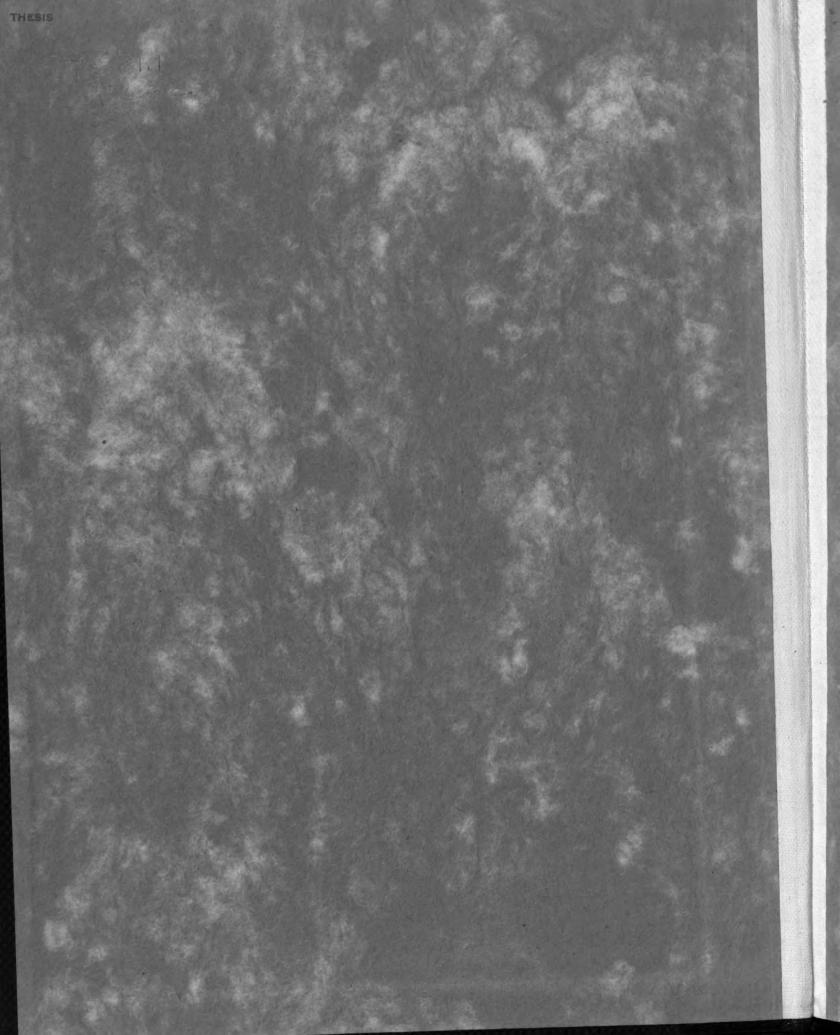
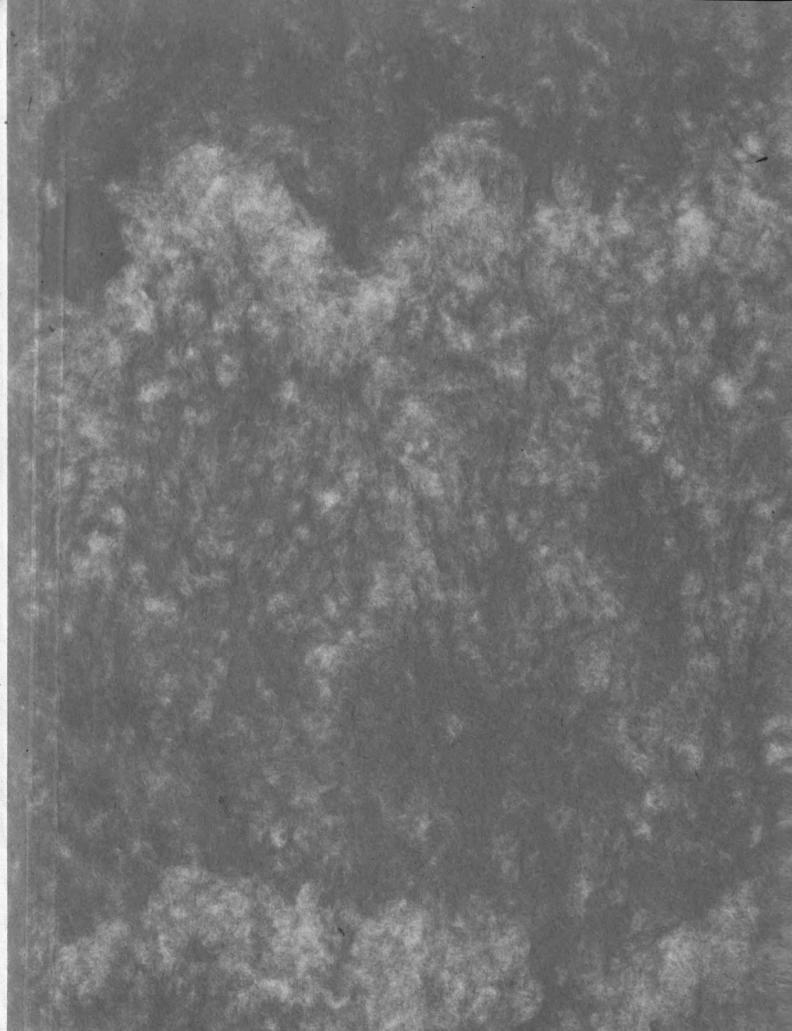


EFFECT OF INDOLE-BUTYRIC ACID SOLUTIONS ON THE ROOTING OF DORMANT WOODY CUTTINGS

> Thesis for the Degree of M. S. MICHIGAN STATE COLLEGE Elizabeth VanMaren 1939





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EFFECT OF INDOLE-BUTYRIC ACID SOLUTIONS ON THE ROOTING OF DORMANT WOODY CUTTINGS

