HORMONES AND ROOT FORMATION

Thesis by

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"The role of auxins in root formation is a good example of a piece of research in pure physiology which has an immediate practical application."

--Went and Thimann, Phytohormones, 1937.

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I. INTRODUCTION

A. Development of the Root-forming Hormone Concept

The role of internal factors involved in root formation has been recognized since early times. In his treatise on agriculture, Agricola (1716) states that there must be in branches and twigs which initiate roots a quantity of the proper matter to induce roots. The phenomenon of root formation was studied in greater detail by Duhamel (1758) who conceived of a sap being elaborated in the leaves which, after passing downward through the cortex, was used in the nutrition of the roots. In 1880 Sachs set forth a modern version of Duhamel's views and postulated the existence of a root-forming hormone which is formed in minute amounts in the leaves and moves toward the base of stems. Root formation on cuttings was accordingly explained by the accumulation of these hormones near the basal cut surface.

After the time of Sachs, most of the studies on root formation placed emphasis on nutritional factors. A high carbohydrate content increased the tendency to regenerate roots (Goebel, 1903; Curtis, 1918; Reid, 1924). Bouillenne and Went (1933) showed clearly that the production of roots (or any other growth) by etiolated cuttings of Impatiens cuttings, deprived of food reserves in the seed, requires carbohydrate. They pointed out, however, that green plant parts, producing or containing carbohydrates, are usually not dependent on carbohydrate. That extensive changes in composition alone will tend to induce regeneration does not seem to follow,

for McCallum (1905) could induce no such regeneration in his attempts to cause such changes in composition. He concluded that nutrient conditions are not the principal factors governing root formation.

In his experiments on correlations in Bryophyllum, Jacques Loeb (1917) revived Sachs' old hormone theory and suggested that root formation in Bryophyllum is controlled by a root-forming hormone. He subsequently changed his views, however, and emphasized the more general factors of nutrition instead of hormones.

The hormone hypothesis was again revived by van der Lek in 1925. He found that root formation in cuttings of Salix, Populus, Ribes, and Vitis was largely dependent upon the existence of buds, especially strongly sprouting ones. Roots were produced by cuttings with all buds removed, but they were less abundant and smaller than in cuttings with their buds present. From these results van der Lek assumed that hormones were formed in sprouting buds and were transported basepetally to stimulate root formation. Where abundant root formation occurred in cuttings with excised buds, he was able to demonstrate the existence of root primordia, which were likely to develop without hormones.

Success in obtaining an active root-forming substance from leaves was first achieved by Went (1929). He found that substances which were active in inducing roots on Acalypha cuttings diffused out of leaves of Acalypha and Carica papaya, when placed with their petioles in water. Since sugar solution was found to have no root-forming effect, the action was not one of nutrition. Went named this root-forming substance "rhizocaline".

Later Thimann and Went (1934) found that tests on a number of pollens and other natural products showed that the rhizocaline occurred almost always together with auxin. They used etiolated pea cuttings as test objects for rhizocaline and employed the Avena technique (Went, 1928) for the determination of auxin. However, there was not always a very good quantitative parallelism between root-forming and growth-promoting activity. Nevertheless, the extract of Rhizopus medium, rich in auxin, was also rich in root-forming hormone. They found that the root-forming substance was extractable by organic solvents only from acid solutions. The distribution among different solvents was the same as that of auxin. The activity was readily destroyed by oxidizing agents and followed that of auxin throughout the various stages of purification. Finally Thimann and Koepfli(1935) and Kögl (1935) found that synthetic indole-3-acetic acid (hetero-auxin) and auxin b were as active in root formation as the purest Rhizopus preparation. This provided final proof that at least one of the hormones causing root formation was identical with auxin. The fact still remains, however, that the root-forming and growth-promoting activities of various natural preparations were not quantitatively parallel. The explanation for this may lie in the influence of substances other than auxin, necessary for root formation.

