old wood and 115 on two-year-old wood. Sphagnum was prepared by soaking in water for 24 hours and extracting the water at the end of this period with a hand-operated wringer. The extracted sphagnum was then mixed thoroughly by repeated tumbling and weighed into individual lots of 20 grams. The pre-weighed sphagnum lots were then wrapped in the similar sheets of polyethylene which were used previously and stored in a polyethylene-lined box. The branches were prepared by removing the leaves from five- to six-inches of stem, wounded by making a v-shaped notch on the abaxial side of the stem completely removing a piece of bark one-quarter inch wide at its widest point, and wrapping the wound with the sphagnum as previously described (Figure 1). Each air-layer was treated with a solution of growth-regulator or with water by injecting with a hypodermic syringe five ml of an aqueous solution through the polyethylene film into the sphagnum (Figure 1).

The following is a list of the growth-regulators used: *Pinus sylvestris*, 2, 4-D, 2, 4, 5-T, IAA, NAA, BNA, IPA and IBA at the rate of 1, 100 and 1, 000 ppm, and on *Picea glauca*, 1 and 100 ppm of 2, 4-D and 2, 4, 5-T and 1, 100 and 1, 000 ppm of IAA, NAA, BNA, IPA, IBA and a new chemical, 4-trinaphtheneacetic acid (4TNA)[1/]. Distilled water was used for comparative treatments. Ten air-

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[1/] Supplied through the courtesy of the Upjohn Company, Kalamazoo, Michigan.

layers were prepared for each treatment, five on one-year-old wood and five on two-year-old wood for each species and applied at random over six trees of each species. Buds in both species were beginning to elongate at the time of treatment. Amount of rooting, wound proliferation and type of injuries were recorded 75 and 90 days after treatment in Picea glauca and Pinus sylvestris, respectively.

Marcots were again applied July 16, 1958 on Picea glauca and July 24, 1958 on Pinus sylvestris in the same manner as before, but differing in that wounding was accomplished by removing a complete ring of bark two mm wide in Picea glauca, and three mm wide in Pinus sylvestris, using the girdling tool described previously. Only one-year-old wood was treated in each species and the treatments used were as follows: in Picea glauca, 0.5 and 1 ppm 2, 4-D, 2, 4, 5-T; 1 and 100 ppm NAA, BNA and 4TNA; and 1, 100 and 1, 000 ppm IBA, IAA and IPA; in Pinus sylvestris, 0. 5, 1 and 100 ppm 2, 4-D and 2, 4, 5-T; and 1, 100 and 1, 000 ppm IBA, NAA, BNA, 4TNA, IPA and IAA. Control treatments in each species consisted of distilled water as before.

The amount of rooting, wound-tissue proliferation and injury was recorded 75 and 90 days after treatment of Picea glauca and Pinus sylvestris, respectively.

On August 4, 1958, eighteen 12-year-old Picea glauca trees were air-layered applying ten air-layers per tree. Treatments included 30 each of 100 ppm BNA, 1, 000 ppm IBA, 1, 000 ppm IAA, 100 ppm NAA, 100 ppm 4TNA and distilled water.

Similar treatments were made on twenty-five eight-year-old *Pinus sylvestris* trees August 14, 1958 treating with 1,000 ppm NAA, IAA and IPA, 100 ppm of 2,4-D, IBA and 2,4,5-T and distilled water. Thirty air-layers were made for each treatment, totaling 240 air-layers, ten per tree with ten additional air-layers on the 25th tree to be used for periodic observations.

Fifteen air-layers of each treatment and of each species were removed 75 and 90 days after treatment in *Picea glauca* and *Pinus sylvestris*, respectively. The remaining air-layers were left on the trees over winter and removed on June 1, 1959. Data were recorded in the same manner as for the July experiment.

1959 Growing Season

To obtain more information concerning the effect of the time of year on rooting, and to determine the effect of position of the air-layers on the trees, experiments were conducted during the 1959 growing season. Air-layers were applied on the first of April, May, June, July and August, using eight-year-old *Pinus sylvestris* and nine- to twelve-year-old *Picea glauca* trees growing on a game preserve in Shiawassee county, near Perry, Michigan. Each month fifteen trees of both species were air-layered applying the air-layers to branches of the lower, middle and top whorls designated as position one, two and three, respectively. To each of the three positions 1 ppm 2,4-D, 100 ppm NAA, IBA and 4TNA; 1,000 ppm IPA; and distilled water were applied to Pinus sylvestris; 0. 5 ppm 2, 4, 5-T; 100 ppm NAA and 4TNA; 1, 000 ppm IPA and distilled water were applied to Picea glauca. The sphagnum and air-layers were prepared as in the earlier studies, girdling the one-year-old stems two and three mm in width on Picea glauca and Pinus sylvestris, respec-pectively. The treatments were injected into the sphagnum with a hypodermic syringe as previously, using only two ml per air-layer.

All air-layers were removed for observation 100 days after treatment and the trees were labeled for future observations.

After carefully removing the sphagnum from around the stems of each marcot, the number of roots, root length in mm, degree of wound-tissue proliferation, and observations of injuries and unusual callus formations were recorded. The degree of wound-tissue proliferation was scaled from one to seven, one indicating no proliferation and seven indicating maximum tissue proliferation. The ratings were first determined by observing random samples of the April 1 treatments and combined with observations made in previous experiments, thus establishing a standard for each of the seven values.

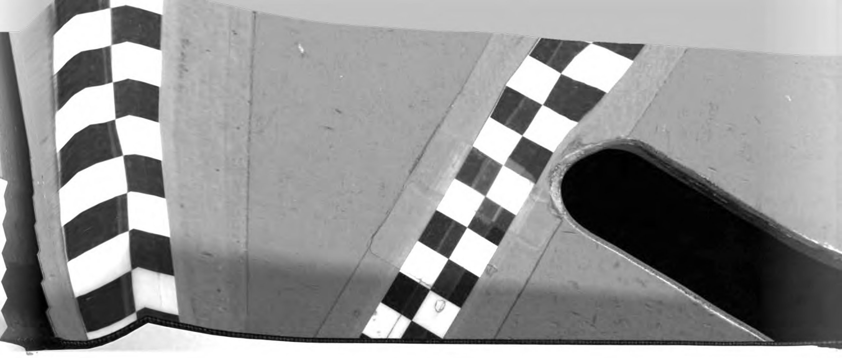
The rooting data was analyzed by first rating the rooting values obtained according to the number of roots produced per stem as follows: no roots - a value of one; one to five roots, inclusive - three; six to ten roots - five; 11 to 15 roots - seven; 16 to 20 roots - nine; 21 to 25 roots - 11; 26 or more roots - 13. All data were transformed to the square root of x + 1 as described by Goulden (1952) and subjected to analysis of variance.

Temperature Studies

To determine the temperature differences between the sphagnum medium and the surrounding air medium, thermocouples made of copper constantan wire were inserted into the sphagnum media of three air-layers located on the southeast, north and west exposures of a six-year-old *Picea glauca* tree. The tree was placed in an unprotected area to simulate field conditions. Two thermocouples were used to obtain temperatures of the surrounding air, one housed in standard U. S. Weather Shelter and the other attached to the tree and unprotected. Temperatures were recorded by a Honeywell-Brown temperature recorder[1/] once each hour from 12:00 noon until 4:00 p. m. on September 6, 1958 and from 9:00 a. m. to 4:00 p. m. on September 7 and 8, 1958.

A similar experiment was conducted for 48 hours starting on November 11, 1958. Six thermocouples were placed in the media of air-layers of which three were located on the southeast and three on the northwest exposure of the tree. Two thermocouples were inserted so that the tip of the wires were located immediately under the polyethylene film, two midway between the stem and polyethylene film and two at the stem surface. Outside air temperatures were recorded from thermocouples placed as in the first study. Temperatures were recorded once each four hours throughout the two-day period.

1/ Honeywell-Brown Instruments, Minneapolis, Minnesota.



Methods of Anatomical Study

After the examination of the marcots as described in the previous air-layering experiments of 1958 and 1959, stem pieces exhibiting characteristics observed in each chemical treatment and in the two species were immediately placed in a killing and fixing solution consisting of 70 percent ethyl alcohol, formalin and glacial acetic acid (FAA). Dehydration was accomplished with a tertiary butyl alcohol series and the specimens were embedded in Parlodin[1/] by gradually adding small pieces of the celloidin material to a 1:1 ether-alcohol solution after placing the sample jars in an oven at 55° C for 24 hours or more between each addition of Parlodion. To prevent the corks from being forced from the bottles by the pressure produced from ether-alcohol vaporization, the corked bottles were tightly clamped between two sheets of heavy gauge steel with eight 1/4-inch bolts. After the Parlodion was thickened to a "stringy" consistency, the infiltrated sample and a surrounding mass of the Parlodion medium was poured into a paper mold placed over a hard-wood block. The specimen was then quickly moved into a position suitable for sectioning and the entire block and mold submerged into absolute chloroform for 12 hours or more to ensure complete hardening of the Parlodion. A surface of the specimen was exposed with a sharp razor blade into a solution of two parts 95 percent ethyl alcohol and one part glycerine for 12 hours for softening.

[1/] Parlodion is the trade name for a purified pyroxylin manufactured by Mallinckrodt Chemical Works, Philadelphia, Pennsylvania.

Some specimens were sectioned longitudinally or transversely with a sliding microtome and others with a rotary microtome at 10 to 20 microns in thickness. Sections were stained with safranin and fast green, as outlined by Johansen (1940) and mounted in Piccolyte[1/] on microscope slides. Free hand sections and sections made with a freezing microtome at 50 microns were also prepared from fresh material obtained after harvesting and observing the marcots applied in the 1959 experiments. These preparations were stained with fast green or Delafield's hematoxylin and fast green, accomplishing dehydration with an ethyl alcohol series. Stained sections were mounted in Piccolyte as previously.

All slides prepared were examined microscopically to study the presence of root primordia, origin of roots, characteristics of the tissues and other observations which might be peculiar to a given treatment, or an untreated marcot.

To determine what changes in cell growth occurred after girdling and treating the stems, detailed camera lucida drawings were made of non-girdled stems for comparison. Particular attention was given to those marcots which had not rooted after the period of treatment to determine whether root primordia existed and whether the lack of root development was due to anatomical barriers, such as fiber bundles, or from injury resulting from chemical treatment or girdling.

1/ Piccolyte is the trade name for a pure resin product of the General Biological Supply Company.

Planting of Rooted Air-Layers

Rooted stems of both Picea glauca and Pinus sylvestris obtained from the exploratory experiments and not selected for anatomical study, were planted in three-inch clay pots containing a soil mixture consisting of three parts sandy loam soil and one part peat, and placed out-of-doors in a partially shaded area. The number of roots present at the time of planting and the survival, after one or more months, was recorded for each stem.

