On the rooting of the cuttings of two conifer species.

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summary

Outdoor cuttings studies on two coniferous species, pitch pine and needle fir, have been made at Suwon, Korea extending from March to early May, 1956. The purpose of these studies was to find the effects of ages of parent trees, topophysis, dilute hormone solution treatments and the time at which the cuttings were collected on the rooting response of the cuttings. On September 2, rooting results were examined.

Pitch Pine (Pinus rigida)

The total of 1335 cuttings were collected from 3- and 10-year old trees. The cuttings treated were planted on March 13 and April 20.

1. In the cuttings collected from 3-year-old seedlings, the best result (34 percent rooted) was obtained by treating ends of cuttings with a mixture of 50 ppm IBA and 50 ppm NAA.

2. No rooting was observed in lammas shoots.

3. The cuttings from 3-year-old seedlings gave better rooting than those from 10-year-old trees.

4. The root origins were developed from the lower part of the cutting and the cut surface.

Needle Fir (Abies holophylla)

One hundred twenty-five cuttings were collected from a 30-year-old tree. The cuttings treated with any one of four mixed hormone solutions were planted on March 23.

1. Fifteen cuttings out of 75 cuttings from the lower half of the crown were rooted. The cuttings obtained from the upper half of the crown did not root at all.

2. The cuttings treated by the hormone solution made up of 20 ppm IAA, 20 ppm IBA, 20 ppm NAA, 20 ppm thiamine and 5 percent sucrose gave the best rooting (22 percent).

3. All roots originated from callus tissues and irregularly arranged parenchymatous tissues near the cut base.

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introduction

In vegetative propagation we perpetuate the hereditary constitution existing in a tree, whereas in the case of seedlings produced by sexual reproduction, a new individual is formed. It has aquired inherent tendencies from both the maternal and paternal trees. Although many studies on forest tree cuttings have been reported, more investigations providing information on other species and environments are needed. This study on the rooting of conifer cuttings was made at Suwon as one means of forest tree improvement.

Results of various investigations are in agreement with regard to the effect of age of tree on the rooting ability of the cuttings. Thimann and Delisle (1939, 1942) concluded that the most important single factor in rooting is the age of the tree from which cuttings are taken. Mitchell et. al. (1942) studying **Pinus caribaea,** Kirkpatrick (1940) with cuttings of **Cedrus** and **Pinus** species, Snow (1940) and Thomas and Riker (1950) investigating **Pinus strobus** cuttings, Deuber (1942) studying the cuttings of **P. strobus, P. monticola, P. parviflora, P. flexilis, P. koraiensis, P. peuce, P. cembra,** and **P. lambertiana** have demonstrated the effect of age on the rooting of cuttings. Larsen (1946) believed that the rooting of his softwood cuttings was the result of the physiological condition of the cuttings. Gardner's opinion (1929) was that the marked morphological differences which often exist between one-year old and older trees, may be expressions of just as pronounced nutritional and anatomical differences within the plant which in turn may be responsible for the differences in rooting response.

Clones, i.e., individual trees and their vegetatively propagated progeny, may differ in rooting capacity. Snow (1940) found that **P. strobus** shows considerable inherent clonal variation and Deuber(1942) has verified this by observing **P. strobus** and other five-needled pines. Doran(1945), Duffield and Liddicoet (1949), and Fielding(1954), studying **P. strobus**, the **hybrid Pinus attenuradiata**, and **P. radiata**, respectively, verified clonal differences in ability to root.

Snow's work (1942) has shown that **Acer rubrum** of different clones vary in the case with which they may be propagated by means of green wood cuttings. Larsen (1946) points out that although variations in the rooting capacicty of different trees can be explained on the strenght of individual variations, it may be conditioned by differences in the stage of development at the time of making cuttings. More recently, Mergen (1955) observed strong evidence of clonal variation in **P. caribaea.** Zinnai and Hukuhara (1954) studied the rooting of cuttings from clones of colchicine-induced tetraploid **Cryptomeria japonica**. Rooting percentage was the same as for diploids but the tetraploid cuttings rooted earlier, their roots were thicker, and their lateral roots were fewer in number. On the other hand, in Thomas and Riker's work(1950), several years' trials, involving some 13,000 **Pinus strobus** cuttings from over 150 different clones, did not produce any consistent rooting response from any of the selections. The authors believed the rooting response to be more likely the result of some temporary condition within the parent tree than to any inherent difference in rooting ability.