B. Object of the Present Work

The primary object of the present study was to investigate the processes by which indole-3-acetic acid is able to bring about root formation on cuttings. The problem was studied by investigating the following:

1. Response of cuttings of a number of different plants to treatments with indole-3-acetic acid.
2. Transport of indole-3-acetic acid in cuttings.
3. The effect of excising the treated base of the cutting and subsequently treating the new base.
4. The correlation of leaves with root formation on treated cuttings.
5. Extraction of root-forming substances other than auxin from leaves.

II. GENERAL REMARKS ON MATERIALS AND METHODS

Eureka lemon (Citrus lemonia) stem cuttings were used as test plants in most of these experiments. The cuttings, about 12 cm. long with two full-sized leaves at the apex, were carefully selected from mature terminal growth. All experiments between December, 1934 and September, 1936 were made with cuttings taken from a lemon grove in Claremont, California, referred to as grove A. Experiments between September, 1936 and May, 1937 were made with cuttings from a grove in Sierra Madre, California (grove B), and those made after May, 1937 came from Pomona, California (grove C).

Other species of plants used in these experiments consisted of:

Camellia japonica

Dianthus Caryophyllus (carnation)*

Ilex cornuta (holly)

Chrysanthemum hortorum

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* Common names will be used throughout this paper in referring to the species. Where no common name is given, only the generic name will be used.

Acalypha hispida

Calothamnus

Rosa multiflora (rose)

Pelargonium zonale (geranium)

Macadamia ternifolia (Queensland nut)

Mimosa pudica (sensitive plant)

Hedera helix (English ivy)

Oenethera

Lantana lilacina

Coleus Blumei var. verschaffeltii

Pyrus malus (apple)

Impatiens Balsamina

Citrus nobilis var. unshiu (Satsuma orange)

Leafy cuttings were used in all instances, and they were made up according to the standard methods for these species as described by Bailey (1935).

The variation in response within a given sample was for the most part small. In some instances individual root counts are presented, and in all cases the standard error is given.

Synthetic indole-3-acetic acid from Merck and Co. was used in all experiments except in a few of the earlier ones in which a product made by the chemistry department of the California Institute of Technology was used. The method of application of the hormone will be described in connection with the presentation of results.

The morphological base of all cuttings was inserted about 3 cm. deep in sand in sash-covered propagating frames. Unless noted otherwise, the sand was heated with #19 G.E. lead-covered nichrome heating wire, and the

temperature was thermostatically controlled at near 30°C. The propagating facilities of the U. S. Department of Agriculture Field Station at Torrey Pines, California, were used for all experiments between September, 1935 and October, 1936, and those at the California Institute of Technology at all other times.

III. EFFECT OF INDOLE-3-ACETIC ACID ON ROOT FORMATION

A. Lanoline Paste Method

Laibach (1933) found that Tradescantia internodes were induced to form roots by the application urine and orchid pollen to the tip of the cuttings in the form of lanoline paste. The effect was doubtless due to the auxin contained in the urine and pollen. Went (1934) mixed indole-3-acetic acid with lanoline and found the resulting paste quite effective in inducing roots on pea cuttings. Accordingly, the lanoline technique was used in the present experiments in the first attempts at rooting cuttings of commercially important plants.

The mixture used consisted of 1 part indole-3-acetic acid to 2000 parts pure lanoline. A small portion of this paste, roughly about 10 mg., was smeared on a small area of one side of the cutting near the top, which had previously been scraped to remove the epidermis and the outer cortical layers. The paste was left on the cutting throughout the experiment.

A summary of the results obtained by the lanoline paste method is found in table I. Apple cuttings failed to respond to the treatment. Results with lemon cuttings were variable. In one experiment treated