Rooted stems of Picea glauca obtained from the 1959 experiments, not used in anatomical studies, were planted in shredded sphagnum in three-inch clay pots and fed periodically with an aqueous solution of a 4-8-8 fertilizer. Records were maintained in the same manner as in the previous study with soil-planted material.

RESULTS

Exploratory

Experiments with six-year-old greenhouse-grown plants of _Picea glauca_ conducted February 23 through May 3, 1958 resulted in the rooting of two of the ten stems treated with 100 ppm NAA and one stem each of those treated with five additional substances (Table I).

Twenty-five days after treatment, the current growth of many of the marcots wilted, and after three to five days the stem and subtending leaves became dry. During this same period, a yellowing of the leaves on the one-year-old stems occurred above the wound, followed by browning and eventual leaf abscission. One week after leaf abscission, the stems became brittle and were then considered dead. Later, one stem exhibited the same symptoms as those which were observed initially.

Calculations based on the weighted average of the number of surviving stems 45 days after treatment and every eighth day thereafter until harvest, showed that although death occurred in all treatments, 2, 4-D, 2, 4, 5-T and GA treated stems resulted in the greatest coefficient of non-living stems when compared with the remaining treatments (Table I).

An examination of the girdled area of the dead twigs showed that in the GA treatments the stem tissue was killed below and above the wound, but in the remaining treatments, death of stem tissue occurred only distal to the girdled area.

TABLE I

Coefficient of Stem Death*, Total Survived and Number Rooted of Picea glauca Air-layers 70 Days After Treatment in a 60°F Greenhouse (10 Plants per Treatment)

Treatment	Concentration (ppm talc)											
	0			100			1000			5000		
	No. Rtd.	Coef.	Surv.	No. Rtd.	Coef.	Surv.	No. Rtd.	Coef.	Surv.	No. Rtd.	Coef.	Surv.
2, 4, 5-T	-	-	-	0	8.33	0	0	11.71	0	0	22.60	0
2, 4-D	-	-	-	0	9.93	0	0	22.22	0	0	13.15	1
GA	-	-	-	0	13.15	1	0	9.12	1	0	13.15	0
IBA	-	-	-	1	7.32	4	0	6.71	1	1	7.75	3
NAA	-	-	-	2	7.28	3	1	7.05	6	0	7.70	5
BNA	-	-	-	0	6.87	5	1	7.05	5	0	7.72	5
IPA	-	-	-	0	6.90	4	0	8.69	5	0	7.94	4
IAA	-	-	-	1	7.05	2	0	6.99	7	0	6.65	4
Dist. H_2O	0	7.35	3	-	-	-		-	-		-	-

*Increasing values indicate a greater rate of stem death.

Proliferation of stem tissue occurred along the upper rim of the girdled wound in all of the stems which were alive 70 days after treatment. No differences in the degree of proliferation between treatments could be detected.

The use of talc was an unsatisfactory method of treatment as it was difficult to control the quantity applied and even the application of the growth regulator.

Aqueous injection with a hypodermic syringe was found to be a superior method of applying the growth-regulators. It was possible to observe the diffusion of a one percent aqueous solution of safranin 0 in preweighed sphagnum of a known moisture content. After four hours, a 5 ml injection had stained 90 percent of the sphagnum and after 12 hours all of the sphagnum was stained. Two-ml injections stained the same quantity of sphagnum in 24 hours.

Field experiments on both _Picea glauca_ and _Pinus sylvestris_ in May, using five ml aqueous injection of the treatments and wounding with a v-shaped notch on the abaxial side of the stem resulted in no rooted stems in any treatment of _Pinus sylvestris_ and in _Picea glauca_, one stem each of 100 ppm NAA and of 100 ppm 4TNA rooted when applied to one-year-old stems. Treatments of 1000 ppm 2, 4, 5-T, NAA, BNA and 4TNA resulted in death of the stems above the wounded area in _Picea glauca_. Since leaves became chlorotic in 100 ppm 2, 4-D and 2, 4, 5-T treatment of the same species, these treatments were eliminated from further experimentation.

Stunting of new growth and a slight yellowing occurred in the 1, 000 ppm

2, 4, 5-T treatments in Pinus sylvestris. Treatments of 1, 000 ppm 2, 4-D of the same species caused tip curling of the current growth and considerable stunting of the leaves. These treatments were therefore eliminated from further study.

No proliferation of the stem tissue was observed in the wounded area in either species. In all of the treatments, the notched area in the stem was completely healed over and, except for the absence of leaf bases in the case of Picea glauca and brachyblasts in Pinus sylvestris, appeared like an unwounded stem.

Air-layers applied in July to one-year-old stems of Picea glauca resulted in five stems rooted when treated with 1, 000 ppm IBA, three stems rooted when treated with 0. 5 ppm 2, 4, 5-T and one and 100 ppm NAA (Table II). Two stems rooted in the 1, 000 ppm IAA treatments and no rooting occurred in this species when treated with one ppm IPA and 100 ppm IAA and IPA. All of the remaining treatments and the control resulted in the rooting of one stem each (Table II).

The average number of roots produced per rooted stem and the average length of the roots varied considerably between treatments (Table II). Of the treatments in which only one stem rooted, one ppm IAA resulted in nine roots, one ppm IBA - three roots, and the remainder averaged 1. 5 roots per stem and of the treatments which yielded three rooted stems, 0. 5 ppm 2, 4, 5-T averaged 6. 3 roots per rooted stem (Table II).

The degree of tissue proliferation in the living stems of Picea glauca

TABLE II

Rooting Results of <u>Picea glauca</u> Air-layers. Ten Stems per Treatment, July 1958; Examined After 75 Days

Concentration (ppm)		Treatment: 2, 4, 5-T	2, 4-D	4TNA	IBA	NAA	BNA	IPA	IAA	H_2O
0.0	No. stems rooted	-	-	-	-	-	-	-	-	1
	Avg. no. roots/ rooted stem	-	-	-	-	-	-	-	-	1.0
	Avg. root length (mm)	-	-	-	-	-	-	-	-	10.5
0.5	No. stems rooted	3	1	-	-	-	-	-	-	-
	Avg. no. roots/ rooted stem	6.3	1.0	-	-	-	-	-	-	-
	Avg. root length (mm)	4.7	24.0	-	-	-	-	-	-	-
0.1	No. stems rooted	1	0	1	1	3	1	0	1	-
	Avg. no. roots/ rooted stem	2.0	0.0	1.0	3.0	2.7	1.0	0.0	9.0	-
	Avg. root length (mm)	12.0	0.0	11.0	10.3	6.3	17.0	0.0	1.1	-
100	No. stems rooted	*	*	1	1	3	1	0	0	-
	Avg. no. roots/ rooted stem	-	-	1.0	2.0	3.3	2.0	0.0	0.0	-
	Avg. root length (mm)	-	-	12.0	2.2	6.0	2.5	0.0	0.0	-
1000	No. stems rooted	*	*	*	5	*	*	1	2	-
	Avg. no. roots/ rooted stem	-	-	-	3.8	-	-	2.0	6.0	-
	Avg. root length (mm)	-	-	-	10.8	-	-	1.5	8.8	-

*Omitted from treatments because of injury or death to stems as indicated in previous studies.

varied but no differences were apparent among treatments. Stem death occurred in all treatments of this same species 20 to 35 days after applying the air-layers and at the end of 70 days, 65.5 percent of the air-layers were dead. There were no differences in survival as a result of treatments.

Rooting in Pinus sylvestris occurred in three stems treated with 1,000 ppm IPA and two stems treated with one ppm 2,4-D and 100 ppm IBA, BNA and IPA. One ppm 2,4,5-T and BNA, IBA and IAA were similar in their action to the use of distilled water, rooting one stem each. No rooting occurred in three remaining treatments (Table III).

Proliferation of tissues above the wound was observed in many treatments (Figure 2), and varied according to the moisture content of the sphagnum medium. Some water had evaporated from sphagnum in many of the air-layers as a result of exposure when grasshoppers chewed holes in the polyethylene. Sphagnum surrounding rooted stems also tended to become dry as a result of water uptake by the newly-formed roots. In those air-layers in which the sphagnum was nearly dry no proliferation occurred, but in those which had moist, and in some instances extremely wet sphagnum, the proliferation was abundant. Extremely wet sphagnum occurred in air-layers which, when wrapped with polyethylene, were not secured sufficiently with theplastic-coated wires, thus allowing water entry between the polyethylene and the stem during heavy rains.

Stems having proliferated parenchyma were also larger in diameter than comparable stems not exhibiting proliferations.

TABLE III

Rooting of *Pinus sylvestris* Air-layers. Ten Stems per Treatment, July 1958; Examined After 90 Days

Concentration (ppm)		2, 4, 5-T	2, 4-D	4TNA	IBA	NAA	BNA	IPA	IAA	H_2O
0.0	No. stems rooted	-	-	-	-	-	-	-	-	1
	Avg. no. roots/ rooted stem	-	-	-	-	-	-	-	-	3.0
	Avg. root length (mm)	-	-	-	-	-	-	-	-	0.8
	No. root-like knobs	-	-	-	-	-	-	-	-	2
0.5	No. stems rooted	0	0	-	-	-	-	-	-	-
	Avg. no. roots/ rooted stem	0.0	0.0	-	-	-	-	-	-	-
	Avg. root length (mm)	0.0	0.0	-	-	-	-	-	-	-
	No. root-like knobs	0	0	-	-	-	-	-	-	-
1.0	No. stems rooted	1	2	0	1	0	1	0	1	-
	Avg. no. roots/ rooted stem	4	1.5	0.0	5.0	0.0	1.0	0.0	1.0	-
	Avg. root length (mm)	2.7	5.3	0.0	1.6	0.0	1.0	0.0	2.5	-
	No. root-like knobs	4	4	1	4	0	3	2	5	-
100	No. stems rooted	0	0	1	2	0	2	2	1	-
	Avg. no. roots/ rooted stem	0.0	0.0	2.0	2.5	0.0	2.0	1.0	1.0	-
	Avg. root length (mm)	0.0	0.0	0.8	0.1	0.0	1.0	2.7	1.5	-
	No. root-like knobs	3	2	4	3	4	1	4	3	-
1000	No. stems rooted	*	*	0	1	0	0	3	1	-
	Avg. no. roots/ rooted stem	-	-	0.0	1.0	0.0	0.0	4.0	1.5	-
	Avg. root length (mm)	-	-	0.0	1.5	0.0	0.0	5.2	0.6	-
	No. root-like knobs	-	-	1	2	1	0	5	2	-

*Omitted because of injury as indicated in previous experiments.

Proliferating tissues from the upper rim of the girdled area occurred in all stems air-layered except four which were treated with 1, 000 ppm NAA, and one stem treated with one ppm 4TNA and another with 100 ppm 2, 4-D.