Molisch (1938) used the term of "topophysis" first to mean "the variation of the nature of the shoot according to its location on the plant". Thimann and Delisle (1939) found that in **Pinus** and **Picea**, lateral shoots root more readily than terminal, and in **Quercus** and **Acer**, that bases of the young plants root more readily than the apices. Deuber(1940) also observed that cuttings from medium-sized twigs which grew on lateral branches rooted better than those made from terminal shoots. Doran and Holdsworth (1940) also observed the effect of position. According to Thomas and Riker's report (1950) on **Pinus strobus** 24 percent of the cuttings rooted from the bottom third of the tree, while only 4 percent rooted from the top third of the tree. Toda (1953) reported that **Cryptomeria** cuttings collected in 1948 rooted more easily when taken from the lower part of the crown.

Quintin (1953) found that cuttings from leading shoots rooted less well than those from lateral branches for both **Abies alba** and **Picea abies**. Doran (1946) found that **Pinus strobus** cuttings rooted in slightly larger percentages if obtained from the north side of the tree crown. In general, the shade shoot or the lowest shoot in the crown rooted considerably better than sun shoots.

Indole-3-acetic acid, indole-3-butyric acid, naphthaleneacetic acid, 2-4-dichlorophenoxyacetic acid, some vitamin groups, some mineral elements, sugar, some fungicides, traumatic acid, rhizocaline and others known as root-inducing substances have been tried in an effort to improve the rooting of cuttings. The voluminous literature on auxins and the rooting of cuttings (Zimmerman and Wilcoxon, 1935. Hitchcock and Zimmerman 1936. 1937. 1939. White, 1937. Bonner 1937. Plank, 1939. Bonner and Greene, 1939. Thimann and Delisle, 1939. Doran, 1940. 1946. 1955. Griffith, 1940. Kirkpatrick, 1940. Snow, 1940. Deuber, 1942. Mitchell, et al., 1942. Thimann and Delisle, 1942. Mirov, 1944. Dorman, 1947. Duffield, 1949. Thomas and Riker, 1950. Quintin, 1954. Waterman and Aldrich, 1954. Wells, 1954. Mergen, 1955.), furnishes few clues as to the basic processes involved, but the beneficial effects of treatment are apparent.

methods and materials

Pitch Pine (Pinus rigida)

The preparation of methods of pitch pine cuttings varied with each test, that is, the methods depended on the age of the mother tree from which the cuttings were taken, and the development of shoots. For example, the cuttings used on the first of February were collected from 3 (1-2 stock) year old seedlings which were growing in the nursery. The needles were yellowish green and 5 to 8 cm. long. Each cutting had 4 to 5 buds on the tip. The needles on the portion of stem which was inserted in the medium were taken off. The tip of each cutting appearing above the ground was 2 cm. long with needles on it. Those cuttings obtained on the 20th of April were collected from the same seedlings mentioned before, but terminal buds had already started growing. However, all cuttings collected before the 20th of April had brownish dormant buds. In subsequent collections, the tender green tissue could be seen between the bud scales.

In one experiment, lammas (the summer or second growth shoot which starts to grow in mid-summer) were used as cuttings. In contrast with normal shoots which show green or dark green, the lammas have pale green color, soft tissues and a much shorter needle length, ranging from 1-2 cm. In some cases, there were no fully developed needles at all on the lammas.

