Asexual Propagation of Pinus by Rooting Needle Fascicles

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(Received for publication January 6, 1964)

Introduction

The place of asexual propagation in a forest biology research program is well documented. Besides the practical advantage of this type of propagation, e. g., multiplication of selected germ plasm for progeny testing and the establishment of clonal seed orchards, the use of genetically uniform trees for fundamental physiological and ecological studies is of great importance. Most of the work on vegetative propagation with pine trees has been done with cuttings, air-layers, or graftings. From the experiments with cuttings it has been established that the rooting ability of cuttings decreases with an increase in the age of the tree from which they were obtained, thus limiting the multiplication of older trees. Another obvious disadvantage of cuttings is the small number of cuttings that can be obtained from a tree, especially if the tree is only a few years old.

Another technique of asexual propagation – the rooting of needle fascicles – shows the possibility of producing a large number of propagules with the same genetic composition. A needle fascicle of pine is formed from a bud in the axil of a scale leaf and consists of a short stem, a shoot apex, and from 1 to 5 needles. Morphologically it is a spur shoot, and it can be stimulated to form a long shoot. Successful rooting of a limited number of needle fascicles has been observed in Pinus strobus (THIMANN and DELISLE, 1942), Pinus densiflora and Pinus densiflora X P. thunbergii (TODA, 1948 a), Pinus resinosa (JECKALEJS, 1956), and in Pinus *echinata* and Pinus elliottii (ZAK and MCALPINE, 1957).

In this report are described experiments that were conducted to determine some of the physiological factors that influence the rooting of needle fascicles. Included were experiments to study the effects of various pre-treatments given to the trees and/or to the needle fascicles on their ability to root. In addition, the sequence of the anatomy of callusing and subsequent root formation was followed with the aid of serial microtome sections that were prepared from fascicles that were collected at periodic intervals. The morphology of root development is described. and the subsequent growth of some of the rooted fascicles was observed for a period of up to 6 years. The species used was primarily Pinus elliottii ENGELM., although fascicles from Pinus taeda L. and Pinus radiata D. DON were each used in one experiment.

Review of Literature

Definition of a "needle fascicle": –

There is some discrepancy in the literature in the use of the word "fascicle". FOSTER and GIFFORD (1959) referred to "leaf fascicles" as a misleading term that obscures the real morphological nature of the structures, which in a morphological sense are spur shoots. DOAK (1935) in defining "dwarf shoot", used "spur shoot", "short shoot", and "brachyblast" as synonyms and reserved the term "fascicle" for the tuft of needles on a dwarf shoot. THOMSON (1914) stated that a "spur shoot" is a specialized branch which is of limited (primary and secondary) growth and bears a limited number of specialized and cyclically arranged leaves, and like ordinary branches, arise either in the axils of primordial leaves or scale leaves on the stem. Recent investigators have employed the term "leaf bundle" or "needle bundle" in reference to the needle leaves enclosed by a sheath, or the scale leaves (TODA, 1948 a, 1948 b; JECKALEJS, 1956; and YIM, 1962). Throughout this paper the term "fascicle~'refers to the needle leaves held together by the scale leaves (sheath) and contains a base and a diminutive shoot apex, which is similar to the "spur shoot" as described by FOSTER and GIFFORD (1959).

Factors that influence rooting: –

Experiments on factors that influence the rooting of needle fascicles of pines can be broken into 5 categories: 1) age of the parent tree (ortet); 2) position of fascicle on tree; 3) time of planting; 4) chemical treatment; and 5) planting medium.

1) Age of the ortet. – The majority of experiments was conducted with fascicles from 1½ to 4-year-old trees. When fascicles from both young and old trees were included, those from the younger trees produced roots more readily than those from the older trees (THIMANN and DELISLE, 1942; and TODA, 1948 a). One exception to this was the report by YIM (1962) who obtained better rooting on fascicles from a 10-year-old tree than from a 3-year-old tree; the difference, however, was small (4.2% vs. 2.4%). In general the results with fascicles paralleled those from studies with cuttings; namely, that it is most difficult to obtain rooted cuttings from old trees (NIENSTAEDT, CECH, MERGEN, WANG, and ZAK, 1958).

2) Position of fascicle on tree. – TODA (1948 a) suggested that the position of the fascicle on the parent tree might affect rooting ability, and he found it more effective to collect the fascicles from side branches than from the leader; the lower positions were also more favorable. These differences, however, were not statistically significant, and YIM (1962) working with Pinus rigida found no difference in rooting between fascicles collected from the main leader and the lateral shoots.

JECKALEJS (1956) cut off the distal portion of Pinus *resinosa* shoots to produce leaf bundles with well defined buds, but he presented no evidence or results to indicate the effect of this pre-treatment on the ability of the fascicles to root.