Tissue proliferation in Pinus sylvestris did not develop in equal amounts in numerous stems, but produced root-like projections and irregularly shaped knobs (Figure 2). Five unrooted stems exhibiting this characteristic were observed in 1000 ppm IPA and one ppm IAA, four in one ppm IBA, 2, 4, 5-T and 2, 4-D; 100 ppm 4TNA, NAA and IPA. No tissue protrusions were observed in 0. 5 ppm 2, 4, 5-T and 2, 4-D treatments, one ppm NAA or in 1, 000 ppm BNA treatments. All other treatments having this characteristic varied from one to three stems (Table III).

Treatments applied to air-layers made in August[1] and removed in November resulted in no rooting of Pinus sylvestris and in Picea glauca, three stems rooted when treated with 100 ppm BNA. Two stems rooted when treated with 100 ppm 4TNA and 1, 000 ppm IAA and one stem rooted with 100 ppm NAA and 1, 000 ppm IBA.

One root was observed in 1, 000 ppm IAA treatments of Picea glauca air-layers which were allowed to remain on the trees over winter and until June 1, 1959. This single root was dead and had disintegrated considerably at the tip. Death of stems occurred in 83. 3 percent of the 90 air-layers applied.

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[1] Since the results of the July treatments could not be ascertained at this time and little rooting had occurred in earlier treatments, the concentrations used in both species were selected on the basis of the highest concentration which did not cause stem death or the treatments that indicated root development in previous studies.

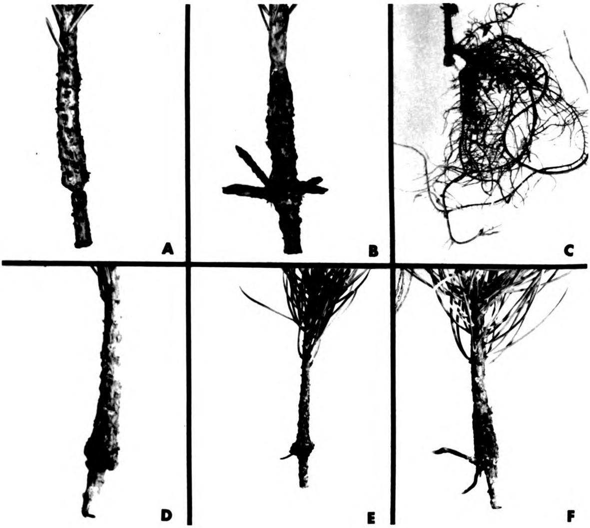


Figure 2. A-C. Air-layered stems of Picea glauca. D-F. Air-layered stems of Pinus sylvestris. A - the average amount of wound proliferation after 100 days. Note the larger stem diameter above the girdled area. B - root development after 100 days. C - amount of root growth 90 days after planting; four roots similar to those in (B) were present at the time of planting. D - the average amount of wound proliferation after 100 days. E - root arising from wound proliferation; note the knobby appearance of the proliferations. F - root development after 100 days.

No rooting occurred in the treatments of *Pinus sylvestris* which were allowed to remain on the trees until June 1, 1959. All stems treated with 100 ppm 2, 4-D had not resumed growth at this time, but all other treatments showed current growth averaging four to five inches in length.

Callus development in *Picea glauca* treatments removed in November appeared similar to that produced in earlier treatments varying from heavy to light tissue proliferation without regard for treatment.

Death of stems occurred in 24.4 percent of the 90 stems air-layered and was observed in all of the treatments.

Root-like projections similar to those observed in the July treatments of *Pinus sylvestris* were also observed in this same species in the August treatments removed from the source plants in November. Nine of 15 stems treated with 100 ppm 2, 4-D, seven treated with 100 ppm 2, 4, 5-T and six treated with 100 ppm IBA possessed the root-like structures. From two to four out of 15 stems in all other treatments had also developed these root-like structures (Table IV).

In addition to root-like structures, a heavy proliferation of tissues was observed at the wound which was made when the brachyblasts were removed along with the leaves at the time of initial treatment. Thirteen and 12 stems of 100 ppm 2, 4, 5-T and 2, 4-D, respectively, and four stems of 1, 000 ppm NAA exhibited the characteristic brachyblast-proliferation (Table IV). No tissue proliferation of brachyblast wounds was observed in the remaining treatments (Table IV).

TABLE IV

Number of Pinus sylvestris Stems Exhibiting Root-like Tissue Proliferations and Tissue Proliferations of Brachyblast Wounds in the August to November and August to June Treatments (Number out of 15 Each)

Treatment	Root-like Proliferations Month of Examination November	 June	Brachyblast Proliferations Month of Examination November	 June
100 ppm 2, 4, 5-T	7	8	13	11
100 ppm 2, 4-D	9	5	12	12
100 ppm IBA	6	7	0	0
100 ppm BNA	2	3	0	0
1000 ppm NAA	2	2	4	0
1000 ppm IPA	2	3	0	0
1000 ppm IAA	4	5	0	0
Distilled water	2	1	0	0

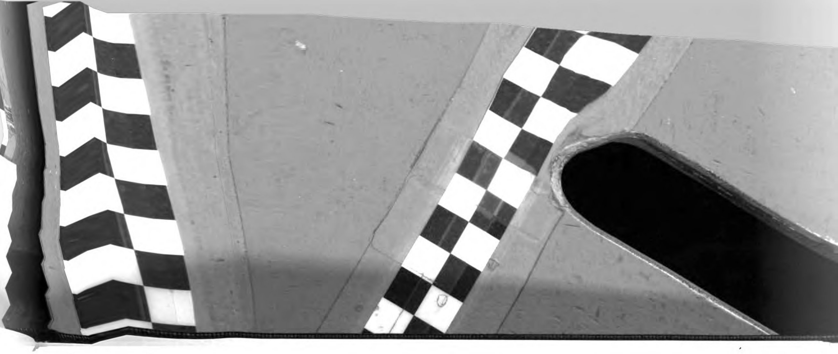
Treatments of Pinus sylvestris which were left on the plants over winter and until June 1, 1959 showed brachyblast-wound proliferations similar to those in the August to November treatments of this same species. Twelve stems of 15 air-layers treated with 100 ppm 2, 4, 5-T, 11 with 100 ppm 2, 4-D and four with 1, 000 ppm NAA exhibited the characteristic but wound proliferations were not observed in any of the remaining treatments (Table IV).

Root-like proliferations of tissue from the girdled area occurred in eight stems treated with 100 ppm 2, 4, 5-T, seven with 100 ppm IBA, five with 100 ppm 2, 4-D and 1, 000 ppm IAA, three with 100 ppm BNA and 1, 000 ppm IPA, two with 1, 000 ppm NAA and one in the control treatment (Table IV).

Despite the low rooting responses in air-layers treated with growth-regulators during the exploratory experiments, further testing in 1959 was warranted. Growth regulators to be used again were selected on the basis of the total number of stems rooted, the average number of roots per rooted stem and the average length of the roots per rooted stem (Tables I, II, and III). In spite of the low rooting response, four-thianaphtheneacetic acid was included in the treatment series of both species because this chemical was relatively new in the field of plant propagation.

Temperature Studies

Thermocouple recordings of temperatures in the sphagnum of air-layers located on southeast, west and north exposures of a Picea glauca tree, when compared with recordings of the temperature of the surrounding air provided



widely varying temperature differences depending upon the time of day and the amount of cloud cover present at the time temperatures were recorded. The highest temperature (105.5°F) was recorded in an air-layer located on the southeast exposure of the tree at 10:00 a.m., during which time the sky was clear, the air temperature was 79°F, and the air-layer was in direct exposure to the rays of the sun (Table V). When a cloudy sky prevailed, temperature differences between the sphagnum and the surrounding air-temperature were not as great as when the sky was clear. Rain falling on the air-layers caused a rapid drop of temperature in the sphagnum, resulting in temperature recordings 10°F lower on the average than that of the surrounding air temperature (Table V).

Temperatures recorded on November 11 and 12, 1959, at the stem surface, in the middle of the sphagnum, and directly beneath the polyethylene of air-layers, resulted in no significant differences when compared with the temperatures of the surrounding air and between thermocouple positions. The sky was overcast on both days with a mean high of 55.5°F and a mean low of 31.5°F during that period.

TABLE V

Temperature in Proximity of, and Inside Air-layers

Date and Time of Recording	Sky Condition	Location of Thermocouples: Outside Air Medium: Protected (°F)	Unprotected (°F)	Mean (°F)	Sphagnum: S. E. Exposure °F	Dev. from outside mean	W. Exposure °F	Dev. from outside mean	N. Exposure °F	Dev. from outside mean
September 6, 1958										
12:00 noon	Scattered clouds	70.5	70.5	70.5	79.0	+ 8.5	80.5	+10.0	77.5	+ 7.0
1:00 p. m.	Scattered clouds	71.0	71.0	71.0	76.0	+ 5.0	77.0	+ 6.0	76.0	+ 5.0
2:00 p. m.	Scattered clouds	72.5	72.5	72.5	76.0	+ 3.5	77.0	+ 5.0	74.5	+ 2.0
3:00 p. m.	Scattered clouds	74.0	74.0	74.0	80.0	+ 6.0	83.5	+ 9.5	78.9	+ 4.9
4:00 p. m.	Scattered clouds	75.0	75.0	75.0	80.5	+ 5.5	82.0	+ 7.0	78.5	+ 3.5
September 7, 1958										
9:00 a. m.	Clear	75.0	75.0	75.0	85.5	+ 10.5	80.0	+ 5.0	80.5	+ 5.5
10:00 a. m.	Clear	79.0	79.0	79.0	105.5	+ 26.5	87.5	+ 8.5	87.0	+ 8.0
11:00 a. m.	Clear	82.0	82.0	82.0	104.5	+ 22.5	92.5	+10.5	93.0	+11.0
12:00 noon	Cloudy	80.0	80.0	80.0	85.0	+ 5.0	85.0	+ 5.0	84.5	+ 4.5
1:00 p. m.	Cloudy	78.5	78.5	78.5	83.0	+ 4.5	83.0	+ 4.5	83.0	+ 4.5
2:00 p. m.	Light rain	79.5	79.0	79.2	78.5	- 0.7	78.5	- 0.7	78.5	- 0.7
3:00 p. m.	Scattered clouds	75.5	75.5	75.5	82.5	+ 7.0	82.0	+ 7.5	79.0	+ 3.5
4:00 p. m.	Rain	75.5	75.5	75.5	65.5	- 10.0	64.5	-10.0	64.5	-10.0
September 8, 1958										
9:00 a. m.	Cloudy	62.0	62.0	62.0	64.0	+ 2.0	64.0	+ 2.0	63.5	+ 1.5
10:00 a. m.	Cloudy	62.5	63.0	62.7	66.0	+ 3.3	68.0	+ 5.3	70.5	+ 6.8
11:00 a. m.	Cloudy	64.5	64.5	64.5	66.5	+ 2.0	66.5	+ 2.0	66.5	+ 2.0
12:00 noon	Cloudy	66.5	66.5	66.5	74.0	+ 7.5	76.0	+ 9.5	74.0	+ 7.5
1:00 p. m.	Clear	66.0	66.0	66.0	83.0	+ 17.0	77.0	+10.0	74.0	+ 8.0
2:00 p. m.	Clear	69.5	68.5	68.7	92.0	+ 23.3	82.5	+13.8	75.5	+ 6.8
3:00 p. m.	Scattered clouds	68.5	68.0	68.2	75.0	+ 6.8	77.0	+ 8.8	76.0	+ 7.8
4:00 p. m.	Scattered clouds	67.5	67.5	67.5	71.0	+ 3.5	72.0	+ 4.5	71.5	+ 0.5
	Total mean	-	-	72.08	79.66	+ 7.58	77.90	+ 5.82	76.52	+ 4.44

1959 Experiments

Rooting

After 100 days of treatment there was no significant influence of growth-regulators or of the position of the girdled branch in the rooting of Picea glauca and Pinus sylvestris air-layers applied at the beginning of April, May, June, July and August.