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TABLE OF CONTENTS

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Introduction	Page 1
Review of literature	3
Methods	6
Tables and discussion of data	11
General conclusions	27
Summary	31
Bibliography	33

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LIST OF TABLES

•

Table		Page
I.	Names and dilutions of growth substances used.	9
II.	Rooting response of <u>Salix</u> cuttings taken in November.	12
III.	Rooting response of <u>Thuja</u> cuttings taken in November.	14
IV.	Rcoting response of <u>Syringa</u> cut- tings taken in November.	15
V.	Rooting response of <u>Salix</u> cuttings taken in December.	17
VI.	Rocting response of <u>Thuja</u> cuttings taken in December.	18
VII.	Rooting response of <u>Syringa</u> cut- tings taken in December.	20
VIII.	Rooting response of <u>Malus</u> cuttings taken in December.	21
IX.	Rooting response of <u>Salix</u> cuttings taken in January.	22
X.	Rooting response of <u>Thuja</u> cuttings taken in January.	23
XI.	Rocting response of Thuja cuttings taken in early April.	26

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 - · · · · · · · · · · · ·
 - .

LIST OF FIGURES

Figure		I	Plate
1. <u>Salix</u> cuttin the end	ngs (January) show nd of eleven days	ving roots at •	I
	ng s (January) show nd of eleven days		I
	ngs (Janua ry) s how nd of eleven days		II
	ngs (January) show nd of eleven days		II
5. <u>Salix</u> cutting the end	ngs (January) shown nd of five weeks.	wing roots at	III
	ngs (January) shown of five weeks.	ving roots at	III
	ngs (December) sho en weeks after Aug		IV
sixte	ngs (December) sho en weeks after ind treatment.		IV
	<u>huja</u> cuttings (Dec allus at the end c		v

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INTRODUCTION

Plant hormones and synthetic growth substances, such as indole-butyric acid, indole-acetic acid, and naphthalene acetic acid, have attracted much attention during the past few years. While various effects which they may have on plants have been studied, one of the most important applications is in the initiation of roots on cuttings. Several commercial preparations of these synthetic substances have been placed on the market and quite widely advertised. This has created considerable interest for people engaged in plant propagation.

Rooting of dormant cuttings of most species of woody plants has never been very successful. The usual practice followed with hardwood cuttings taken in the late fall or early winter is to bury them in sand in a well drained place and keep them cold, so that callusing may take place. The following spring they are removed and set in propagating boxes to allow rocts to develop. This method is successful with several species, but with others, such treatment will not cause the cuttings to strike root.

The term dormancy is used in this paper to apply to the entire period of inactivity which plants undergo during the winter months. It is known that cuttings of many woody plants will resume their activity after a short period of cold weather, if they are brought into a greenhouse and kept well watered. However, when kept out-of-

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doors, these same plants remain inactive until spring. The peach is a particularly good example of this. A few warm days in winter will cause the buds to become active, and during the next cold period they are frozen, causing serious damage. Such observations would lead one to suppose that environment is mainly responsible for dormancy. However, some other plants (e.g., lilies and tulips) become dormant during the summer, and do not renew their growth until fall or the following spring. This would indicate a hereditary cause. Still other causes have been suggested for the dormancy of some seeds. When all of these things are considered, it seems reasonable to think of dormancy as resulting from a combination of environmental and hereditary factors.

It has been suggested by some workers that the lack of success in rooting cuttings during their dormant period might be due to a lack of a necessary hormone in the twig when the plant is inactive (2). The experiments described in this paper were started with the idea that if treatment with synthetic growth substances could be made to induce the rooting of dormant cuttings, such a method would be distinctly advantageous. At the time when these experiments were started, most work favored the use of indole-butyric acid for the rooting of cuttings. For that reason and because of the fact that most of the market

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preparations were solutions of indole-butyric acid in alcohol, it was decided to use different concentrations of indole-butyric acid as the test solutions. Some of the recent work (22) shows better results from the use of indoleacetic acid.

REVIEW OF LITERATURE

As early as the middle of the eighteenth century, the presence of a substance in plants, which was capable of initiating callus and root growth, was postulated by du Monceau. Sachs also presented a theory that plants contain a root forming substance which moved toward the base of the stems. He considered that when "an obstacle for further downward movement" such as a ring or girdle about the stem existed, this substance gathered above the wound and started the formation of roots at that place. The "growth enzymes" of Beijerinck's publications were closely related (26).

The works of Boysen Jensen (1914), Went (1926), and van der Lek (1925) were influential in bringing the hormone concept into studies of root formation in recent times. During the last ten years the amount of literature concerning phytohormones and their effects on plants has increased very rapidly. In this country, Zimmerman, Hitchcock, and other workers at the Boyce Thompson Insti-

-3-

 tute have been instrumental in starting the work on root initiation in cuttings.

Phytohormones or synthetic growth substances are, by no means, the panacea for all of the difficulties of the plant propagator. It seems evident that such a growth substance is not the limiting factor in cuttings made from some plants (8, 22). A theory has been presented, principally by Went (25) and Cooper (9), that the absence of some other hormone-like factor in the plant is responsible for the lack of rooting. This substance, which they call "caline" or "rhizocaline", when present, is stimulated by the action of auxins, and the cuttings root successfully.

It has been found that the treatment with synthetic growth substances will make some cuttings root while untreated cuttings kept under the same conditions fail to form any roots. In other cases, however, no treatment so far discovered will induce the rooting response desired. One explanation that has been offered is that some plants in themselves, have plenty of the auxin present, and the treatment will not be advantageous in any way; the others have the auxin present in insufficient quantity, and they benefit by the treatment.

That a growth hormone is not present in the tissues during the dormant season has been postulated by Avery and others (2). They also state that the centers for the

-4-

production of this hormone are in the terminal buds. These workers have attempted to show that the supply of growth hormone increases during the time when the terminal buds are swelling, and reaches the maximum just prior to the period of most rapid expansion of the current season's shoots (2, 12). Evidence seems to indicate that the auxin is formed from some inactive precursor, the chemistry of which is not thoroughly understood (21, 26).