3) Time of planting. – No results of studies on the effect of time of planting could be found in the literature; however, one can assume that fascicles behave similarly to cuttings, and thus rooting is influenced by the season at which they were collected (THIMANN and DELISLE, 1942; DORAN, 1946; MERGEN, 1955; and NIENSTAEDT, et al., 1958).

[1]) The authors are Professor of Forest Genetics and Assistant in Research, respectively. This report is part of a comprehensive program supported by a grant from the National Science Foundation for basic research in forest genetics (G-8891). Support for MERGEN'S salary for the academic year was provided by the JOHN A. HARTFORD Foundation, Inc. Y. B. LINHART assisted with one phase of the greenhouse experiments.

4) Chemical treatment. — THIMANN and DELISLE (1942) obtained an increase in rooted brachyblasts from 3-year-old white pines after treatment with IAA (0, 50, and 100 mg. per Liter) for 24 hours (7.5% control *vs.* 74% treated). TODA (1948 a) dipped the bases of leaf bundle cuttings of *Pinus densiflora* × *P. thunbergii* and *Pinus densiflora* in solutions of alpha-naphthalenacetic acid (2, 4, and 8 mg. per cc.), and obtained an increase in the number of rooted fascicles in the 1-, 3-, and 4-year-old trees, but a decrease in the 2- and 7-year-old ones. In all cases there was low survival in the fascicles treated at the higher concentrations. In another experiment, TODA (1948 a) treated the fascicles after they had been planted for 7 weeks without obtaining a beneficial effect.

JECKALEJS (1956) treated leaf bundles of *Pinus resinosa* in 10^{-6} M and 10^{-7} M concentrations of methoxone (2-methyl, 4-chlorophenoxyacetic acid) for 12 and 24 hours, and he reported best success for those cuttings that were treated at a 10^{-7} M concentration for 24 hours. ZAK and MCALPINE (1957) dipped needle bundles of *Pinus elliottii* into 0.8% indolebutyric acid in talcum but they did not report on the effect on rooting. YIM (1962) treated leaf bundles of *Pinus rigida* with 0.3% IAA in talcum, and after planting they were sprayed at 5-day intervals with solutions of either 0.25% urea or 0.25% mineral nutrients (Hyponex). Rooting and survival were lower for those sprayed with urea, with little or no difference between those sprayed with minerals or water.

5) Medium. — In most studies the medium for planting was either sand or mixtures of sand and peatmoss, with the exception of "Kunuma" soil, a weathered pumice, that was used by TODA (1948 a). YIM (1962) treated the medium with a mercury fungicide before planting the fascicles but none of the studies discussed the effect of medium on subsequent survival or rooting.

Anatomy of root formation: —

Several investigations have been made on the origin of callus tissue and the subsequent development of roots in *Pinus*, but most of these reports dealt with cuttings or graft unions, and only a few isolated papers deal with needle fascicles. The descriptions of callus formation and subsequent development of roots vary from species to species, and, with but one exception, they consider the process of root formation as endogenous in nature.

TODA and SATOO (1948) reported that the first callus tissue in cuttings of *Pinus densiflora* × *P. thunbergii* originates from the cambium and phloem cells, but that subsequent callus is produced from the phloem and from the pith. A study of 5 species of *Pinus* by SATOO (1956) showed a similar sequence, and he also noted the contribution of the cortex cells. REINES and MCALPINE (1959) studied the anatomy of callused and rooted dwarf shoots, and they observed that the cortex and pith contributed largely to callus formation, but that cambial cells, and xylem and phloem parenchyma were also capable of proliferation. In slash pine graft unions, the first callus tissue was largely derived from the medullary rays (MERGEN, 1954).

After callus formation, root primordia are initiated and differentiated within the callus tissue. REINES and MCALPINE (1959) reported the formation of a spirally oriented vascular tissue within the callus, and this unoriented tissue elongated and produced a root with normal protostelic arrangement. DELISLE (1942), however, reported more active division of the cambium in certain places, resulting in the formation of cushions of meristematic cells with virtually no wood or phloem tissue. After further division, a root "anlage" (initial) is formed "... which by further division pushes its way through the phloem, cortex, and periderm." SATOO (1956) described the development of roots from parenchymatous tissue that had not yet differentiated into cambium-like layers.

A comparison of the root origin in cuttings and air-layers of *Pinus elliottii* showed a great similarity, and these roots had arisen in the callus or wound tissue and were endogenous in nature (MERGEN, 1955).