Significant differences in the rooting capacity of individual Picea glauca trees treated April 1 were observed. Out of 15 trees treated, on one tree six air-layers rooted, on two trees five rooted, on two trees four rooted, on four trees one rooted, and on six trees no air-layers rooted. Treatments applied to Pinus sylvestris on July 1 also showed a significant difference in rooting capacity of individual trees as follows: on one tree seven of 18 air-layers rooted, on one tree two rooted, on four trees one rooted, and on nine trees none rooted.

An analysis of the effect of the time of year on the rooting of Picea glauca showed that when air-layers were applied on May 1, a significantly greater number of stems rooted at the end of the 100-day period than those applied on the first day of April, June, July and August, and that although the rooting difference between April and June treatment was not significant, applications of air-layers at the beginning of these months resulted in a significantly greater number of rooted stems than those applied July 1 and August 1 (Table VI). Time of application did not significantly influence the number of roots per rooted stem. The total number of air-layered Pinus sylvestris stems rooted after 100 days of

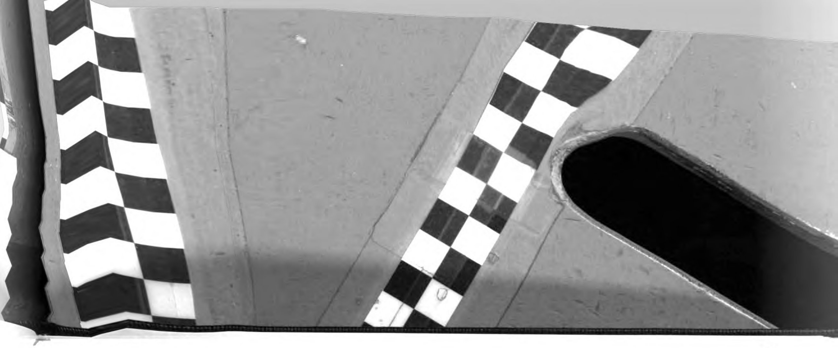


TABLE VI

Rooting Results (After 100 Days) of Picea glauca Air-layers

Date of Application and Type of Observation	Treatment in ppm (45 stems each): Dist. H_2O	0.5 2, 4, 5-T	100 NAA	1000 IBA	100 4TNA	Monthly Mean (225 stems only)
April 1						
Total rooted	6	5	6	6	5	5.6*
% rooted	13.33	11.11	13.33	13.33	11.11	12.44
Avg. no. roots/ rooted stem	4.33	2.40	2.16	2.33	3.20	2.82
Avg. root length (mm)	9.73	10.58	12.76	11.38	9.75	10.84**
May 1						
Total rooted	5	6	8	8	14	8.2*
% rooted	11.11	13.33	17.77	17.77	31.11	18.21
Avg. no. roots/ rooted stem	2.40	2.33	5.50	2.50	4.14	3.60
Avg. root length (mm)	9.58	16.28	16.41	11.20	13.71	13.43**
June 1						
Total rooted	5	2	9	8	5	5.8*
% rooted	11.11	4.44	20.00	17.77	11.11	12.88
Avg. no. roots/ rooted stem	3.80	5.50	4.22	4.50	5.00	4.60
Avg. root length (mm)	24.21	21.09	16.86	22.94	19.70	20.96**
July 1						
Total rooted	0	1	0	1	1	0.6*
% rooted	0.00	2.44	0.00	2.44	2.44	1.33
Avg. no. roots/ rooted stem	0.00	1.00	0.00	6.00	2.00	3.00
Avg. root length (mm)	0.00	1.00	0.00	8.83	10.50	4.06**
August 1						
Total rooted	1	2	3	5	4	3.0*
% rooted	2.44	4.44	6.66	11.11	8.88	6.66
Avg. no. roots/ rooted stem	1.00	6.50	6.00	1.75	3.50	3.75
Avg. root length (mm)	2.00	8.30	5.94	3.75	2.92	4.58**

*Differences significant at the 5% level.
**Differences significant at the 1% level.

Multiple Range Tests

Number of Roots						Root Length (mm)				
Expected F: 1%, 14.15; 5%, 5.84						1%, 14.15; 5% 5.84				
Observed F: 11.12						20.34				
Monthly mean:	April	May	June	July	August	April	May	June	July	August
	5.6	8.2	5.8	0.6	3.0	10.84	13.43	20.96	4.06	4.58

Note: Any two means not underscored by the same line are significantly different.
Any two means underscored by the same line are not significantly different.

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treatment when applied on July 1 was 13; on June 1, five; August 1, four, and no rooting occurred at the end of 100 days when air-layers were applied on the first of April and May. Because of the effect of individual trees in the July treatments, there was no significant effect of time of year on the rooting of this species.

Mean root length was not significantly influenced by chemical treatments or by the position of the air-layer on the tree within any given 100-day period. Air-layers applied to this species on the first day of June, however, resulted in a significantly greater mean root length at the end of 100 days of treatment than those applied in April, May, July and August. The mean root length was significantly greater when air-layers were applied April 1, and May 1 than on the July and August applications (Table VI).

Because of the low rooting response of *Pinus sylvestris*, no analysis was made of the mean root length between monthly treatments.

Tissue Proliferations

The degree of tissue proliferation at the wounded area in *Picea glauca*, as indicated by numerical ratings of one to seven did not vary significantly within four of the five 100-day treatment periods.

Indolebutyric acid treatments caused a significantly greater amount of tissue proliferation than 2, 4, 5-T, NAA, 4TNA and control treatments applied August 1, but in the April, May, June and July treatment periods no significant differences between treatments were obtained (Table VII).

TABLE VII

Tissue Proliferation (After 100 Days) of Picea glauca Air-layers

Date of Application	Average of Numerical Ratings[1/] (45 stems each) Treatment (ppm)					Monthly Mean (225 Stems)
	Dist. H_2O	0.5 2, 4, 5-T	100 NAA	1000 IBA	100 4TNA	
April 1	2.13	2.24	2.11	2.06	2.13	2.13*
May 1	1.91	2.00	2.02	1.78	2.17	1.97*
June 1	2.02	1.57	2.35	2.24	1.75	1.98*
July 1	1.17	1.20	1.04	1.60	1.28	1.26*
August 1	1.54	1.64	1.95	2.35*	1.87	1.87*

*Difference significant at the 5% level.

1/ Numerical ratings were given according to a standard of values from 1 to 7; a value of 1 indicated no proliferation with increasing values indicating a given quantity of tissue proliferation at the wounded area.

Multiple Range Test

Expected F: 1%, 14.15; 5%, 5.84
Observed F: 5%, 10.97

	April	May	June	July	August
Monthly mean:	2.13	1.97	1.98	1.26	1.87

Note: Any two means not underscored by the same line are significantly different.
Any two means underscored by the same line are not significantly different.

A monthly mean of tissue proliferation ratings obtained at the end of the April 1 treatment period was significantly greater than the monthly means of May, June, July and August treatments, and the May, June and August monthly means were significantly greater than that of the July treatment period (Table VII).

Proliferations of wound tissue in stems of *Pinus sylvestris* were greater, on the average, than those of *Picea glauca*, but did not vary significantly between any two monthly means, nor was there a significant difference between treatments (Table VIII).

A pronounced difference in the general appearance of the wound proliferations was observed between *Pinus sylvestris* and *Picea glauca.* In *Picea* proliferations were generally smooth-surfaced and evenly distributed in the wounded area, but in many stems of *Pinus sylvestris*, regardless of chemical treatment, root-like projections from wound proliferations were observed. These observations compared with those of the exploratory experiments (Page 48). Treatments applied to *Pinus* in April resulted in 142 (52.58 percent) of the stems with root-like proliferations; in May, 154 (57.03 percent); June, 204 (75.55 percent); July, 166 (61.48 percent) and in August, 144 (53.33 percent) of the stems had root-like outgrowths of proliferated tissues.

Tissue proliferations from wounds which resulted from removal of the brachyblasts in the air-layered region occurred in 2, 4-D, NAA and 4TNA

TABLE VIII

Tissue Proliferation (After 100 Days) of Pinus sylvestris Air-layers

Date of Application	Average of Numerical Ratings[1/] (45 Stems Each) — Treatment (ppm): Dist. H_2O	1.0 2,4-D	100 NAA	100 IBA	1000 IPA	100 4TNA	Monthly Mean (270 Stems)
April 1	2.42	2.53	3.02	2.40	2.54	2.97	2.66
May 1	2.97	3.17	3.17	3.02	2.91	3.15	3.06
June 1	3.15	3.17	2.88	3.08	2.88	3.33	3.08
July 1	2.77	2.88	2.57	2.91	2.75	3.00	2.81
August 1	2.80	3.04	2.68	2.80	2.80	2.80	2.82

1/ Numerical ratings were given according to a standard of values from 1 to 7. A value of 1 indicated no proliferation with increasing values indicating a given quantity of tissue proliferation at the wounded area.

treatments and the frequency was significantly greater in NAA treatments of April, May and June than with 2, 4-D and 4TNA treatments in these same periods. The lowest frequency of brachyblast-wound proliferation occurred in the treatments applied in July (Table IX).

Injuries to Air-layered Stems

The leaves on some air-layered *Picea glauca* stems frequently became yellow, gradually turned brown and dehisced. Yellowed leaves and dead stems were observed in all treatments at nearly the same frequency, but variations occurred between times of application. The greatest frequency of stem death was observed in the air-layers applied June 1, but the greatest frequency of air-layers with yellowed leaves occurred in those applied July 1 (Table X). The lowest total number of dead stems was observed in the August treatments with two stems dead, followed by 32 dead stems in the treatments applied April 1.

Anatomical Studies

Studies of transverse and longitudinal sections of non-air-layered *Picea glauca* and *Pinus sylvestris* stems from one-year-old shoots at the beginning of June showed structural differences between the two species. Current growth at this time was still in the elongation stage; *Pinus sylvestris* averaging approximately 10 cm in length, and *Picea glauca* averaging approximately six cm in length.