Needle Fir (Abies holophylla)

In this study, cuttings made on the 23rd of March from a 30 year old tree were used. Some of the cuttings were collected from the upper half of the crown, the remainder from the lower half of the crown. The purpose was to find whether position on the parent tree affects the rooting percentages of the cuttings. The diameter of cuttings from the lower part of the crown, were smaller than those from the upper part of the crown. Shoots on the upper part of the crown received more sunlight and had comparatively large terminal buds.

After the cuttings were prepared, they were usually pretreated with tap water. In some instances, shown in tables, this treatment was omitted. Various chemicals were used for treating with cutting as follows: 3-indole butyric acid (abbreviation, IBA), 3-indole acetic acid (IAA), naphthalene acetic acid (NAA), sucrose ($C_{12}H_{22}O_{11}$) (su.), vitamin B_1 (thiamine) (B_1), vitamin B_6 (pyridoxine) (B_6), nicotinic acid ($C_6H_5O_2N$) (N), glycine (amino acetic acid, $C_2H_5O_2N$) (G), and control (no hormone treatment, soaked in tap H_2O).

Soaking the basal ends of the cuttings using the chemical in liquid form was adapted for this study as it has been for most others. The soaking method consisted of standing the basal ends of the cuttings to a depth of about 2.5 cm. in a solution of the chemicals. Contact of the chemicals with the needles was avoided. The author thought that warming the solution might be helpful so the temperature was maintained at between 20° and 25° C. When the soaking treatment was continued 24 hours or more, all solutions were renewed because they had become contaminated by oleoresins or other extracts from the cuttings.

After the original top soil in the cutting beds had been taken off to a depth of 8 inches, clean, coarse river sand was spread over the beds. The cutting beds were of the common out-door type and had 4-by-18-foot wooden frames partially sunk in the ground. The river sand medium was treated with an 0.8 percent formalin solution to control soil fungi 20 days before the cuttings were planted. Immediately before the cuttings were planted, the medium was treated with Usplun (an organic mercurial) solution, which is a fungicide. In order to prevent excessive heating, the beds were covered with a single layer of lath screen during the late spring and summer. Cuttings were watered daily. All beds were sprayed with Bordeaux mixture and Ferbam suspension to control moulds.

RESULTS and DISCUSSION

Regardless of the species tested, the cuttings were not disturbed until the results of rooting were examined. The cuttings were dug and the numbers of rooted cuttings were recorded on September 2, 1956, about 140 days after the begining of the experiment. Very many factors can modify the rooting of cuttings. The age of the mother tree from which cuttings were taken, topophysis, chemical treatment, and the date of collecting cuttings were evaluated in this study. The data obtained from each species were analysed statistically. Since it may be that the percentage of rooting depends to a great extent upon the number of days between treatment and recording results, a relatively long period (140 days) was used. K.V. Thimann and J. Behnke (1950) believe 140 days to be adequate.

Pitch pine (**Pinus rigida**)

The two factors, the effect of age of parent trees, and the effect of hormone on the rooting of pitch pine cuttings, were examined. The results obtained from the total of 1335 cuttings are presented in table 1.

Experiment 1.

In experiment 1, the age of parent trees was tested as a variable affecting rooting percentages. Of the 300 cuttings from 10 year old trees, none rooted, but 25 cuttings out of 205 cuttings from 3 year old trees did root. With hormone treatment, the mixed solution containing 25 ppm. IAA, 25 ppm. IBA brought 28 percent rooting and the solution containing 50 ppm. IBA resulted in 5 percent rooting (cf. Test No 1 and No. 2). The calculated t-value indicates that the IAA, and IBA treatment with a concentration of 50 ppm. exceeds the IBA treatment with the same concentration at the 1 percent level of significance (t-value 2.69, df. 178).

Experiment 2.

Here also young trees produced better results than older. The difference in rooting ability between the cuttings from 3-yeat-old seedlings and those from 10-year-old trees was significant at the 5 percent level. A non-significant difference has been calculated between the two compound hormone uses in test Nos. 1 and 2, and Nos. 1 and 3 in experiment 2.

Experiment 3.