The single reference to the exogenous origin of roots is the one by TODA and SATOO (1948) for cuttings of *Pinus densiflora* and *Pinus densiflora* × *P. thunbergii.*

Growth of rooted fascicles: —

Although adequate root systems to support the life of the fascicles have been observed, no reports of subsequent height growth are available. JECKALEJS (1956) did not report budding and growth of his rooted fascicles, although TODA (1948 b) presented a longitudinal section through a growing bud in a leaf bundle of *Pinus densiflora.* DELISLE (1942) noted that untreated rooted brachyblasts did not produce terminal buds and that they eventually died. If, however, they were treated with auxin some produced terminal buds and developed into normal plants. Dwarf shoots of *Pinus elliottii* produced shoots from their apical meristems and on rare occasions these ends formed branches with apical dominance (REINES and MCALPINE, 1959).

Experimental Procedures

Rooting of fascicles

Two series of experiments were conducted, one being a preliminary study to determine the effect of: 1) position of fascicle; 2) chemical treatment; and 3) medium. The second series of experiments utilized the results from the first ones, and consisted of refinements and/or enlargements of these experiments.

Preliminary studies: —

Needle fascicles were collected from the upper or lower stems and branches of 1- and 3-year-old *Pinus elliottii* seedlings, from rooted air-layers of 15-year-old trees, and from the upper part of stems from 3-year-old seedlings from which the terminal bud had been cut off. The buds in the latter treatment exhibited obvious swellings at the time of collection. The fascicles were sterilized for 10 minutes in 10% chlorox to which a wetting agent (Laboratory Aerosol) had been added. After this, they were rinsed in distilled water, and planted individually in culture tubes under the following conditions: 1) planting to a depth of 1 cm. in agar in individual test tubes; 2) placing the fascicle on top of agar in which a well had been gouged to hold a small water reserve; 3) covering the bases of fascicles with sand, peatmoss, or vermiculite (or combinations), and watering with either a nutrient solution or distilled water.

Two types of agar were used: 1) 1% industrial grade agar-agar, sterilized and filtered; and 2) Bacto-Yeast Morphology Agar.[2]) The nutrients added to the agar-agar were either soluble starch, 1% dextrose, or KNOP's solution.

Chemical treatment consisted of dipping the sterilized bases into talcum powder to which various concentrations

[2]) Bacto-Yeast Morphology Agar is a commercial trade name by DIFCO Laboratories for agar preparation containing a carbon source, amino acids, vitamins, trace elements, and major elements.

of α naphthalenacetic acid or indolebutyric acid had been added.

The culture tubes were placed in humidity chambers under a 16-hour photoperiod in an incubator held at approximately 30° C.

All of the fascicles cultured in agar died either from contaminations, or from lack of oxygen and moisture before they produced roots. A mixture of sand and vermiculite was a more favorable medium than either material used alone, and the fascicles from seedlings where the top had been cut off rooted better than those from untreated seedlings. The chemical treatment did not show any statistically significant effect on rooting, nor on subsequent growth of the rooted fascicles, but a larger number of rooted fascicles was obtained after treatment with indolebutyric acid.

Because the overall number of fascicles that rooted was low and variable, no statistical analysis of the data was made. However, the best results were obtained when the fascicles were collected from seedlings where the terminal bud had been cut off prior to collecting the fascicles, by treating the fascicles with indolebutyric acid, and placing their bases in a well aerated medium.

Main experiments: —

Experiment 1. — For this experiment a $2 \times 2 \times 2$ factorial design was used to test the effect of clipping of the terminal bud, location of fascicles on stem, and chemical pre-treatment. Two levels of the clipping treatment were tried during October on 3- and 4-year-old *Pinus elliottii* trees to stimulate bud growth in the fascicles before they were planted in the rooting medium. One treatment consisted of decapitation of the terminal bud, and collecting the fascicles from the upper internode 1 month after the buds had been cut off. For the second level of treatment the upper internode was cut off at the first whorl, and the fascicles were collected from the second internode 1 month after pre-treatment. After decapitation the plants were placed in a greenhouse under a 16-hour photoperiod. A total of 32 trees were pre-treated and the fascicles were cut from the trees in such a manner that a sliver of wood and bark remained attached to their base.

For the chemical pre-treatment the bases of half the fascicles were dipped in 1 : 100 indolebutyric acid (IBA) mixture in talcum prior to planting; the fascicles used as controls were dipped in talcum.

The planting medium was a 3 : 1 mixture of sand and peat, and the fascicles were planted to a depth of approximately 0.5 cm. A total of 3840 fascicles were planted in a randomized block design in the greenhouse, and the individual fascicles were kept in an upright position by strings. The planting flats were covered with transparent plastic boxes to maintain a high relative humidity around the needles.