Some workers have obtained results showing that the age of the tree from which the cutting is taken is an important factor. They also hold that the position of the cutting on the parent tree (whether it is terminal or lateral, and whether it comes from the basal or apical portion of the tree) affects greatly the rooting response of the cutting (22).

In cuttings which invariably fail to root, auxin supply is probably not the limiting factor, and it has been suggested that other factors, such as supply of carbohydrate, vitamin B_1 , bios, or some other hormone-like substance may be insufficient (5, 18, 9, 25).

Treatment with growth substances has been found to inhibit the growth and development of buds. In many plants the development of the terminal bud will inhibit the growth of lateral buds, and if the terminal bud is removed, one or more of the lateral buds will develop.

-5-

Thimann (20) has found that the inhibition of the development of lateral buds in <u>Pisum</u> seedlings by pure auxin is not compensated by growth elsewhere, and involves a decrease in dry weight. Also the extent of the inhibition is not dependent on the distance between the bud and the point of application of the auxin. Thimann and Skoog (23) have found that this inhibition is not due to injury.

-6-

That auxin is present in larger amounts during the period just preceding most rapid shoot development has been previously stated (2, 12). It would seem more reasonable then, to expect that the addition of auxin to a cutting would cause more rapid development of the buds. Since just the opposite is true, the problem of explanation becomes very difficult. It seems that the same substance can both inhibit and promote bud development, and the conditions under which each of these reactions takes place is a question still to be solved.

METHODS

The cuttings used throughout the experiment represent four species, <u>Syringa vulgaris</u> (lilac), <u>Malus</u> <u>pumila</u> (apple), <u>Thuja occidentalis</u> (arbor vitae), and <u>Salix babylonica</u> (weeping willow). Of these, the first two are considered difficult or impossible to propagate from cuttings. The arbor vitae is not so difficult to

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root, while willow roots very readily under most conditions.

The cuttings were taken periodically during the dormant season of 1937-1938 and during the early part of the 1938-1939 dormant season. An interval of three weeks was usually allowed between dates of sampling, in an attempt to include any changes which might occur during the period commonly spoken of as dormancy.

All of the cuttings were taken from branches at the basal part of mature plants, only the terminal parts of the preceding season's growth being used. On the willow, this meant using the pendant twigs, while on the other species the position of the cutting on the parent plant would have been upright or somewhat horizontal. The cut was made close below a node and at an angle. Each cutting was approximately ten centimeters long.

The cuttings considered here were not treated with wax or any such material to cut down transpiration. Also when leaves developed they were allowed to remain on the cutting. Checks were run on one set to determine whether or not the removal of several buds, before these had an opportunity to develop, would be beneficial to the rooting, since such treatment would reduce the surface for transpiration. The results were not considered indicative of difficulties of that nature, so other sets

-7-

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were all run on cuttings whose buds were not removed.

All of the solutions used were forms of indolebutyric acid. One is sold under the trade name of "Auxilin". This material is already in solution form and the dilutions used were suggested by the direction sheet furnished with the preparation. The second material goes under the trade name, "Hormodin A", and is also in solution in alcohol. Again the directions accompanying the bottle were considered in making the dilutions. Pure crystals of indole-butyric acid were obtained from the Eastman Kodak Co. for use in another set. Because of the insolubility of these crystals in water, they were first put in solution in ethyl alcohol. For each preparation 0.5 grams of the indole-butyric acid crystals were dissolved in 20 cc. of 95% ethyl alcohol, and then 5 cc. of glycerin were added. This was further diluted by adding a measured quantity of the mixture to a definite quantity of water. To avoid impurities found in ordinary tap water, dilutions of all three of the materials were made with distilled water.

Table I gives the concentrations of each dilution.

Fresh solutions were prepared each time any cuttings were to be treated. The cuttings were placed in beakers of the solution so that approximately the lower two centimeters of each cutting was immersed. They remained

-8-

TABLE I

Solution Concentration	
Auxilin A	40 mg. per L.
Auxilin B	SO mg. per L.
Hormodin A	20 mg. per L.
Hormodin B	40 mg. per L.
Indole-butyric acid A	20 mg. per L.
Indole-butyric acid B	40 mg. per L.

TRADE NAMES AND CONCENTRATIONS OF GROWTH SUBSTANCES USED

in the solutions for twenty-four hours, during which time they were kept at room temperature and away from direct sunlight. The controls were immersed to the same distance in distilled water and kept under identical conditions. At the end of the twenty-four hour treatment, all of the cuttings were removed from the solutions, rinsed in tap water and placed in the rooting medium. This rooting medium was made by mixing two parts (by volume) of sand with one part of peat. It was placed in standard size greenhouse flats, and watered thoroughly before planting.

The cuttings were placed in the flats at an angle of about 45°. Though the spacing varied somewhat with the type of cutting, most of them were placed about one inch apart in rows that were about three inches apart. Such spacing was, of course, impossible in the case of the <u>Thuja</u> cuttings because of their large spreading tops. These were placed at sufficient distance to prevent crowd-ing.

The flats were placed on the bench in a greenhouse and, during the first two weeks or more after planting, they were kept covered with newspapers to prevent rapid drying out of the soil and to keep the air about the cuttings very moist. The relative humidity in the greenhouse varied from about 50% to 80% and the temperature was so regulated that there was a day and night variation which, during the 1937-1938 season was about 65° F. for night and between 75° and 80° F. in the daytime. During the 1938-1939 season the temperature was more constant, remaining mostly between 65° and 70° F.