TABLE I

Effect of Mixture of Indole-3-Acetic Acid and Lanolin on Root Formation

Species	Date set in sand	No. of cuttings treated	Time in sand	Average number of roots per cutting		
				Indole-3-acetic acid-lanolin	Pure lanolin	Difference
			weeks			
Eureka lemon	4/4/35	10	3	1.4 ± 0.6	0.3 ± 0.2	1.1 ± 0.6
	4/4/35	10	4	6.0 ± 0.8	1.9 ± 0.5	4.1 ± 0.9
	5/28/35	10	5	5.2 ± 1.0	2.4 ± 0.6	2.8 ± 1.2
	9/3/35	10	6	4.8 ± 0.5	2.5 ± 0.9	2.3 ± 1.0
	9/19/35	10	6	4.0 ± 0.8	4.3 ± 0.6	0.3 ± 1.0
Acalypha	3/19/35	5	1	70.4 ± 9.0	11.0 ± 0.6	59.4 ± 9.0
Lantana	3/19/35	10	4	25.9 ± 9.0	3.5 ± 1.7	22.4 ± 9.2
Impatiens	12/4/35	10	$3\frac{1}{2}$	11.1 ± 1.7	7.5 ± 1.4	3.6 ± 2.2
Coleus	12/4/35	10	$3\frac{1}{2}$	46.1 ± 6.8	23.3 ± 5.2	22.8 ± 8.6
Chrysanthemum*	6/4/36	10	2	6.2 ± 1.6	1.9 ± 0.8	4.1 ± 1.8
Delicious apple	1/2/35	1000	3	0	0	0
			6	0	0	0
			10	0	0	0

* Variety, Mary Pickford

Figure 1. Root formation on lemon cuttings. Upper row, treated near apex with lanoline; lower row, treated near apex with mixture of lanoline and indole-3-acetic acid. Photographed 4 weeks after treatment.

Figure 2. Root formation on Lantana cuttings. Upper row, treated near apex with pure lanoline; lower row, treated near apex with mixture of lanoline and indole-3-acetic acid. Photographed 5 weeks after treatment.

Figure 3. Lemon cuttings showing roots appearing at the base of cuttings and at the point of application of the mixture of lanoline and indole-3-acetic acid. Photographed 4 weeks after treatment.

B. Water Solution Method

1. Comparison with Lanoline Paste Method

In January, 1936 it was found that a high concentration of indole-3-acetic acid in water solution is very effective in inducing roots on lemon cuttings when applied at the base of the cuttings. The method was found to give results far superior to those of the lanoline paste method. In the January, 1936 experiment 3.7 ± 0.9 roots per cutting were obtained on the tap water controls, 3.1 ± 0.9 on the cuttings treated by the lanoline method, and 19.7 ± 2.8 on cuttings treated at the base for 24 hours with a 0.50 mg. per cc. water solution of indole-3-acetic acid. The root count was made 6 weeks after the treatment. Pictures of another lemon experiment similar to the above are shown in figure 4. The method also was found to be equally effective on Chrysanthemum cuttings (figure 5).

At about the same time Hitchcock and Zimmerman (1936) independently discovered that the water solution method was superior to the lanoline technique. They found that basal applications of water solutions of indole-3-acetic acid were quite effective in inducing roots on cuttings of Ilex, Taxus, Hibiscus, and Pachysandra.

The water solution method as used in the experiments described in the present paper was as follows: The basal ends of the cuttings were placed in beakers containing the minimum amount of the test solutions required to cover about 1 cm. of the stem. They were left in a nearly saturated atmosphere of the sash-covered propagating frames for 6 to 40 hours, as mentioned specifically for each experiment. On removal from the test solution, the cuttings were rinsed with tap water and the bases were inserted about 3 cm. deep in sand in the sash-covered frames. Root counts were usually made at intervals of three and six weeks after the treating period.