TABLE IX

Proliferations of Brachyblast Wounds and the Total Number of Stems with Knob-shaped Proliferations at the Girdle Area in Pinus sylvestris

Date of Application	Total Number with Brachyblast Proliferations						Monthly Total (270 Stems)	Monthly Total of Stems with Knob-shaped Proliferations (270 Stems)
	Treatment (ppm)							
	Dist. H_2O	1.0 2, 4-D	100 NAA	100 IBA	1000 IPA	100 4TNA		
April 1	0	4	30	0	0	10	44	142
May 1	0	5	29	0	0	20	54	154
June 1	0	1	14	0	0	9	24	204
July 1	0	0	5	0	0	6	11	166
August 1	0	3	11	0	0	11	25	144

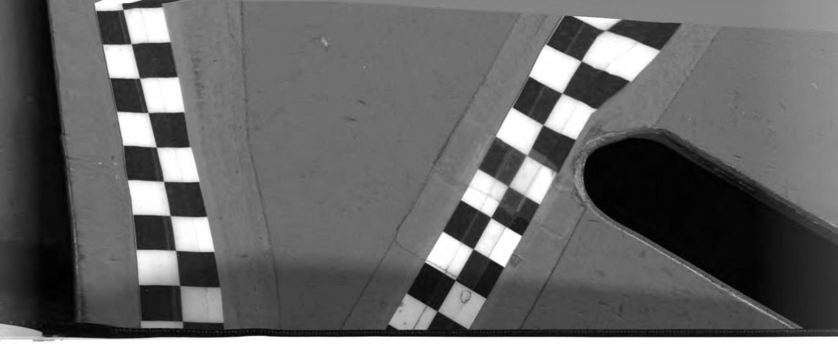
TABLE X

Injury and Stem Death of Air-layered Picea glauca After 100 Days

Date of Application	Total of 225 Stems Each		
	No. Dead Stems	No. Stems with Yellow Leaves	Total Injured
April 1	32	23	55
May 1	103	9	112
June 1	117	22	139
July 1	29	140	169
August 1	2	68	70

Stems of Picea glauca were characterized by a definite layer of epidermal cells with heavily cutinized walls. These cells were rectangular in shape being two or three times as long radially as transversely. Beneath the epidermis was an irregular layer one to several cells deep consisting of collenchyma. The periderm from seven to nine cells in depth was composed of large parenchymatous cells with slight suberization. At frequent intervals on the surface of the stem were concave longitudinal bands consisting of lamellar collenchyma. The cortical region comprized almost one-third of the stem, and was made up of irregularly-shaped, isodiametric parenchyma cells many of which contained tannin deposits. Large resin canals were present in the cortex and were surrounded by one to several layers of relatively thick-walled secretory cells containing dense cytoplasm. The pericyclic and phloem regions were difficult to delimit because they coalesce. Only occasional bundles of pericyclic fibers were in evidence.

A region of cambial cells clearly separated the phloem from the xylem, and consisted of lanceolate-shaped tracheids with numerous bordered pits on their radial walls. At relatively even intervals throughout the xylem were radially arranged uniseriate rays consisting entirely of parenchyma cells. Vertical parenchyma was largely confined to discrete areas along the perimeter of the growth ring. The pith was composed of large thin walled cells, and chambered by means of layers of simply pitted sclerenchymatous cells (Figures 3 and 4).



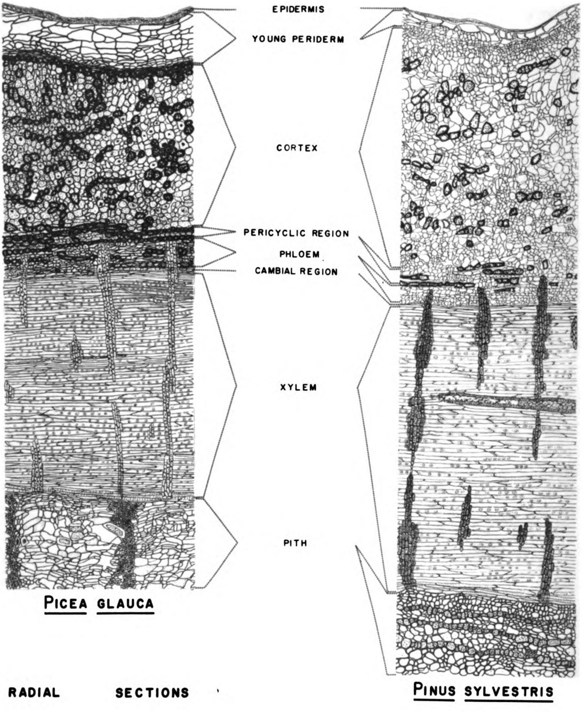


Figure 3. Comparative anatomy of Picea glauca and Pinus sylvestris stems in radial view. Semi-diagramatic. Approx. 40 X.

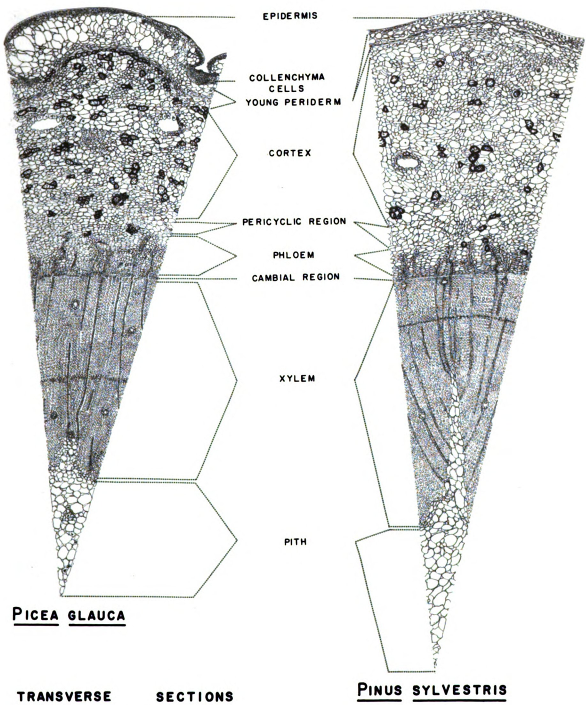


Figure 4. Comparative anatomy of Picea glauca and Pinus sylvestris stems in transverse view. Semi-diagramatic. Approx. 40 X.

Stems of *Pinus sylvestris* were characterized by a single row of epidermal cells nearly isodiametric in shape and covered with a thin cuticle. The young periderm consisted of a slightly suberized layer two to four cells deep. Beneath the periderm was deep cortex consisting of irregularly-shaped parenchyma cells, many containing tannin. Large evenly-spaced resin canals were found in the cortex and were bordered by secretory cells containing dense cytoplasm. The pericyclic region was not well defined in *Pinus*, having cells about the same shape as in the cortex, but in general, slightly smaller in size. Phloem tissue consisted almost entirely of sieve cells with occasional phloem parenchyma. Phloem rays were heavily cytoplasmic and contained prominent nuclei. Xylem and phloem were clearly delimited by the cambium. Xylem tissue was characterized by lanceolate tracheids with numerous bordered pits on the radial walls and radially arranged rays consisting of densely cytoplasmic parenchyma bordered on their transverse walls with tracheid-like cells having half-bordered pits on the wall surface adjacent to ray parenchyma. This arrangement of tracheids gave the ray the appearance of being multiseriated, but in most stems, uniseriate secondary rays dominated. Resin canals, smaller in diameter than those of the cortex were relatively evenly dispersed, bordered by densely cytoplasmic cells, and often found in association with a secondary ray.

Primary rays were conspicuous in this species, consisting of comparatively large parenchyma cells which were indistinguishable from cells of the pith. The tracheids adjacent to the pith did not contain circular bordered pits but instead,

4

5.

6.

7.

flatly oval-shaped bordered pits. No vertical parenchyma existed in the xylem of this species (Figures 3 and 4).

The outstanding differences observed between stems of Picea and Pinus are summarized below:

Picea	Pinus
1. Collenchyma layer beneath epidermis.	1. No collenchyma layer beneath epidermis.
2. Bands of lamellar collenchyma in vertical grooves on the surface of the stem.	2. Vertical collenchyma bands absent.
3. Vertical parenchyma at the perimeter of a growth ring in the xylem.	3. No vertical parenchyma in the xylem.
4. Heavy tannin deposits in cortex.	4. Tannin deposits in cortex approximately one-third less in quantity when compared with Picea.
5. Occasional fiber groups in the pericycle.	5. No fibers present in the pericyclic regions.
6. Primary xylem rays not conspicuous.	6. Conspicuous primary xylem rays.
7. Uniseriate secondary rays composed of parenchyma only.	7. Secondary rays bordered on the radial walls of ray parenchyma with tracheid-like cells. Uniseriate rays present, rarely multiseriate.

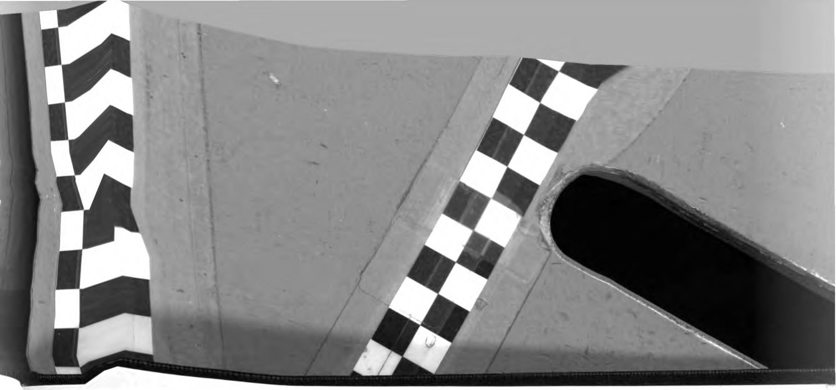
After wounding the stems of both Picea glauca and Pinus sylvestris by removing a ring of bark, the sequence of anatomical changes which took place at and near the wound were as follows: approximately one mm above the upper rim of the girdled area, a meristematic zone was formed from dedifferentiated

parenchyma cells of the cortex, existing phellogen and phloem. The new periderm was continuous with that of the non-wounded stem area and parenchyma cells formed by periclinal and anticlinal divisions of the cambium (Figure 5). Two meristems, therefore, simultaneously took part in the production of wound proliferation - a meristem produced by the dedifferentiation and the existing cambium. The wound phellogen continued to divide, developing parenchymatous tissue basipetally and a phellem acropetally. As the phellem developed, the cortex and phloem tissues most proximal to the girdle rim began to break up and eventually became sloughed-off (Figure 5).

The cambium increased in activity first producing loosely arranged isodiametric parenchyma cells followed centripetally by shortened but lanceolate tracheids and a well defined phloem tissue centrifugally. Numerous secondary rays were also produced by the cambium, following an irregular course in the wound xylem and less irregularly in the phloem. The tracheids of the wound xylem were characterized by the presence of numerous, large bordered pits on all wall surfaces and in many instances with more than one longitudinal row of these pits on each wall (Figure 5).