At intervals during the test period the soil was loosened around one or two of the cuttings in order that they might be removed and checked for callus or root development and then replaced without injury. Aside from such inspection the cuttings were not disturbed until time for making the final count. That time differed with the species under consideration. The time for each set is given in the tables following. In the species where no rooting results were obtained, the cuttings

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were left in the rooting medium until the whole shoot was definitely dead. Whenever any cuttings in any set died, they were removed and recorded without disturbing any of the others.

TABLES AND DISCUSSION OF DATA

The following tables show the results from the treatment of nearly 2000 cuttings. Since the weather during October and early November of 1938 was unseasonably warm, the results given in tables II to IV represent work with cuttings in the very early part of the dormant season, or possibly before all of the changes usually considered as accompanying dormancy had occurred. Tables V to VIII represent results from the experimental work with cuttings taken in late November and early December of 1937 when the trees were very definitely inactive. Tables IX and X express results for the month of January -- about the middle of the dormant season. Table XI is the record of a group of Thuja cuttings taken in early April 1938 when dormancy had probably been overcome. No tables are given for the latter part of the dormant season but the results of the experiments are discussed following table X.

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TABLE II

ROOTING RESPONSE OF SALIX CUTTINGS TAKEN IN NOVEMBER*

No. treated	Treatment	Dead; not rooted		Dead; rooted		Living; rooted	
		no.	%	no.	0/0	no.	62
40	Auxilin A	13	32.5	27	67.5	0	0.0
40	Hormodin A	10	25.0	30	75.0	0	0.0
40	Control	3	7.5	4	10.0	33	82.5**

* Cuttings were removed from rooting medium after 6 1/2 weeks.

** These living and rooted cuttings were in poor condition when removed.

The treatment of willow cuttings during the very early part of the dormant season was not beneficial. The temperature preceding the collection of this set had been warm, with only a few frosty nights to initiate the changes which accompany dormancy. The leaves of the current season were dry and dead but many of them were still clinging to the twigs when they were brought in. The buds on these cuttings did not develop repidly as was common for the willows treated later in the season. Very minute leaves did develop on most of the cuttings, but on some even the swelling of the buds was never noticeable. Soon after being set, the treated cuttings began to show signs of death, and this observation was substantiated by

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the fact that the roots which did form were very few and small, in contrast to the abundant root systems formed when the cuttings were taken later in the season.

All of the treated plants were dead when they were removed from the rooting medium at the end of the six and onehalf weeks period. Of the controls, a large percentage were still alive, and though their root systems were relatively small, in comparison with those formed on cuttings taken at other times, it seems reasonable to believe that practically all of them would have survived if they had been transplanted and kept in favorable conditions. These facts would appear to indicate some injurious effect due to the treatment. The same concentration of solutions at other times did not show any harmful effects, so it is doubtful that the solutions were too strong, unless the cutting might be considered much more sensitive to the addition of growth substance at the period when the plant was first becoming inactive. To apply the facts previously mentioned concerning the small quantity or lack of auxins in the dormant buds of many woody plants, it would seem that the added supply of synthetic growth substance should be of greater value then than at any other time of the year. Judging from the above data, however, this cannot be the case.

A smaller proportion of the controls survived at

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this time than at any other time during the winter months. Such results would imply that November is a very poor time to start willow cuttings. There would be no advantage in having started them this early, since their own slowness to develop roots would allow cuttings taken at a later time to surpass them before the time would come for transplanting to out-of-doors conditions. Just what physiological conditions are responsible for such slow development is a question which would involve many factors and be difficult to answer completely without very thorough analysis of the material at frequent intervals during these changes.

TAELE III

No. treated	Treatment	Dea not	ad; rcoted		ring; .used		ing; ted
		no.	0%	no.	0 <u>7</u> 0	no.	%
25	Auxilin A	20	80.C	0	0	5	20.0
50	Auxilin B	48	96.0	0	0	2	4.0
25	Hormodin A	22	88.0	0	0	3	12.0
50	Hormodin B	44	88.0	0	0	6	12.0
50	Control	34	68.0	7	14.0	9	18.0

ROOTING RESPONSE OF THUJA CUTTINGS TAKEN IN NOVEMBER*

* Cuttings were removed from the rooting medium after 14 weeks.

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Arbor vitae cuttings, taken from the trees in November, showed poorer rooting response when treated with the indole-butyric acid solutions than when untreated. Those which had rooted, especially the controls, had vigorous root systems and the tops were bright green and showing new growth at the tips, when the plants were removed from the rooting medium in February. Fourteen percent of the control plants had formed abundant callus at the cut surface, and several of these probably would have rooted if they had been allowed to remain for a longer time in the rooting medium. It is worthy of notice that none of the treated specimens which had failed to root, showed any indication of callus. The tops of all of these non-rooted, hormone-treated specimens, were dead when the records were taken.

The growth substance, again, seemed to show an injurious effect when used on Arbor vitae cuttings taken in the very early dormant season. While some of the controls rooted very slowly, the percentage of rooting was sufficiently higher than that on the treated cuttings, to be significantly in favor of the non-treated groups.

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TABLE IV

ROOTING RESPONSE OF SYRINGA CUTTINGS TAKEN IN NOVEMBER*

No. treated	Treatment		ead; rooted	Dea call	used .		ving; oted
		no.	°/0	no.	<i>6</i> /0	no.	0/0
50	Auxilin A	47	94.0	2	4.0	1	2.0
50	Auxilin B	46	92.0	4	٥.٥	0	0.0
25	Auxilin 20**	22	88.0	1	4.0	2	8.0
25	Auxilin 160**	22	88.0	1	4.0	2	٥.8
50	Control	47	94.0	3	6.0	0	0.0

* Records were taken at the end of fourteen weeks.
** Auxilin 20 represents a solution containing 20 mg.
per liter and Auxilin 160 contains 160 mg. per liter of
indole-butyric acid. These concentrations of the Auxilin
were not used in the other series.