When the fascicles were lifted after 1 year, 30 had produced roots; all of these were from the 3-year-old trees, and no roots were produced on fascicles that had not been pre-treated with IBA. There was no statistical difference between the fascicles from the upper and lower internode. A chi^2 test indicated that the "clipping" 1 month prior to collecting the fascicles increased the number of rooted fascicles at the 1% level of significance.

Experiment 2. — In this test efforts were also made to stimulate bud swelling in the fascicles while still part of the tree. During November incisions were made with a scalpel into the stems above the fascicles on the main stem of 4-year-old *Pinus elliottii* trees (60 fascicles per tree on 10 trees). The trees were placed under a 16-hour photoperiod and after 2 months 480 fascicles were collected and planted, and the remaining 120 were planted after 3 months. The fascicles were dipped in IBA in talcum and the planting procedure was the same as for Experiment 1.

Although many of the fascicles had swollen buds at the time they were planted, none of them produced roots during the 12-month period the experiment was under observation.

Experiment 3. — Twenty 4-year-old *Pinus taeda* trees were used in this experiment to observe the effect of change of axis (orientation) of the needle fascicles on subsequent rooting. The stem of the upper internode was split along the vertical axis during November and the resulting 2 halves were bent across each other and held in a horizontal position so that the orientation of the individual fascicles was changed by 90°. After being split and tied, the trees were placed under a 16-hour photoperiod. It was hoped that the growth of the buds inside the fascicles would be stimulated. Ten weeks after this pre-treatment, 950 fascicles were collected, dipped in IBA and planted under conditions similar to those described under Experiment 1.

This treatment did not stimulate the growth of the buds in the fascicles and when the experiment was discontinued after 1 year none of the fascicles had produced roots.

Experiment 4. — *Pinus radiata* trees, 6 years old, were used in this experiment because they had 2 types of fascicles along the main stem: the typical fascicles with 2 or 3 needles; and the larger fascicles with a visible bud or small shoot emerging from the center of the fascicle. A total of 500 fascicles were collected and half of them were treated with IBA before being planted in the peat-sand medium.

After 8 months, there were 4 fascicles with roots, all of which were of the large fascicle type, and 1 of these had been treated with IBA.

Anatomy of callusing and root formation

Materials and methods: —

Fascicles from the main stem of 3- and 4-year-old slash pine trees were collected and planted in vermiculite. The humidity around the fascicles was kept high by placing a transparent plastic box over the planting flat. No treatment was given to the trees or to the fascicles prior to planting. To obtain representative samples of the various stages during callusing and root formation, fascicles were collected at 1-week intervals, the first series being collected after they had been 1 week in the planting medium. The fascicles were fixed in FAA, embedded in paraffin, sectioned longitudinally at a thickness of 8 microns, stained with Safranin and counter-stained with Fast Green.

Results: —

In the first fascicles that were collected 1 week after planting no callus formation had occurred *(Fig. 1 B)*. Even though they appeared similar to the controls *(Fig. 1 A)* there were mitotic divisions in the pith, indicating that callus cells are derived initially from the parenchymatous cells of the pith *(Fig. 1 C)*. In the fascicles collected after 2 weeks there was considerable proliferation of callus cells, mostly from the pith *(Fig. 1 E)*, and mitotic divisions also were common within the callus tissue at this time *(Fig. 1 D)*.