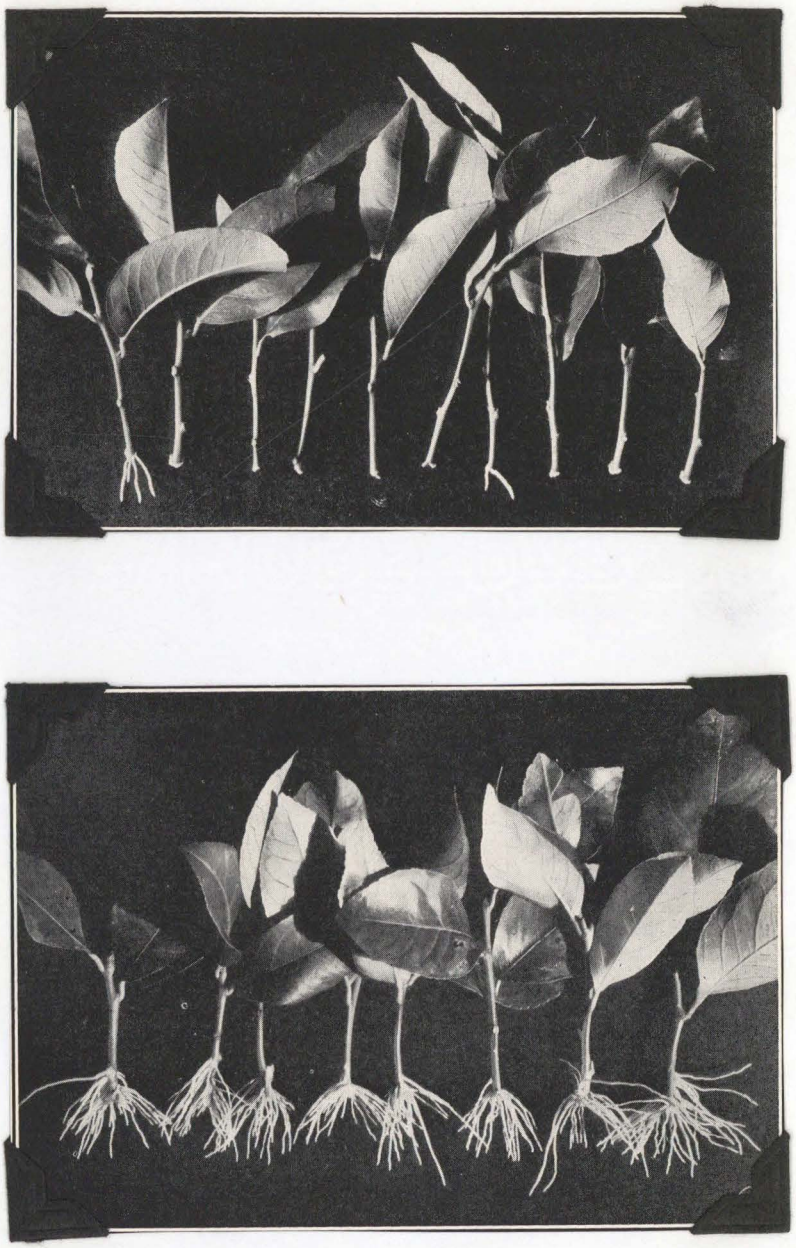

Figure 4. Root formation on lemon cuttings. Upper row treated at base with tap water for 8 hours; lower row, treated at base with 0.50 mg. per cc. water solution of indole-3-acetic acid for 8 hours. Photographed 17 days after treatment.

Figure 5. Root formation on Chrysanthemum cuttings. Upper row, treated at the base with tap water for 15 hours; middle row, treated near apex with a mixture of indole-3-acetic acid and lanoline; lower row, treated at base for 15 hours with a water solution of indole-3-acetic acid (0.10 mg. per cc.) Photographed 2 weeks after treatment.

2. Relation of Concentration of Solution to Number of Roots Formed

Results given in figure 6 for leafy lemon cuttings show clearly that for 10 and 20-hour treatments only the solutions of 0.20 mg. per cc., or higher, were effective in increasing significantly the number of roots as compared with tap water controls. Forty-hour treatments on the other hand showed a significant increase in root formation for the 0.10 solution.

Treatments with solutions stronger than 0.20 mg. per cc. are of doubtful value. Not only are the increases in number of roots of no statistical significance, but also the treatment is quite likely to cause injury to the base of the cutting. The 0.20 mg. per cc. solution can, therefore, be considered as the best solution for root formation on lemon cuttings.

Of the three treating periods tested for the 0.20 solution, the 20-hour period gave the best results. Severe injury at the bases of the cuttings resulted from a 40-hour treatment, while a 10-hour period gave only about half as many roots as twenty hours.