In the lilac cuttings taken during November, rooting seemed very difficult, as it was during all of the dormant season. Here the untreated cuttings showed no rooting at all after fourteen weeks, while the variously treated groups showed from two to eight percent rooting. The number callused but not rooted was about the same in both the treated and control groups. In practically all cases these calluses were turning dark brown and softening and the cutting appeared to be dead. Therefore it is unlikely that a longer period in the rooting medium

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would have appreciably altered the percentage of rooting. These callused cuttings are not comparable to the callused arbor vitae cuttings mentioned in the preceding table, since in those the callus appeared healthy, and the plant still would have a chance of rooting successfully.

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ROOTING RESPONSE OF SALIX CUTTINGS TAKEN IN DECEMBER*

No. treated	Treatment	Dead; not rooted		Liv: roo	ing; ted
		no.	70	no.	¢'jo
10	Auxilin A	4	40.0	6	60.0
10	Auxilin B	3	30.0	7	70.0
10	Hormodin A	4	40.0	6	60.0
10	Hormodin B	2	20.0	క	80.0
10	Indole-but. A	3	30.0	7	70.0
10	Indole-but. B	5	50.0	5	50.0
20	Control	రో	40.0	12	60.0

* Records taken after cuttings had been in rooting medium 13¹/₂ weeks.

The samples represented in the above data are smaller than those involved in most of the other tables. The fact that the controls showed approximately the same amount of rooting as any of the treated cuttings would indicate a lack of noticeable effect from the treatment. This is in contrast to the results obtained on the November cuttings where the indole-butyric acid showed an injurious effect. Again, in contrast to the samples taken earlier in the dormant season (November) the December cuttings which rooted, all survived for more than three months and probably would have suffered no mortality in a longer time. The length of the roots on these successfully rooted specimens was short, even at the end of the thirteen and one-half weeks.

TABLE VI

ROOTING RESPONSE OF THUJA CUTTINGS TAKEN IN DECEMBER*

No. treated	Treatment	Dead; not rooted		Living; callused		Living; rooted	
		no.	%	no.	0%	no.	%
10	Auxilin A	3	30.0	3	30.0	4	40.0
10	Auxilin B	4	40.0	0	0 .0	6	60.0
10	Hormodin A	6	60.0	1	10.0	3	30.0
10	Hormodin B	6	60.0	2	20.0	2	20.0
10	Indole-but. A	9	90.0	0	0.0	1	10.0
10	Indole-but. B	6	60.0	0	0.0	4	40.0
20	Control	15	75.0	5	25.0	0	0.0

* Records taken at the end of 132 weeks.

For the cedar cuttings taken in December, the controls had formed no roots after thirteen and one-half weeks in the

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rooting medium, while some cuttings in each of the treated groups had rooted successfully. These percentages of successful rooting varied considerably in the different groups, and since each sample was rather small, the variations cannot be highly significant. Nevertheless, it might be noted that the highest percentage of rooting occurred where the strongest concentration of indolebutyric acid was used; namely, in the Auxilin B which represents 80 milligrams of indole-butyric acid per liter. The treatments with 40 milligrams per liter also show a better average of rooting response than the treatment with 20 milligrams per liter.

Twenty-five percent of the controls had formed callus when the cuttings were removed. Had all of these formed roots later, they still would not have brought the percentage of successful rooting as high as that for most of the treated groups. Since in three of the treated groups there was also a fair percentage of callus formation, one might expect a slight increase in the number of plants rooted, if they had been allowed to remain longer in the rooting medium.

The mortality rate was high, both in treated groups and control. To the propagator this would mean handling a large number of cuttings which would never make salable plants. For practical purposes, then, December could not

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be considered a good month to take cuttings of arbor vitae.

TABLE '	VII
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ROOTI	ROOTING RESPONSE OF <u>SYRINGA</u> CUTTINGS TAKEN IN DECEMBER*									
No. treated	Treatment	Dead; not rooted				Living; rooted				
-		no.	61 10	no.	¢; 13	no.	¢; 10			
10	Auxilin A	9	90.0	0	0.0	1	10.0			
10	Auxilin B	ర	٤0.0	2	20.0	0	0.0			
10	Hormodin A	9	90.0	1	10.0	0	0.0			
10	Hormodin B	9	90.0	1	10.0	0	0.0			
10	Indole-but. A	9	90.0	1	10.0	0	0.0			
10	Indole-but. B	8	80.0	2	20.0	0	0.0			
20	Control	20	100.0	0	0.0	0	0.0			

* Records taken at the end of 19 weeks.

Only one cutting in the lot taken in December showed any rooting. It was one treated with 20 mg. of indolebutyric acid per liter, in the form sold as Auxilin. The callus which formed in a small percentage of the other specimens had, in nearly all cases, become soft and showed no evidence of being able to root if given more time. The leaves on these cuttings developed to a small extent but soon became dry and dead, and, when the cuttings were removed, there remained no buds which showed any indication of life. ROOTING RESPONSE OF MALUS CUTTINGS TAKEN IN DECEMBER*

No. treated	Treatment	Dead; not rooted		Dea callu	
		no.	ojc	no.	0/0 /0
10	Auxilin A	0	0.0	10	100.0
10	Auxilin B	0	0.0	10	100.0
10	Hormodin A	2	20.0	ర	٥٥.0
10	Hormodin B	l	10.0	9	90.0
10	Indole-but. A.	0	0.0	10	100.0
10	Indole-but. B	1	5.0	9	90.0
20	Control	1	5.0	19	95.0

*Records taken after 19 weeks.

Nearly all of the apple outtings formed callus, but this had already softened and become dark-colored when the cuttings were removed from the sand-peat mixture. No roots formed on any of these cuttings and all of the cuttings were dead when the records were taken.

The work of Cooper (8) and others is in agreement with these results, for they too, found it impossible to root apple cuttings. In accordance with this fact apple stems are supposed to lack "rhizocaline" according to their theory. Whether this is sufficient explanation is questionable. In view of the work done it would appear reasonable that not the auxin supply, but the supply of

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some other substance or group of substances, may be the limiting factor in the failure of apple cuttings to root.