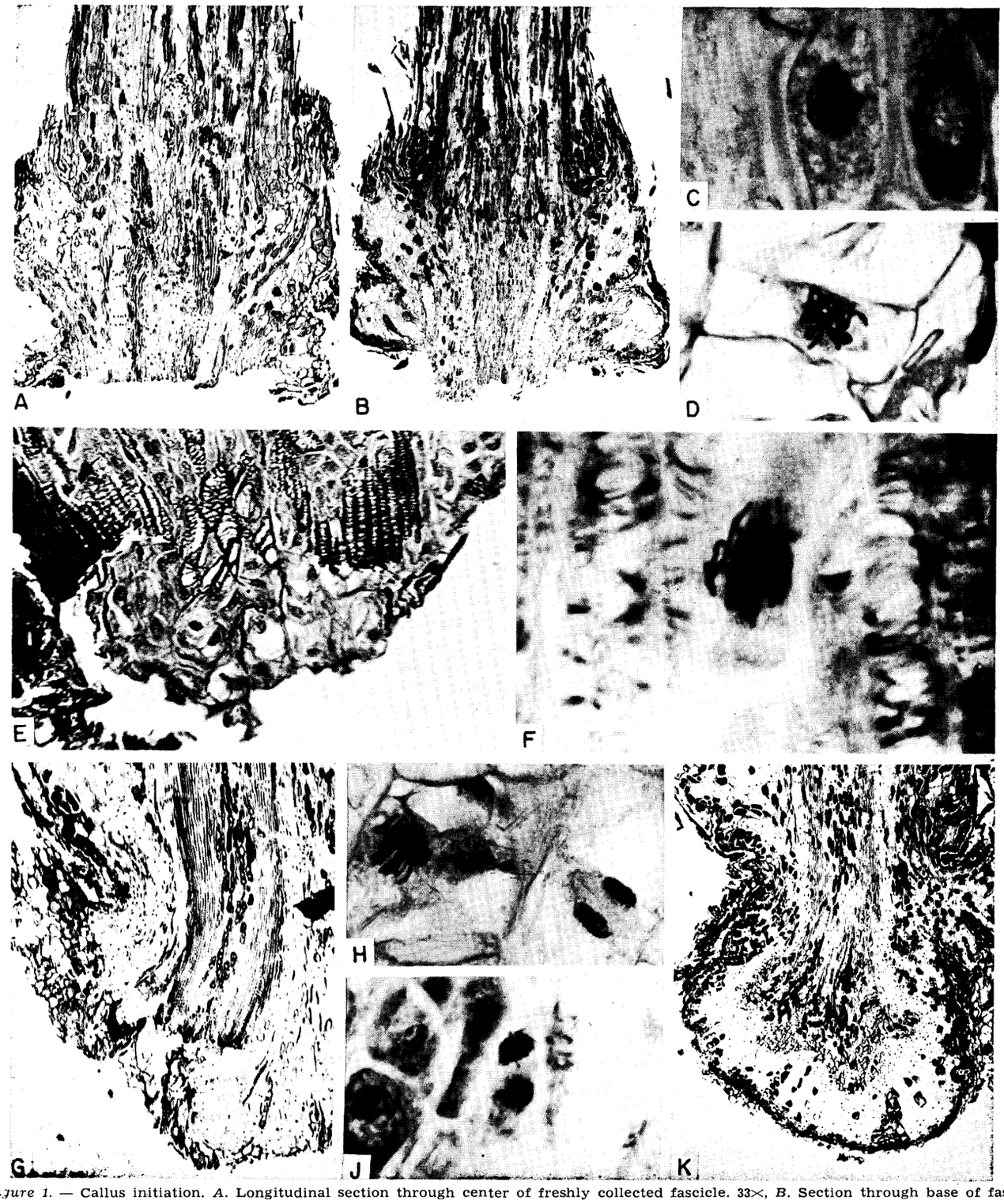

Figure 1. — Callus initiation. *A.* Longitudinal section through center of freshly collected fascicle. 33×, *B.* Section through base of fascicle (1 week). 20×, *C.* Mitotic division in parenchymatous cells of pith (1 week). 530×, *D.* Mitotic division in callus tissue (2 weeks). 590×, *E.* Callus cells and "wound xylem" derived from pith (2 weeks). 140×, *F.* Mitotic division in the phloem (3 weeks). 950×, *G.* Extensive callus formation and callus cells proliferating from the cambium (3 weeks). 36×, *H.* Mitotic divisions in callus tissue (3 weeks). 500×, *J.* Mitotic division in the pith (3 weeks). 600×, *K.* Callus with differentiated tissues (4 weeks). 28×.

Meristematic activity of the phloem and cambium was noted after 3 weeks, and mitotic divisions within the phloem suggest its contribution to callus formation *(Fig. 1 F)*. A large part of the callus that was present after 3 weeks was derived from the cambium, as well as from the cells within the callus as evidenced by the mitotic activity within the callus *(Fig. 1, G and H)*. At this time the pith continued to contribute to callus formation as demonstrated by mitotic divisions *(Fig. 1 J)*.

After 4 weeks a well defined callus had developed *(Fig. 1 K)* and covered the base of the fascicle. Tissues within the callus were differentiated, and the wound xylem was