Opposing experiments 1 and 2 in which the cuttings from 10-year-old trees did not root, experiment 3 showed two rooted cuttings from 10-year-old trees. The effect of age variation was significant between test No. 2 and No. 4 in this experiment. Both had identical hormone treatment. Judging by the results of a t-test between No. 1 and No. 2 solution (t-value=2.2, df=73), the latter (containing 50ppm. IBA, 20 ppm. thiamine, 20 ppm. pyridoxine, 20 ppm amino acetic acid, and 3 percent sucrose) gave significantly better rooting.

Experiment 4.

In order to find whether a certain hormone treatment provides better rooting than other treatments, this experiment was undertaken. Two simple hormone treatments and three mixtures were used as shown in table 1. The same concentration and duration of soaking were employed for each. The fact that some rooting was obtained from each of the tests employed may have been the results of the young age of parent trees.

The fact that highly significant difference was calculated between test No. 1 and No. 3 (t-value 3.3678 and df. 98) suggests that NAA gives better rooting success than IAA. However, no significant difference was found between the results of test No. 2 and No. 4. This suggests that NAA treatment does not always result in better success. It is impossible to give a reasonable explanation within the limits of this experiment.

No significant differences were found between test No. 2 and No. 3 (t-value 2.125 and df. 98). Considering the above, one can not conclude that any special hormone treatment always result in better rooting than others. Further studies are needed.

Experiment 5.

A total of 230 lammas collected from 10 year old trees between Mach 23 and April 14 were treated with mixed hormones as shown in table 1. Complete failure resulted. Soon after planting in the beds, some swelling and elongation resulted from the absorption of soil water. The lammas were attacked early by fungi. The tender tissues probably made invasion by fungi easier.

The Origin of Roots

In order to investigate the origin of roots, basal portions of the removed cuttings were cut 40 microns in thickness in young cuttings by a sliding microtome. These sections were stained wtih safranin and hematoxyline. Within these limited observations, adventitious roots in pine cuttings develop from following tissues.

1. Cambial and phloem portions of ray tissues.

2. Callus tissues.

Almost all adventitious roots from stem tissues at the cut bases originated in cambial and phloem portions of ray tissues presumably developing meristematic activities of these tissues. Primary tissues played a greater role in root formation than secondary ones. Root forming rays were limited to primary ones having started from leaf gaps in cuttings of pitch pines.

A small colony was formed at the end of primary uniseriate rays. The cells of the colony initiate mitotic divisions, and as a results of the pressure of the adjacent dividing cells, the diameter of the cutting was increased.

Table 1. Effect of age of parent trees, and of the type of treatment on the rooting of Pitch pine cuttings.

Experiment 1.

Test No.	Date	Age of tree	Cuttings Length (cm)	Cuttings Condition	Pretreatment by water soaking (hrs.)	Auxin treatment Materials and concentration in p.p.m.	Auxin treatment Hrs.	Cuttings No. taken	Cuttings No. Rooted	Percent of rooting
1	3-15	3	12	Normal shoot	16	IBA 50	24	100	5	5
2	〃	〃	〃	〃 〃	〃	IAA, IBA 50	〃	80	22	23
3	〃	〃	〃	〃 〃	〃	Tap water	〃	25	0	0
4	〃	〃	〃	〃 〃	〃	IBA 50	〃	60	0	0
5	〃	〃	〃	〃 〃	〃	IAA, IBA 50	〃	120	0	0
6	〃	〃	〃	〃 〃	〃	Tap water	〃	120	0	0
Totals								505	27	5.3

Experiment 2.

1	3-26	3	12	Normal shoot	24	IAA, IBA, 80, B_1, B_6, G.N. 40, Su.2.5%	24	25	4	16
2	〃	〃	〃	〃 〃	〃	NAA 80, B_1 B_6 N.G.40, Su.2.5%	〃	〃	2	8
3	〃	〃	〃	〃 〃	〃	B_1, B_6, N.G.40 Su. 2.5%	〃	〃	2	8
4	〃	10	〃	〃 〃	〃	IAA, IBA, 80, B_1, B_6, G.N.40, Su. 2.5%	〃	〃	0	0
5	〃	〃	〃	〃 〃	〃	NAA, 80, B_1, B_6 N.G.40, Su.2.5%	〃	〃	0	0
Totals								125	8	6.4

Experiment 3.