Pinus sylvestris stems produced a greater amount of wound proliferation than Picea glauca, and it often took the form of knob-shaped proliferations which were identified as areas of greater meristematic activity at the ends of primary rays and closely paralleled secondary rays of the xylem. The knob-shaped proliferations consisted of a well defined phloem developed centrifugally

Figure 5. A - wound proliferation from upper rim of a girdled Picea glauca stem; Pe - periderm formed over wound tissues; Fc - former cortex which eventually becomes sloughed-off, X 55.
B - wound-xylem in Pinus sylvestris, X 300.



from a cambium that was continuous with the vascular cambium of the non-proliferated stem area, a very irregularly arranged mass of tracheids interspersed centripetally with occasional parenchyma cells and a wedge-shaped area of parenchyma tissue adjacent to the end of a primary ray.

Root initials arose from the ends of phloem rays of wound tissues in both species. In <u>Pinus</u>, however, the initials were invariably found at or near the apex of a knob-shaped tissue proliferation described above. The initials in both species were first evident as bulbous-shaped masses of cells which were densely cytoplasmic and contained prominent nuclei (Figures 6 and 7). Further development of the embryonic root was characterized by cell elongation basipetally and continued cell divisions acropetally, pushing into the cortex of the stem (Figures 6 and 7). No dissolution and only light crushing of interfering cells of the cortex was observed in either species as the root developed.

At the time of emergence of the root from the stem in both <u>Pinus</u> and <u>Picea</u>, a well defined plerome was present. A definite calyptrogen, dermatogen and periblem were not distinguishable, but appeared to be a relatively homogeneous group of cells (Figures 6 and 7).

Root emergence in both species was accomplished by a rupture of the periderm and epidermis (Figures 6 and 7). A well developed adventitious root contained a central core of undifferentiated tissue and centrifugally, primary vascular strands, a pericycle, a prominent endodermis, thick cortex and a suberized epidermis (Figure 6).

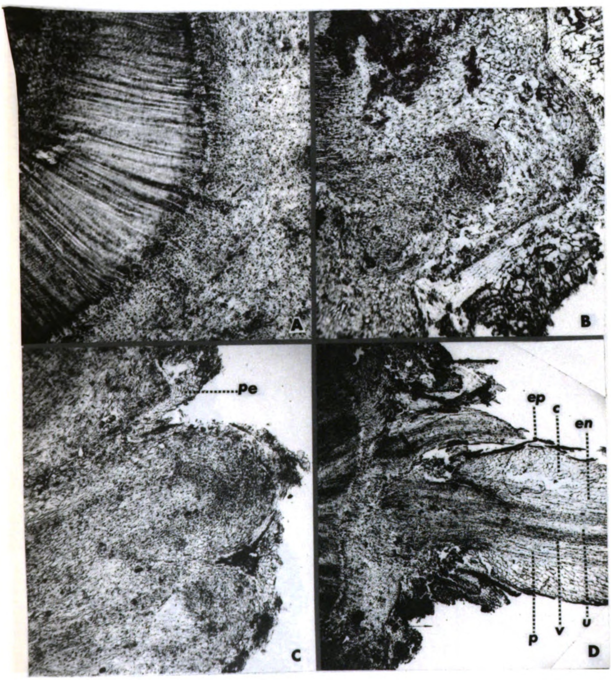


Figure 6. Transverse sections of air-layered Picea glauca stems.
A - root initial arising from phloem ray (arrow) X 55;
B - root histogen (arrow) projecting further into the cortical region X 75;
C - root emerging through periderm (pe) X 75;
D - base of a well developed root; ep - epidermis, c - cortex, en - endodermis, p - pericycle, v - young vascular tissue, u - undifferentiated tissue X 55.

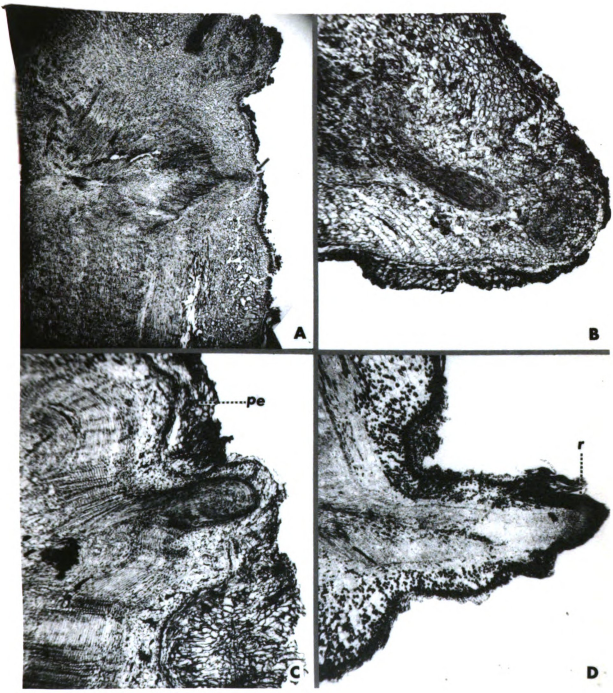


Figure 7. Transverse sections of air-layered Pinus sylvestris stems.
A - root initial (arrow) arising from a phloem ray at the apex of a tissue proliferation X 55;
B - further elongation and differentiation of a root histogen X 75;
C - emergence of root histogen through periderm (pe) X 75;
D - young root (non-median) after emergence from stem, (r) root-tip X 75.

Proliferations from wounds made by removing the brachyblasts from the stems of Pinus sylvestris were similar in structure to the knob-shaped proliferations produced in the wounded area, but differed in the relative amounts of wound tissues produced and were not associated with vascular rays. In comparison, the brachyblast wound proliferations consisted of a thicker periderm and thinner cortex, phloem and xylem than the proliferations of the girdle wound. Wound xylem and phloem was produced by divisions of vascular cambium of the brachyblasts and the periderm and cortex from dedifferentiated cells of the surrounding cortical areas and existing periderm.

Anatomical studies of longitudinal sections cut through the girdled area of dead Picea glauca stems were conducted in an effort to determine whether girdling deeper than the cambium was the cause of stem death. After detailed observations of sections from six randomly selected stems, one showed the presence of cuts from girdling in the xylem of the previous seasons' growth. Similar cuts, however, were observed in living stems of this species. Further observations of the sections from dead stems showed that no periderm had formed over the wound and the vascular cambium had ceased dividing as evidenced by the amount of new xylem produced when compared with living stems of the same treatment period.

Survival of Rooted Air-layers

Because of the low rooting response obtained in Pinus sylvestris, most of the rooted stems were used in the anatomical study and not planted for further growth. A sufficient number of rooted stems was obtained from Picea glauca treatments to enable a study of the survival of rooted air-layers when planted in soil or in shredded sphagnum.

A period of at least 30 days after planting was found necessary before survival counts could be made because indications that some stems would not survive were not apparent from visible symptoms for approximately 30 days; when cool air temperatures and frequent cloudiness prevailed, a period of at least 45 days after planting was necessary in order to make survival counts. Because of this variation in time requirement, final counts of survival were not made until November 21, 1959, giving a period of 70 days for the last planting of the rooted Picea glauca air-layers which were removed from the source plants on September 10, 1959.

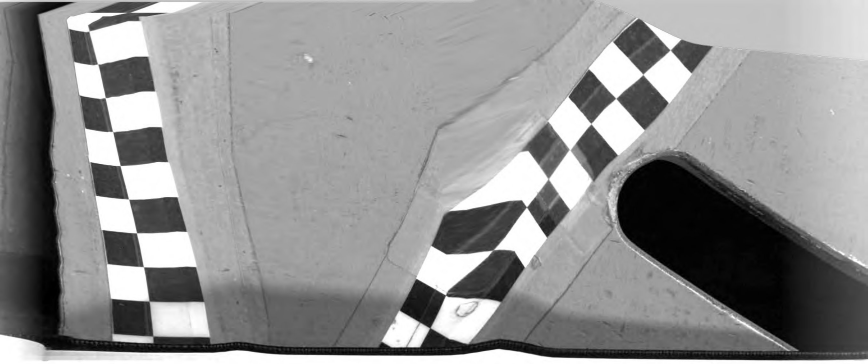
Twenty of the stems rooted in the exploratory experiments were planted in a soil mixture of which seven survived. Survival of rooted air-layers planted in shredded sphagnum July 10, August 10 and September 10 was 52 percent, 91 percent and 100 percent, respectively (Table XI).

Periodic examinations of the amount of root growth of the planted stems showed that lateral roots form within one week after planting and after one month the length of the roots which were present at the time of planting were more than four times greater in length. After three months a very extensive root system was present, but no new roots originating from the stem were observed (Figure 2).

TABLE XI

Survival of Rooted Air-layers of Picea glauca Planted in Two Different Media

Date Planted	Medium	Number Planted	Number Survived	Percent Survival Nov. 21, 1959
October 9, 1958	Soil mix	20	7	35.0
July 10, 1959	Shredded Sphagnum	24	15	52.0
August 10, 1959	Shredded Sphagnum	36	33	91.0
September 10, 1959	Shredded Sphagnum	27	27	100.0



DISCUSSION OF RESULTS

The results of the exploratory experiments conducted in 1958 and the periodic seasonal studies of 1959 have clearly shown that the initiation and development of adventitious roots in nine- to 12-year-old Picea glauca and seven- to eight-year-old Pinus sylvestris trees is physiologically possible by the air-layered method and that no significant benefit was achieved with the use of any of nine growth-regulators in the promoting of root initiation and development. No conclusions could be given for this failure to respond to chemicals in the rooting of the two species, but it might be noted that, to the knowledge of the author, the only previous rooting of these species was observed by Farrar and Grace (1926) and Kirkpatrick (1940) with cuttings of Picea glauca and Komissarov (1938) and Hitt (1955) with cuttings of Pinus sylvestris. The younger plants used by these investigators suggested that the seven- to 12-year-old trees were too old for favorable response to a growth-regulating stimulus. Support for this reasoning was given by Deuber (1940) and Thimann and Delisle (1939) in which rooting of cuttings from Picea abies trees reduced sharply as the age of the source plants increased.

Rooting, however, was found to vary significantly with the time of application of the air-layers in Picea glauca and in both species, some individual trees were significantly greater in rooting response than others. This response indicated that physiological and inherent factors had a greater influence on the rooting of these two species than exogenously applied growth-regulators. In order to secure convincing evidence that individual trees rooted significantly greater than others in these experiments, further air-layering of propagules would be necessary. Wright *et al.* (1958) have pointed out that ".... the genetic variation attributable to species hybridization, geographic variation and individual tree variation are not separable in work done to date on material of unknown provenance." The seed-grown trees used in these experiments were of unknown origin and probably represented progeny from more than one provenance.