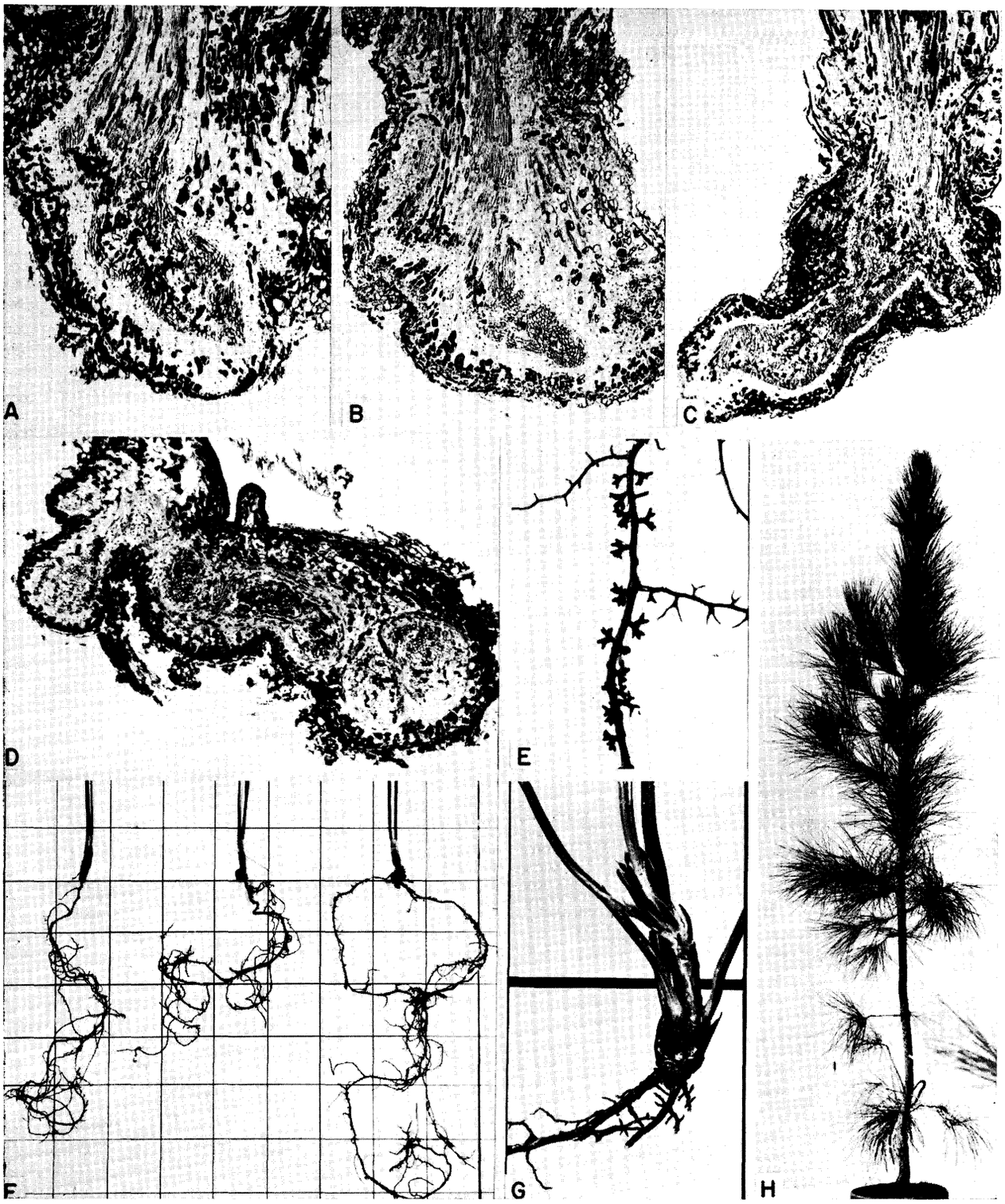

Figure 2. — Root initiation and growth of rooted fascicles. *A.* Callus after 5 weeks showing root protuberance. 30×, *B.* Further root elongation in callus (6 weeks). 28×, *C.* Root on fascicle planted for 12 weeks. 22×, *D.* Root which shows unusual tracheid orientation. 22×, *E.* Mycorrhizae on root of needle fascicle. *F.* Root systems on 3 fascicles (lines are part of 2.5 × 2.5 cm. grid), *G.* Developing bud on rooted needle fascicle (lines are part of 2.5 × 2.5 cm. grid), *H. Pinus elliottii,* 6 years old, grown from needle fascicle (2.3 m. tall).

surrounded by a ring of cambium with undifferentiated callus cells outside. The 4 protuberances in *Fig. 1 K* suggest potential root initials.

The protuberances in the callus of the fascicles planted for 5 and 6 weeks *(Fig. 2, A and B)* show further development of the root, which appears to be endogenous in origin and takes on a characteristic root form by distal elongation. *Fig. 2 C* shows a root which was visible without dissecting and was approximately 1 mm. long when it was collected after 12 weeks. Characteristic of many of the roots formed was the convoluted condition indicated by the tracheid cells which appear both in cross section and longitudinal section in *Fig. 2 D.* Spirally oriented tracheid "nests", which lack orientation, were also observed in many roots.

Extensive root systems developed on many of the fascicles. The longest main root that was produced during a 12-month period was 55 cm., and had a large number of side roots, some having 2 and 3 separate roots. The position of the roots varied; some grew at right angles to the stem, while others grew in a typical downward direction *(Fig. 2 F)*. Mycorrhizae formed on many of the roots, while they were still in the rooting medium, and before there was any activity of the bud *(Fig. 2 E)*. Fascicles that only produced callus, or did not produce callus, remained green for a period of up to 1 year; however, many turned brown and died before this period.