TABLE	I	Χ
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ROOTING RESPONSE	OF	<u>EALIX</u>	CUTTINGS	TAKEN	ΙN	JAHUAPY*
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No. treated	Treatment	Dead; not rooted			ving; oted
		no.	9/c	no.	0/0
20	Auxilin A	0	0.0	20	100.0
20	Auxilin B	0	0.0	20	100.0
20	Hormodin A	0	0.0	20	100.0
20	Hormodin B	0	0.0	20	100.0
20	Indole-but. A	0	C.O	20	100.0
20	Indole-but. B	0	0.0	20	100.0
20	Control	0	0.0	20	100.0

* Records taken at the end of $l\frac{1}{2}$ weeks.

In this series of cuttings rooting and survival was 100%. The roots developed a few days earlier in those which had been treated with any of the concentrations of the synthetic hormone solutions.

These plants were replaced in the rooting medium after the records were taken to determine what effect a longer time might have. Within about three weeks (after the treatment) the control plants had developed enough so that little or no difference was discernible between •

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quantity and length of roots on the controls and on the treated specimens. This would show little or no advantage in the use of auxins, for the few days' difference in speed of rooting would not be valuable and would be overcome as the plants were left longer.

Five cuttings from each treatment of this series were placed in water instead of the sand-peat mixture. This did not alter rooting percentages and therefore all of the results were brought into the one table.

No. treated	Treatment		ad; rooted		ving; oted
1		no.	%	no.	01 10
20	Auxilin A	14	70.0	6	30.0
20	Au xilin B	15	75.0	5	25.0
20	Hormodin A	15	75.0	5	25.0
20	Hormodin B	16	80.0	4	20.0
20	Indole-but. A	18	90.0	2	10.0
20	Indole-but. B	19	95.0	1	5.0
20	Control	19	95.0	1	5.0

TABLE X

ROOTING RESPONSE OF THUJA CUTTINGS TAKEN IN JANUARY*

* Records taken at the end of 9 weeks.

The arbor vitae cuttings taken in January showed a very low percentage of rooting. A small number (five

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from each treatment) were placed in a water medium, to facilitate observation of the time when roots first started.

In the January series no callus formation was noted on the non-rooted specimens when the records were taken. This is quite different from the results shown by the earlier series, where a fair percentage had formed callus. At this time of the season then, the rooting, if it were to take place at all, did occur rapidly. In January the plants out-of-doors have been exposed to many very cold weeks, and it is likely that the dormancy is easily broken in the arbor vitae when that has occurred.

ROOTING RESPONSE OF SYRINGA CUTTINGS TAKEN IN JANUARY.

In the same manner as was used for the willow and arbor vitae, a series of one hundred-forty lilac cuttings were treated with the different concentrations of the auxin solutions. One group of twenty cuttings was kept as controls. From both the treated and control groups there was no rooting development after ten weeks. Neither was there any noticeable callus formation on any of the samples. The buds on the cuttings developed somewhat, but the leaves which were formed, soon dried, in spite of sufficient quantities of water being present

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in the soil at all times. At this time of the year, the cuttings need something more than just added growth substance if any roots at all are to be formed.

ROOTING RESPONSE OF MALUS CUTTINGS TAKEN IN JANUARY.

One hundred-forty apple cuttings were taken in January and divided into groups for the various treatment and the control in the same manner as used for cedar in table X. At the end of ten weeks, when the records were taken not a single cutting had formed any rocts. Again there was callus on nearly every one, but this had become dark and soft and the cutting was dead. The buds had made only a very slight development when the cuttings were first set in the rooting medium.

With apple, in January as well as all other times in the dormant season, it was impossible to induce rooting by the addition of indole-butyric acid solutions.

ROOTING RESPONSE OF SALIX CUTTINGS TAKEN IN FEBRUARY

Groups of ten willow cuttings were treated with each of the six solutions used in January (see table IX). At the end of two weeks in the treated and the control lots every cutting had formed roots. Like the January cuttings.

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those which were treated developed roots a few days earlier, but this advantage was soon overcome.

ROOTING RESPONSE OF THUJA, MALUS, AND SYRINGA CUTTINGS TAKEN IN FEBRUARY

The time in February when the cuttings were taken had been preceded by a period of a few days warm rain. Each series of seventy cuttings was divided into groups of ten, and these groups treated with the different concentrations of auxin mentioned in table IX for the treatment of willow. Records were taken at the end of twelve weeks. At that time all of the cuttings were dead and no roots had formed on any of them. This indicates an inability of any of these three species to root if cut in February.

TABLE XI

No. treated	Treatment	Dead; not rooted		Living; rooted	
		no.	%	no.	%
50	Auxilin A	46	92.0	4	క.0
50	Hormodin A	46	92.0	4	క.0
50	Indole-but. A	47	94.0	3	6.0
50	Control	46	92.0	4	క.0

ROOTING RESPONSE OF THUJA CUTTINGS TAKEN IN EARLY APPIL*

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*Records taken after 15 weeks.

These cuttings represent a group taken very soon after the dormant season was over. The mortality was very high on this group. The tops began to turn brown within a few weeks and when the records were taken they were completely dead. Callus was not noticeable on these dead cuttings, and it is doubtful if they remained alive long enough for any such development to occur. If the auxin supply is the limiting factor in the rooting of arbor vitae, and the supply of natural auxin is more abundant in the spring than at any other time, then this series should have rooted better than those taken in December and January. The conclusion from such an experiment might be that the arbor vitae lacks some other substance which would be conducive to rooting when taken in the winter and early spring.

GENERAL CONCLUSIONS

On the whole, the treatment of cuttings of apple, lilac, arbor vitae, and willow with solutions of indole-butyric acid in an attempt to induce rocting during the dormant season, seemed quite unsuccessful. In a few cases slight differences could be observed, but

these differences were so small that they would be of little value in suggesting some cause for the failure of such cuttings to root during the winter months.