The significant differences in number of roots and of root lengths observed between the five 100-day treatment periods of *Picea glauca* is closely correlative with temperature fluctuations recorded by the local United States Weather Bureau Station during these periods. In April treatments of *Picea glauca*, when effects of individual trees were encountered, 28 out of 225 air-layers rooted. If the effect of individual trees was removed, there was a total of 15 rooted.

The highest rooting (41 of 225 stems) resulted from the air-layers applied on May 1 and removed August 1. At the time of application in May, air temperatures did not exceed 60° F and remained above 41° F, followed by gradually increasing temperatures reaching a high of 89° F at the end of June. The lowest rooting response occurred when air-layers were applied July 1 at a time when the maximum air temperature reached 88° F followed by a 34-day period with a mean maximum temperature of 82.9° F (Figure 8). The greatest number of injured stems also occurred among those treated in July.

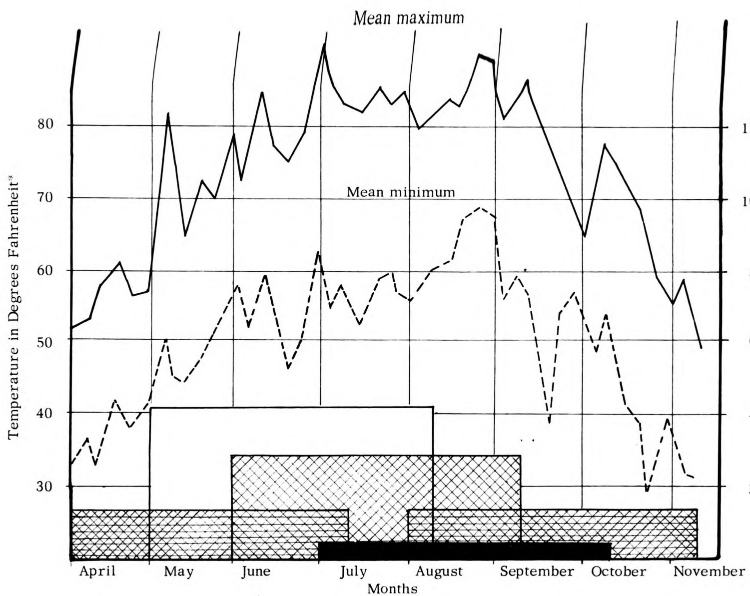
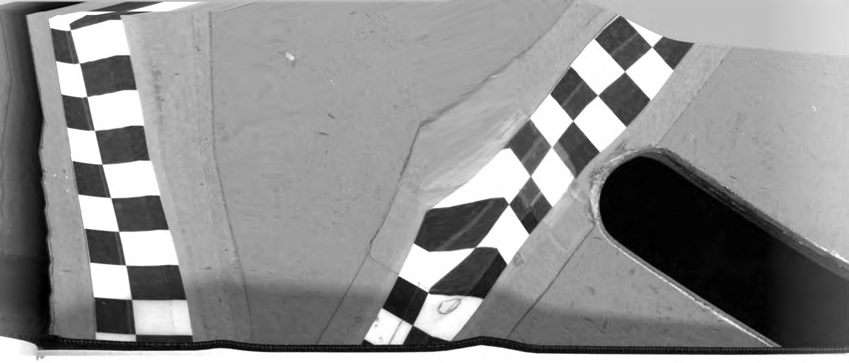


Figure 8. Total number of rooted air-layers of Picea glauca in comparison with five-day maximum and minimum air-temperature means.

*Calculated from the 1959 U. S. Weather Bureau reports, Lansing, Michigan.



Studies of the temperature in the air-layers showed that when the air-temperature in the proximity of an air-layer reached 79.5°F, the sphagnum medium directly exposed to the sun was 105.5°F (Table V). It is probable, therefore, that high temperatures of 105.5°F and higher occurred in some of the air-layers applied at the beginning of July and might have accounted for the low rooting response and high number of injured stems. Higher rooting responses and comparatively little stem injury in other treatment periods were likely a result of more favorable temperatures at the time of treatment.

Observations of longitudinal sections through the girdled area of dead stems showed that no meristematic activity in the proximity of the wound had occurred. Temperature extremes occurring at the time of, or shortly after, wounding may have prevented the formation of a protective periderm layer near the wound surface by killing the functional cambium and preventing dedifferentiation of parenchyma in the cortex and phloem. The failure to correlate stem death with possible injury from girdling too deeply, substantiates this reasoning.

An influence of temperature on root length was suggested from a comparison of root length with air-temperatures during the treatment periods. Cool temperatures (60 to 75°F) seemed to favor rapid root growth after initiation.

Air-layers applied on June 1 resulted in a significantly greater mean root length than those applied the first of April, July and August. The maximum mean temperatures averaged 77.5°F in June, and increased to an average of 84°F until harvest (Figure 9). The lowest mean root length was observed at the end of the

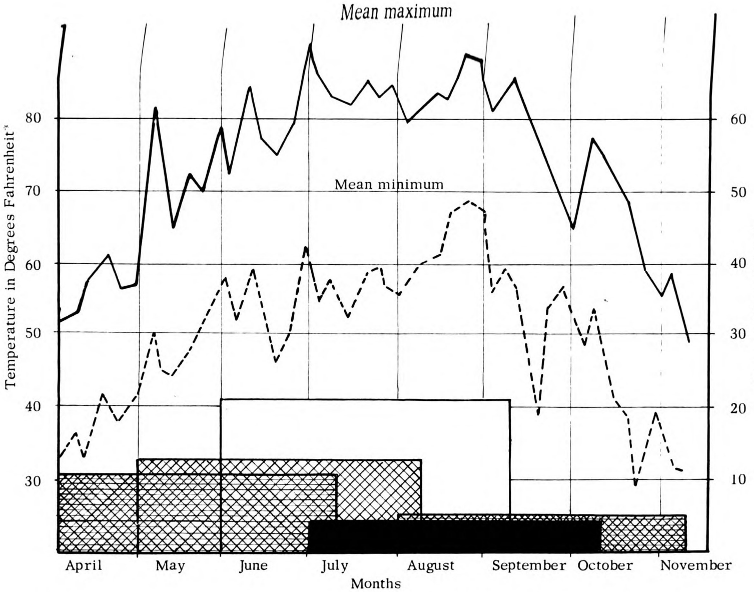


Figure 9. Mean root length of Picea glauca in comparison with five-day maximum and minimum air-temperature means.

*Calculated from the 1959 U. S. Weather Bureau reports, Lansing, Michigan.

July treatment period. At the beginning of this period the maximum mean temperature was 89° F, and continued to reach 80° F or above through the months of July and August. The low mean root length resulting from the August treatments might be attributed to the steady decline in temperatures beginning one month after treatment (Figure 9).

Although the time required for root initiation was difficult to observe in these experiments, evidence was found from anatomical observations that roots initiate relatively early in the treatment period in Picea glauca. Additional evidence of early initiation of roots was observed in the exploratory studies conducted in a greenhouse when well developed roots were present as early as five weeks after the initial application of the air-layers.

These comparisons suggested a critical period for root initiation with respect to air temperature soon after treatment. Temperatures reaching above 80° F or below 60° F tended to hinder root initiation and development, the mean root length at the end of the treatment period was less.

Factors other than tree age, inherent capacities, and temperature, undoubtedly influenced root initiation and development. These other factors were the physiological condition of the stem at the time of initial treatment and during the treatment period, the amount of air space in the sphagnum medium, and the nutritional status of the plants.

The anatomical studies have shown that root initials arose from secondary rays of the phloem which were produced by divisions of ray initials of the cambium.

It was reasonable, therefore, to presume that root initiation occurred more readily during the period of rapid cambial activity and when food reserves in the stem were present in sufficient quantity to support the formation of root initials. Both of these conditions were ideal at the beginning of May in *Picea glauca* (Kienholz, 1934).

Increased moisture and subsequent decreased oxygen in the sphagnum medium as a result of heavy rains may have contributed to a reduction of root initiation and development in both species. In this respect the use of polyethylene films in air-layering was not completely satisfactory without a practical method for sealing the film against the stem and thus preventing the penetration of water.

The position of the air-layered branch with respect to the rank of the whorl on the trees had no significant effect on rooting and callusing of *Picea glauca* and *Pinus sylvestris*. Yet studies of *Albizzia* by Toole (1948), *Pinus strobus* by Doran and Holdsworth (1940), *Picea abies* by Grace (1939) and others have shown that cuttings taken from lower branches root more readily than those from positions higher on the tree.

Reasons for rooting in some stems and not in others on the same tree and within the same treatment period are difficult to discern. The possible factors influencing this variability of rooting are postulated as follows: Because treatments were applied on all sides of the trees, variations in the length of time that the air-layers were exposed to rays of the sun would also vary,

thus causing higher temperatures in the sphagnum medium of some and lower temperatures in others. Thermocouple recordings support this reasoning (Table V). In addition to temperature differences, the degree of stem vigor and the rate of carbohydrate assimilation could be expected to vary with the length of time that the stems were directly exposed to the sun.

Anatomical changes at and near the wound surface of both species were similar to those observed by Hiller (1951) from Taxus cuspidata cuttings and by Beal (1951) from several species of gymnosperms. The initiation and development of adventitious roots from knob-shaped proliferations of the vascular tissues in Pinus sylvestris is unique to this study and has not been observed in Picea glauca and not believed to have been observed previously in other species.

The irregular arrangement of tracheids of the wound xylem and the presence of bordered pits on all of their wall surfaces in Pinus sylvestris (Figure 5) was observed in Taxus cuspidata stems by Hiller (1951). This distortion of xylem in Pinus was observec both in air-layers treated with growth-regulators and in those treated with distilled water.

The appearance of bordered pits on the tangential as well as the radial walls of the tracheids when observed from a longitudinal section of the stem may have been a result of cell distortion occurring in such a manner that cell walls which would normally be in a radial plane were twisted into the tangential plane. Occasional groups of multiseriate bordered pits, however, cannot be explained on the basis of cellular distortion. A possible cause of this phenomenon was suggested from the work by Jeffrey (1917) who postulated that aberrant tissues, formed as a result of wounding, may be a temporary reversion expressing cell characteristics of ancestral plant groups. A comparison of the tracheids of wound xylem in *Pinus sylvestris* with tracheids of the fossil genus *Prepinus* as described by Jeffrey shows a remarkable resemblance.

The proliferation of tissues from wounds caused by removal of dwarf shoots in *Pinus sylvestris* was the most pronounced in treatments of NAA, 4TNA and 2, 4 - D. This suggests that these growth-regulators are more active in stimulating meristematic activity than IBA and IPA. Anatomical examinations of the proliferations showed that the meristematic activity was limited for the most part to periderm formation and there were no indications of root initiation.