Test No.	Date	Age of tree	Cuttings Length (cm)	Cuttings Condition	Pretreatment by water soaking (hrs.)	Auxin treatment Materials and concentration in p.p.m.	Auxin treatment Hrs.	Cuttings No. taken	Cuttings No. rooted	Percent of rooting
1	4-9	3	12	Normal shoot	24	NAA 50, B_1, B_6, G. 60, Su. 3%	35	50	1	2
2	〃	〃	〃	〃 〃	〃	IBA 50, B_1, B_6, G. 60, Su. 3%	〃	25	5	20
3	〃	10	〃	〃 〃	〃	NAA 50, B_1, B_6, G. 60, Su. 3%	〃	50	0	0
4	〃	〃	〃	〃 〃	〃	IBA 50, B_1, B_6 G. 60, Su. 3%	〃	100	2	2
Totals								225	8	3.6

Experiment 4.

1	4-20	3	12	Normal shoot	16	IAA, IBA 100	24	50	4	8
2	〃	〃	〃	〃 〃	〃	IAA 100	〃	〃	8	16
3	〃	〃	〃	〃 〃	〃	IBA, NAA 100	〃	〃	17	34
4	〃	〃	〃	〃 〃	〃	NAA, IAA 100	〃	〃	8	16
5	〃	〃	〃	〃 〃	〃	IBA 100	〃	〃	10	20
Totals								250	47	18.8

Experiment 5.

Test No.	Date	Age of tree	Cuttings Length (cm)	Cuttings Condition	Pretreatment by water soaking (hrs.)	Auxin treatment Materials and concentration in p.p.m.	Auxin treatment Hrs.	Cuttings No. taken	Cuttings No. rooted	Percent of rooting
1	3-26	10	7-10	Lammas	24	IAA, IBA, 50, B_1, B_6 G. N. 40, Su. 2.5%	24	50	0	0
2	〃	〃	〃	〃	〃	NAA, 70, B_1, B_6, N. G.. 40, Su. 2.5%	〃	40	0	0
3	4-9	〃	〃	〃	〃	NAA, 50, B_1, B_6, G. 60, Su. 3%	35	25	0	0
4	〃	〃	〃	〃	〃	IBA, 50, B_1. B_6, G. 60, Su. 3%	〃	20	0	0
5	〃	〃	〃	〃	〃	IBA, NAA, 50, B_1, B_6, G. 60, Su. 3%	〃	20	0	0
6	4-14	〃	〃	〃	〃	IBA, NAA, 40, B_1, B_6 20, Su. 3%	40	25	0	0
7	〃	〃	〃	〃	〃	NAA, 40, B_1, B_6, 20 Su. 3%	〃	25	0	0
8	〃	〃	〃	〃	〃	IBA, 40, B_1, B_6, 20 Su. 3%	〃	25	0	0
Totals								230	0	0

Table 2. Effect of topophysis on the rooting of needle fir cuttings.

Test No	Date	Age of tree	Cuttings		Pretreatment by water soaking (hrs.)	Auxin treatment		Cuttings		Percent of rooting
			Length (cm)	Condition		Materials and concentration in p.p.m.	Hrs.	No. taken	No. rooted	
1	3-23	30	10	Normal shoot, lower crown	2	IAA, IBA, NAA, 40 B_1, 20, Su, 5%	24	50	11	22
2	〃	〃	〃	〃	〃	IAA, NAA, 40 B_1, 20	〃	50	4	16
3	〃	〃	〃	Normal shoot, upper crown	〃	IAA, IBA, NAA, 40 B_1, 20, Su. 5%	〃	25	0	0
4	〃	〃	〃	〃	〃	IAA, NAA, 40 B_1, 20	〃	25	0	0
Totals								150	15	

This cell colony was the root primordium. By further cell divisions it pushes through the phleom, cortex and periderm, to the outside.