Willow cuttings taken very early in the dormant season were very difficult to root, and the synthetic hormone used seemed to have an injurious effect under the conditions of these experiments. It appeared to inhibit root formation and favor a higher mortality rate among the rooted plants. Rooting occurred in a larger percentage of the cuttings taken later in the winter, and the toxic effect of the indole-butyric acid disappeared. In the January and February groups, where all of the cuttings developed roots, the treatments made roots form more rapidly and more abundantly. On remaining about three weeks longer in the rooting media, however, the controls surpassed the treated plants in number, size, and vigor of roots. (See figures 5 and 6.) The small amount of time gained by the use of the hormone solutions in these cases would probably be of negligible value. Similar results showing that the later rooting tends to minimize the effects brought about by treatment, have been shown also by the experiments of other workers (11).

Arbor vitae cuttings taken at any time during the dormant season give very poor rooting response. On the large samples taken during the early part of the 1938-

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1939 dormant season, the percentage of cuttings which formed roots was smaller in the treated sets than in the control sets. While this difference was not great, it was consistent for all treatments and would indicate an injurious effect due to soaking the bases of the cuttings in the indole-butyric acid solutions. Through December and January, cuttings taken at any time showed some benefit from being treated with the synthetic hormone solutions. However the mortality of both treated and untreated groups of cuttings taken during these months was very high. For this reason anyone interested in propagation of large numbers of plants would find the slight advantage due to the treatment of questionable value. Cuttings which were taken at the latter part of the dormant season (February) gave no indication of rooting. If some substance, or group of substances is responsible for the successful rooting at other times, then this substance must have been lacking during the latter part of the dormant season. From the data of these experiments, the evidence indicates that auxin supply may be insufficient in arbor vitae, but that this may not be the only limiting factor. What these other factors may be is not known at the present time.

Lilac cuttings taken at any time during the dormant season showed negligible rooting response. Lilac is con-

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sidered very difficult to root at any time, and for this reason, that type of propagation is little practiced. Callus forms in many cases, but does not indicate that roots will necessarily follow.

Apples, according to the results of this study, would be placed among the plants which are impossible to propagate from cuttings during the dormant season. In practically all cases, callus formed but soon became dark and soft, and no roots developed.

Whether the poor rooting results, particularly with lilac and apple, may be due to a lack of some substance in the cutting itself is a question under debate at the present time, there seems to be some evidence to support such a theory, and it might help to explain why rooting is usually much more successful on cuttings taken during the spring and early summer, for such a substance would probably be more abundant during the seasons of more rapid growth.

There was a small amount of bud inhibition on the treated samples of some of the sets. On cuttings taken during the later part of the dormant season the buds, particularly on lilac, developed very rapidly. This would mean that the dormancy was readily broken at that time. The rapid transpiration caused by this relatively large leaf area undoubtedly had some effect on the

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mortality rate, but the soil was kept moist at all times so that the cuttings could take in water to the full extent of their own ability. That some other factor was of greater importance in their failure to root seems conclusive.

Since all of these cuttings were taken from mature plants, there might be some explanation of their failure to root, according to the work of Thimann and Delisle (22). Their work on difficult plants shows that better rooting results from using the cuttings taken from very young trees. They also have found some correlation between the position of the cutting on the parent plant and its ability to root. These results favor the cuttings taken at the basal part of the tree, and the cuttings used in the experiments described in this paper were from the basal parts of the plants.

SUMMARY

1. Cuttings of willow, arbor vitae, lilac, and apple were made at approximately three week periods during the dormant season. These were treated with indolebutyric acid solutions of different concentrations.

2. During the early part of the dormant season (November) treatment with indole-butyric acid showed an injurious effect on willow and cedar.

-31-

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3. Treatment of willow cuttings later in the dormant season caused more rapid rooting response. This effect was overcome when the cuttings were left about three to five weeks in the rooting medium.

4. Arbor vitae cuttings showed very low percentage of rooting at any time in the dormant season. There is some evidence that treatment aided root formation in the low degree that it occurred.

5. Lilac cuttings were not induced to form roots in any appreciable number of cases. Cuttings taken late in the dormant season leafed out very rapidly, but died without having formed either callus or roots.

6. Apple cuttings formed no roots at any time in the dormant season. Callus was usually formed, but soon became soft and dark colored.

7. Treatment with indole-butyric acid solutions cannot be recommended as of any practical value in aiding root development on cuttings taken during the dormant season in the species of plants used in this series of experiments.

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EXPLANATIONS OF FIGURES

- Figure 1. Salix cuttings (January) showing roots at the end of eleven days. Group on the left was treated with Auxilin, 40 mg. per liter. Group on the right was treated with Hormodin, 20 mg. per liter.
- Figure 2. <u>Salix</u> cuttings (January) showing roots at the end of eleven days. Group on the left was treated with indole-butyric acid, 20 mg. per liter. Group on the right was untreated.
- Figure 3. <u>Salix</u> cuttings (January) showing roots at the end of eleven days. The group on the left was treated with Auxilin, 80 mg. per liter. The group on the right was treated with Hormodin, 40 mg. per liter.
- Figure 4. <u>Salix</u> cuttings (January) showing roots at the end of eleven days. The group on the left was treated with pure indole-butyric acid, 40 mg. per liter. The group on the right was untreated.
- Figure 5. <u>Salix</u> cuttings (January) showing roots at the end of five weeks. The first group on the left was treated with Auxilin, 40 mg. per liter. The second group was treated with Hormodin, 20 mg. per liter. The third group was treated with pure indole-butyric acid, 20 mg. per liter. The group on the right was untreated.
- Figure 6. <u>Salix</u> cuttings (January) showing roots at the end of five weeks. The first group on the left was treated with Auxilin, 80 mg. per liter. The second group was treated with Hormodin, 40 mg. per liter. The third group was treated with pure indole-butyric acid, 40 mg. per liter. The group on the right was untreated.
- Figure 7. <u>Thuja</u> cuttings (December) showing roots sixteen weeks after Auxilin treatment (80 mg. per liter). See Table VI.