Growth of rooted fascicles

After adequate roots had formed on the fascicles, they were planted in a loose mixture (peat-sand-soil) in individual pots. The needles were kept in an upright condition to prevent them from touching the soil and thus minimizing their invasion by fungi. The pots were fertilized with soluble nutrients and placed under a 16-hour photoperiod. The root systems kept on growing under these conditions, and were able to support the needles, which remained green for a period of up to 2 years. In all of the fascicles that were planted only 2 produced terminal shoots and grew into trees. These 2 fascicles had been obtained from decapitated *Pinus elliottii*. These fascicles had exhibited a swelling of the fascicle before planting. *Fig. 2 G* illustrates the growth of 1 of these fascicles just after it was removed from the rooting medium and before it was planted in a pot. The shoot assumed apical dominance, and from this fascicle a normal tree was formed. After 6 years, the tree is 2.3 m. tall *(Fig. 2 H)*, and despite the pot-bound condition it is kept under, it grew 75 cm. during this past year.

Discussion

The production of sizeable isogenic lines of members of the *Pinaceae* presents great challenges, and the rooting of needle fascicles as a potential technique of asexual multiplication is a possibility of producing a large number of trees with an identical hereditary make-up. Experiments have shown that needle fascicles will root and that the resulting propagules can develop into normal plants with an orthotropic growth pattern. These experiments have also pointed out the difficulty in getting a high percentage of needle fascicles that will root. They did show, however, that needle fascicles responded favorably to pre-treatment of the stem by decapitating the terminal meristem, to treatment with IBA, and to planting in a well aerated medium.

Clipping the terminal bud was beneficial to rooting; possibly it stimulated growth in the bud before collection. Further work along these lines where auxins and other chemical treatments are used in conjunction with physical treatments should be encouraged. With the pine species tested, only the uppermost fascicles on the stem showed any pronounced growth activity, and this swelling was especially noticeable in the fascicle closest to the cut. As was observed, some sort of stimulation in the fascicle before collection is beneficial to rooting, but its greatest effect is on the subsequent growth of the fascicle. In excess of 50 rooted fascicles were potted and kept under observaiton for up to 2 years; only those with pronounced bud swellings started height growth.

The consistent beneficial effect of IBA, and the sharp decrease in rooting response between fascicles from trees 3 and 4 years old are most interesting. In Experiment 1 no rooting occurred on fascicles which were not treated with IBA, and none from the 4-year-old trees rooted. These observations are similar to the results with studies on air-layering and rooting cuttings of *Pinus elliottii* (Mergen, 1955). Downs (1949) made a study to determine at what age the rooting capacity falls off in *Pinus elliottii*, and he considered that the dividing line between extensive rooting and poor rooting probably lies between 2-year-old trees and 3-year-old trees. The complete failure that was obtained in Experiments 2 and 3 was with fascicles from 4-year-old trees, which might help to explain these results.

The anatomical study indicated that meristematic cells in the pith initiated callus formation, and mitotic divisions were present in fascicles that had been planted for 1 week. This callus develops at the base of the fascicle and precedes root formation, and is formed by divisions of cells in the pith, the cambium and phloem. In *Pinus densiflora* × *P. thunbergii* a change in the phloem and cambium cells was observed after 3 days, and after 5 days callus appeared to be present (Toda and Satoo, 1948). Considerable variation exists among species in the cells that initiate the callus, but there is general agreement that the pith, phloem and cambium contribute to a great degree (Toda and Satoo, 1948; Satoo, 1956; and Reines and McAlpine, 1959).

Reines and McAlpine (1959) noted that excessive callus formation inhibited rooting, even though organized tissue systems with a lack of orientation and direction were formed. In our experiments many fascicles had formed large knobs of callus when they were lifted from the medium after 1 year; they showed similar differentiation with a lack of visible root development.

Further anatomical and physiological investigations should prove valuable in understanding the relationship between bud development and root formation, and they might lead to a method of inducing bud formation in fascicles that would root and grow into normal trees. The answers might well lie in the use of either auxins or antiauxins, but we might also be confronted with one of nature's well kept secrets, namely the process of "ageing". In these studies we attempt to change the product of a particular meristem by modifying the external and internal factors. All of these studies bear on one of the most fundamental problems in biology — the factors that bring about a change in the physiology during the development of an organism from a juvenile stage to a mature stage. Any new information on this subject will be of interest.

Summary

Experiments were conducted on *Pinus elliottii* Engelm., *Pinus taeda* L., and *Pinus radiata* D. Don to determine some of the physiological factors that influence the rooting and subsequent development of needle fascicles. Factors studied were: 1) age of ortet; 2) position of fascicle on tree; 3) time of planting; 4) chemical treatment; and 5) planting medium. Included, also, were various pre-treatments given to the ortets.