As a result of the relatively small number of rooted stems obtained, an adequate comparison between survival of rooted air-layers planted in sphagnum and in soil could not be made. The data obtained nevertheless suggest the importance of time of planting for the survival of rooted air-layers. All of the rooted stems of *Picea glauca* survived when planted on September 10, 1959 in contrast to 52 percent survival of those planted July 10, 1959 (Table XI). Air temperatures at planting time in September reached a high of 84° F and decreased gradually during this month, while temperatures at the planting time in July reached 82° F, and the daily mean maximum continued to reach above 80° F for 60 days. The cooler temperatures of the September planting may have favored lower transpiration of the plants until a sufficient root system had developed. The stems planted

in this same month, however, also had a greater mean root length at the time of planting than those of the July and August plantings and a considerable influence on survival may have resulted from the amount of root-surface present.

On the basis of the results from these experiments, the use of air-layering to propagate *Picea glauca* and *Pinus sylvestris* commercially on a large scale is not recommended. For the perpetuation of desirable clones, rare specimens and experimental plant material, which is not readily propagated by other vegetative methods, air-layering has considerable merit.

SUMMARY

Vegetative propagation of woody and herbaceous plants is used primarily for the perpetuation of individuals selected for desirable characteristics. Many species of plants are propagated vegetatively by cuttage and graftage; others are reproduced, but with great difficulty, by either method.

Interest in the process of air-layering has stimulated some investigations into the feasibility of this method for propagating plants which do not readily produce roots. Promising results have been obtained from air-layering experiments with several deciduous shrubs and shade trees of the temperate zone (Creech, 1950; Wyman, 1952; Hanger *et al.*, 1954; Ching *et al.*, 1956). Many tropical plants are almost exclusively propagated by air-layerage, principally the mango, *Mangifera indica*, avocado, *Persea gratissima* and the Lychee, *Litchi chinensis* (San Pedro 1935; Fielden 1936; Grove 1947; Singh 1953). Investigations by Lasschuitt (1950), Mergen (1952, 1955), Frolich (1957) and Hoekstra (1957) have shown that rooting by air-layerage is possible in several coniferous species.

On the basis of the results obtained by some of these investigations, the present study was conducted to determine the feasibility of air-layering and some of the factors affecting the rooting of *Picea glauca* and *Pinus sylvestris.*

Exploratory experiments were conducted in the 1958 growing season to establish the value of nine different growth-regulators for the induction of root initials in air-layered branches of seven- to 12-year-old trees of *Picea glauca* and *Pinus sylvestris.*

In March 1958 air-layers were applied to Picea glauca plants growing in a greenhouse and treated with talc-carried growth-regulators. Little rooting occurred in these treatments, and the method of applying the growth-regulators was found to be unsatisfactory. All treatments were thereafter injected into the polyethylene-wrapped sphagnum in aqueous form with a hypodermic syringe.

Experiments using field-grown trees of Picea glauca and Pinus sylvestris were conducted in May, July and August, 1958. Stems of the May treatments were wounded with a v-shaped notch on the abaxial side and those in July and August by removing a complete ring of bark two to three mm wide. Some of the treatments applied in July rooted in both species; up to 50 percent rooting occurred in 1, 000 ppm indolebutyric acid treatments of Picea glauca and 30 percent rooting from indoleproprionic acid treatments of Pinus sylvestris. No rooting resulted from the May treatments, and little rooting resulted from air-layers applied in August, the latter occurring only in Picea glauca.

From the results of the exploratory experiments growth-regulators were selected for use in further investigations conducted in the 1959 growing season. Air-layers were prepared on the lower, middle and top whorls of 15 trees of each species at the beginning of April, May, June, July and August, 1959. The air-layers at each whorl on Picea glauca were injected with aqueous solutions of one of the following treatments: 0. 5 ppm 2, 4, 5-trichlorophenoxyacetic acid, 100 ppm naphthaleneacetic acid, 100 ppm 4-thianaphtheneacetic acid, 1, 000 ppm indolebutyric acid and distilled water and on Pinus sylvestris air-layers were

injected with 1 ppm 2, 4-dichlorophenoxyacetic acid, 100 ppm naphthaleneacetic acid, indolebutyric acid and 4-thianaphtheneacetic acid, 1, 000 ppm indolepro-prionic acid and distilled water.

Results 100 days after treatment showed that there were no significant differences between treatments or between positions of the air-layers on the trees. A significantly greater number of Picea glauca stems rooted, however, when air-layers were applied at the beginning of May, little rooting occurred when applied in July.

Mean root length was significantly greater at the end of the June treatments when compared with April, July and August.

The number of rooted stems and the mean root length were correlative with the mean minimum and maximum air temperatures, which might suggest that temperatures above 80° F occurring at the beginning of a treatment hindered root initiation and increased the incidence of stem death, but that root length was increased by gradually rising daily mean temperatures and hindered by temperatures which prevailed below 60° F.

Outstanding differences in stem anatomy between Picea and Pinus were observed. Picea stems were characterized by a layer of collenchyma directly beneath the epidermis and in vertical grooves on the surface of stem, vertical parenchyma at the perimeter of the growth rings in the xylem, groups of pericyclic fibers, parenchymatous secondary rays and inconspicuous primary rays. Pinus stems, on the other hand, did not have a collenchyma layer beneath the

epidermis and bands of pericyclic fibers, but were characterized by conspicuous primary rays and tracheid-like cells bordering the radial walls of secondary ray parenchyma.

Anatomical examinations of sections cut through the wounded area of Picea glauca and Pinus sylvestris stems showed that root initials arose from secondary phloem rays of tissue proliferations produced by the vascular cambium. Root initials in stems of Pinus sylvestris invariably occurred at the apex of a knob-shaped tissue proliferation.

Anatomical examinations of dead stems of Picea glauca showed that no meristematic activity had occurred at or near the wounded area, and it was suggested that high temperatures at the beginning of the treatment period may have prevented the formation of a protective periderm over the cut surface causing stem death.

Survival of rooted air-layers of Picea glauca planted in September was 100 percent after 70 days in contrast with 52 percent in July when observed after 130 days.

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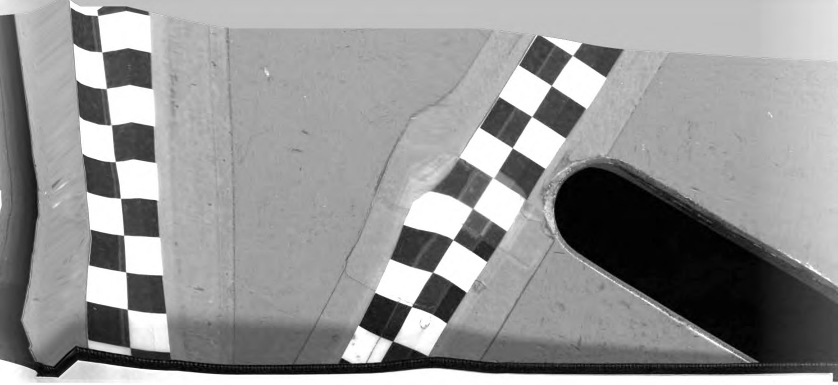

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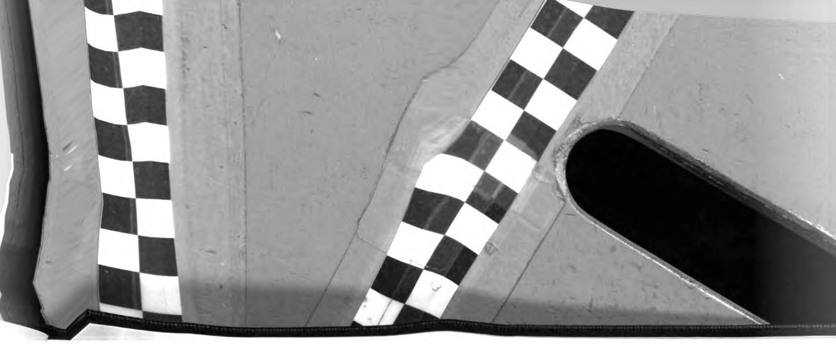

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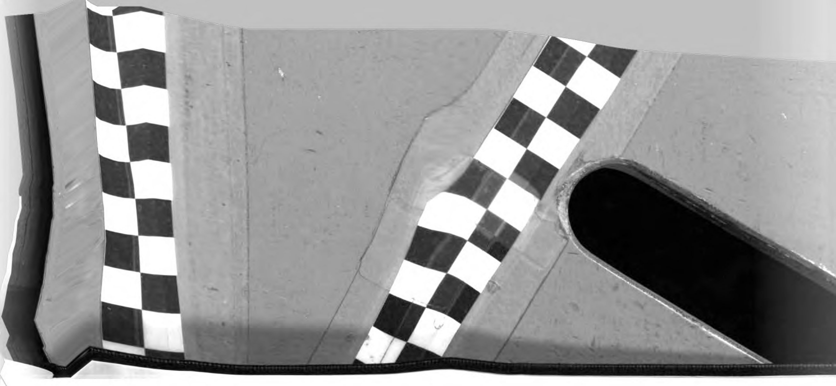

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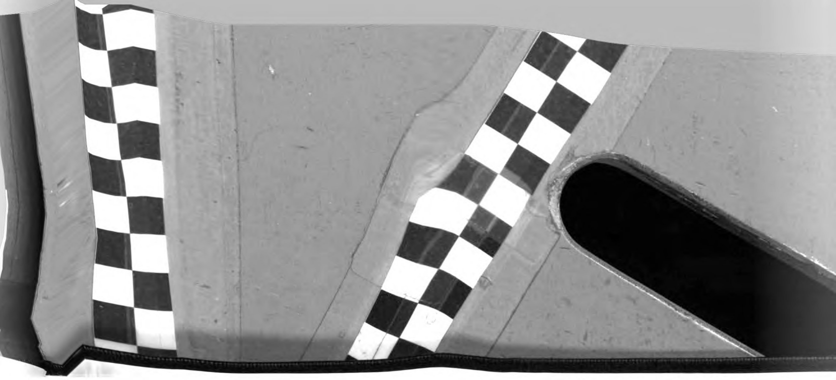

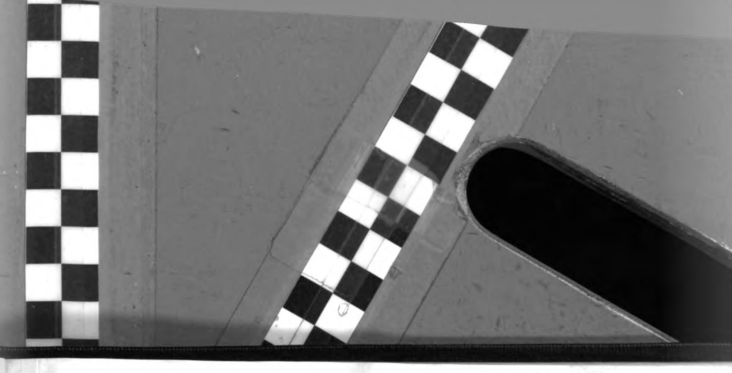

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