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- Figure 8. <u>Thuja</u> cuttings (December) showing roots sixteen weeks after indole-butyric acid treatment (40 mg. per liter). See Table VI.
- Figure 9. <u>Thuja</u> cuttings (December) showing callus but no roots. These were untreated and had been in the rooting medium sixteen weeks. See Table VI.

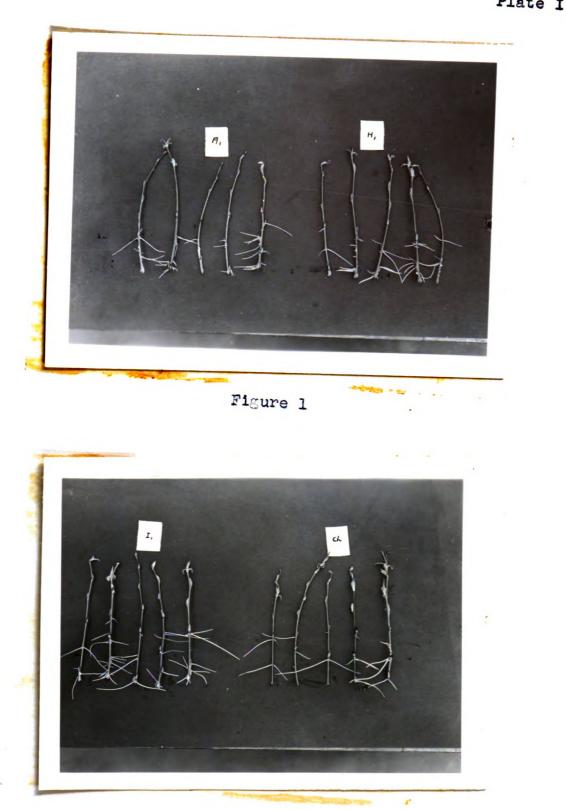


Figure 2

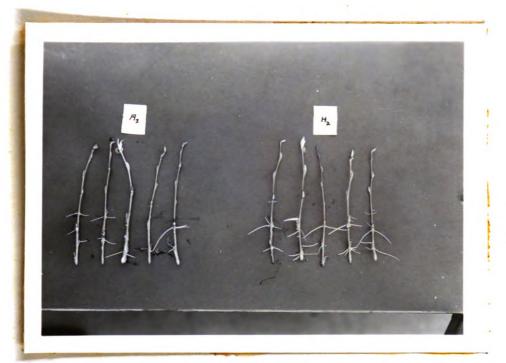


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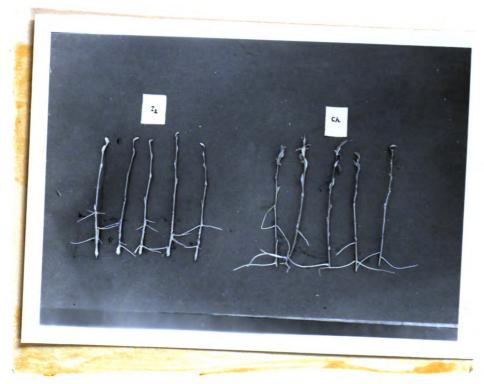


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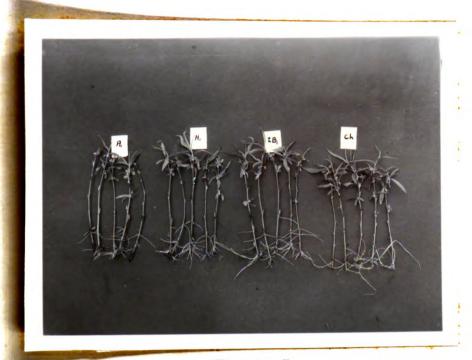
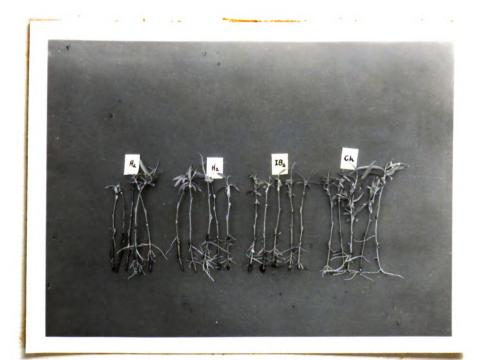


Figure 5



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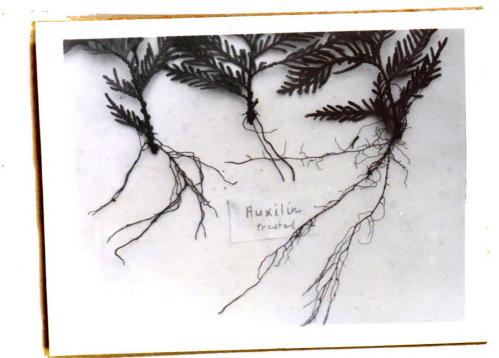


Figure 7

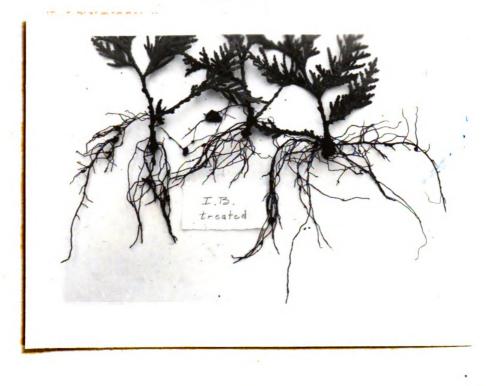


Figure 8



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