A sharp decrease in rooting response between fascicles from trees 3 and 4 years old was observed. Needle fascicles responded favorably to pre-treatment of the stem by decapitating the terminal meristem, to treatment with IBA, and to planting in a well aerated medium. The experiments pointed out the difficulty in obtaining a high percentage of needle fascicles that will root and will grow into a nor-

mal tree. However, once a rooted fascicle starts to grow it can develop into a normal tree.

An anatomical study indicated that meristematic cells in the pith initiated callus formation, and mitotic divisions were present 1 week after planting. After the initial proliferation by the pith, both the cambium and phloem cells contributed to callus formation. The morphology of root development was followed during a 12-month period, and the subsequent growth of some of the rooted fascicles was observed for a period of up to 6 years.

Résumé

Titre de l'article: *Multiplication végétative des Pins par enracinement de fascicules d'aiguilles.*

Des expériences ont été entreprises sur *Pinus elliottii, Pinus taeda* et *Pinus radiata* en vue de déterminer certains des facteurs physiologiques qui influencent l'enracinement et le développement ultérieur des fascicules d'aiguilles. Les facteurs étudiés étaient:
1) l'âge de l'arbre-mère; 2) la position du fascicule d'aiguilles sur l'arbre; 3) l'époque de la mise en végétation; 4) le traitement chimique; 5) le milieu d'enracinement. En outre, les arbres-mères ont été soumis à différents prétraitements.

On observe une diminution rapide du taux d'enracinement entre les fascicules d'aiguilles prélevés sur des arbres de 3 et 4 ans. Les fascicules d'aiguilles répondent de façon favorable dans leur enracinement au prétraitement de l'arbre-mère par décapitation du méristème terminal, au traitement avec l'acide indol butyrique, et à la mise en végétation dans un milieu bien aéré. Les expériences ont montré la difficulté d'obtenir un pourcentage élevé de fascicules d'aiguilles qui s'enracinent et se développent pour donner un plant normal. Cependant, lorsqu'un fascicule enraciné commence à pousser, il peut donner un plant normal.

Une étude anatomique montre que les cellules méristématiques de la moelle sont à l'origine de la formation du cal, et que des mitoses se manifestent une semaine après la mise en végétation. Après la prolifération initiale des cellules de la moelle, les cellules du cambium et du phloème contribuent à la formation du cal. La morphologie du développement radiculaire a été suivie sur une période de 12 mois, et la croissance ultérieure de certains des fascicules enracinés observée sur une période allant jusqu'à 6 ans.

Zusammenfassung

Titel der Arbeit: *Ungeschlechtliche Vermehrung von Pinus durch die Bewurzelung von Nadelbündeln.*

Über einige Versuche an *Pinus elliottii, P. taeda* und *P. radiata* wird berichtet, physiologische Faktoren zu finden, die bei Nadelbündeln die Bewurzelung und ihre weitere Entwicklung beeinflussen können. Folgende Faktoren wurden untersucht: 1) Alter des Zweiges; 2) Position des Bündels am Baum; 3) Zeitpunkt des Steckens; 4) chemische Behandlungsweisen; 5) Medium, in das gesteckt wurde; außerdem auch verschiedene Vorbehandlungsweisen bei den verwendeten Zweigen.

Es wurde eine starke Abnahme der Bewurzelungsfähigkeit zwischen Bündeln von 3jährigen und 4jährigen Bäumchen beobachtet. Günstige Ergebnisse wurden erhalten nach der Vorbehandlung des Stämmchens durch Dekapitation, durch die Anwendung von IBS und durch die Verwendung eines gut durchlüfteten Mediums. Aus den Experimenten geht klar die Schwierigkeit hervor, einen großen Prozentsatz an Nadelbündeln zu bekommen, die sich bewurzeln lassen und auch nachher zu normalen Bäumchen heranwachsen. Wenn allerdings ein bewurzeltes Bündel einmal zu wachsen anfing, dann entwickelte es sich auch zu einer normalen Pflanze.

Die anatomische Untersuchung zeigte, daß im Mark meristematische Zellen die Callus-Bildung einleiten; Mitosen waren dort 1 Woche nach dem Stecken vorhanden. Nach den anfänglichen Markwucherungen beteiligten sich dann auch Cambium- und Phloemzellen an der Callus-Bildung. Die Morphologie der Wurzelentwicklung wurde 12 Monate lang verfolgt und ebenso dann auch das folgende Wachstum einiger bewurzelter Nadelbündel bis zum Alter von 6 Jahren beobachtet.

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