Needle Fir (**Abies holophylla**)

The writer wished to determine whether or not topophysis affected the rooting ability of needle fir cuttings. On March 23, 125 cuttings from a 30 year old tree were treated with hormone and planted (Table 2). Seventy five cuttings were collected from the lower half of the crown and 50 cuttings from the upper half. Although no cuttings obtained from the upper half rootet, 15 cuttings from the lower half showed rootings. A highly significant difference based on origin in the crown was demonstrated in this experiment (t-value=3.6 with df.123). The results indicate that it is possible to increase rooting by collecting cuttings from the lower half of the crown.

Several authors (Doran, et al., 1940, Snow, 1940, Deuber, 1942, Doran, 1946) came to essentially the same conclusions. They are of the opinion that rooting behavior apparently modified by topophysis is the result of some physiological variation. This should be studied further using various techniques because the nature of the effect of location within the crown is not yet proved. All roots originated from callus tissues and irregularly arranged parenchymatous tissues near the cut base.

conclusions

The following generalizations appear to be true on the basis of the evidence submitted here.

An important item is the time of year at which the cuttings were taken. In the present trials the best time of year for pitch pine cuttings to be placed in outdoor cutting beds was during the period in which the new growth is about to begin. Of the factors studied, season of collection appeared to be of greatest significance.

It was evident that the age of the parent tree from which cuttings were taken was a factor in their rootings. The reason for the inability of cuttings from certain aged coniferous trees to form roots is not known. There are many possibilities for the difference in responce. Some hormones may be produced in the young seedlings that is not produced as the trees grow older. Some root-inhibiting substances may be produced in older trees, but not in young seedlings.

Generalizations on the effect of hormone treatment are not possible. It is believed that hormone treatments produce differences in rooting responce only when all other factors are optimum. It is probable that when hormone content of the parent tree is high, hormone treatment of the cuttings taken from it does not help much in producing roots; possibly it is even harmful (Mirov, 1944).

The position from which a cutting was taken has been reported to play an important role in the rooting of cuttings. One trial with needle fir cuttings helped confirm this. Differences probably are due to variations in hormone distribution. But our present knowledge of the content and distribution of plant hormone in forest trees, particularly in conifers, is very limited.

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摘　　要

리기다소나무와 젓나무의 野外揷木이 水原農科大學 林業苗圃에서 1956年 實施되었는데 다음과같은 事實이 確認되었다.

리기다소나무(pinus rigida)에 있어서는 총 1335본의 삽수가 3년생 및 10년생의 모수에서 얻어지고 表에서 보이는바와 같은 處理를 받고 3月 13日 및 4月 20일에 揷木되었다. 이에 있어서는 ① 幼齡木일수록 發根이 높다는것과 50ppm의 IBA 또는 NAA가 가장 좋은 결과 (34 per cent)를 보여주었다. ② lammas shoot에서는 전연 발근이 되지않었다. ③ 揷木時期가 또한 發根率에 영향을 주었다. ④ 發根起元은 揷穗의 下端部位 또는 切口에 생긴 Callus 부터였다.

젓나무(Abies holophylla)에 있어서는 3) 년생의 모수에서 揷穗가 採取되었고 3月 23日에 揷木되었다. 이에 있어서는 ①樹冠의 上半部에서 採取된 삽수는 전연 發根하지않었다. ② 樹冠의 下半部에서 얻어진 揷穗에 있어서는 20ppm IAA, 20ppm IBA, 2)ppm NAA, 20ppm thiamine 및 5 percent sucrose의 혼합용액이 가장 좋은 發根率(22 per ceut)를 보여주었다. ③ 모든 發根은 不規則한 parenchymatous 組織에서 유래했었다.