3. Histological Investigation of the Treated Base

Histological examination of the base of lemon cuttings treated for 20 hours with the 0.20 solution showed a band of meristematic cells, apparently resulting from a proliferation of the cambium, appearing between the phloem and xylem within two days after the treatment. This region usually developed into a band of about 25 cells within six days. At that time numerous root primordia were observed in this proliferated region. It was not determined from just what tissue the roots were differentiated in untreated cuttings. Eames and MacDaniels (1925) state that "adventitious

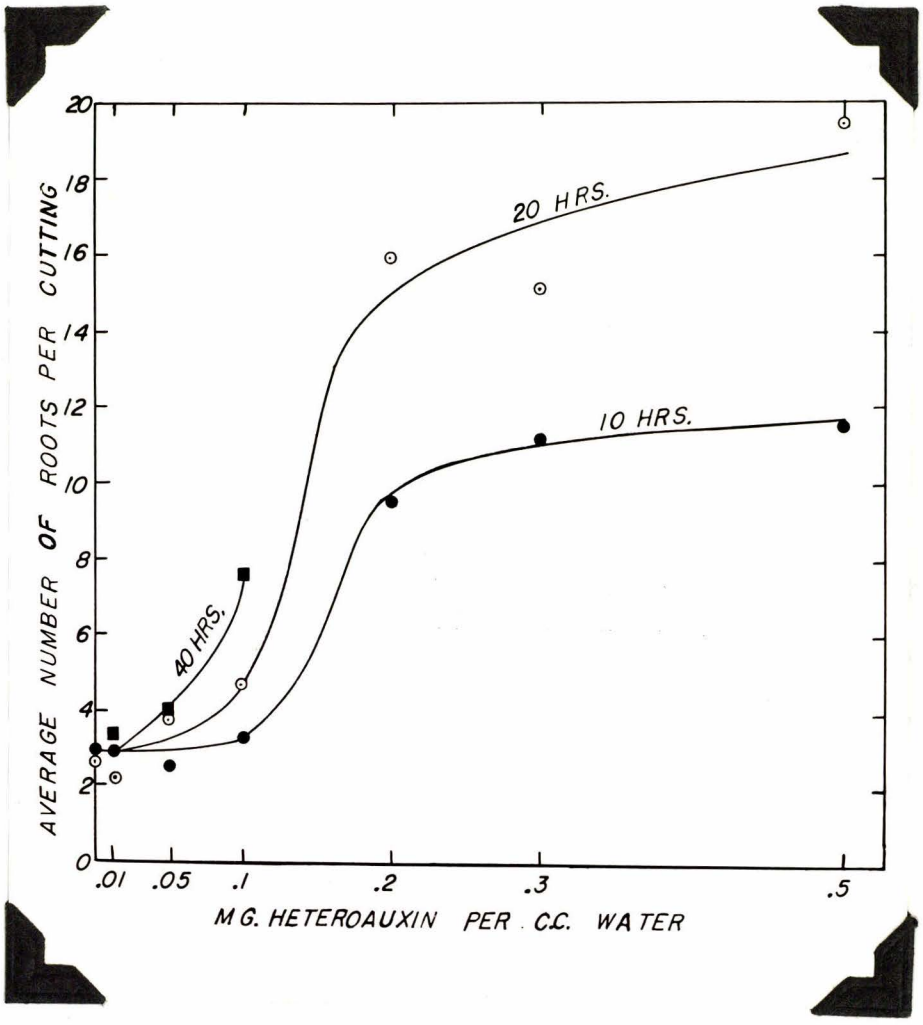

Figure 6. Relation of concentration of indole-3-acetic acid (heteroauxin) solution to number of roots formed on leafy lemon cuttings.

roots develop by the formation of apical root meristems in the pericycle of stems and roots". If this is the normal region of root initiation in the lemon, it follows that the basal application of a strong indole-3-acetic acid solution causes root formation in tissues other than those in which they normally occur. This question, however, requires further investigation. The results presented by the writer are of interest mainly in showing that in treated woody stems a region of proliferated cells develops between the xylem and phloem and that the root primordia occur in this region.

Some few roots usually pushed their way out through the bark by the 8th or 9th day after the treatment, but most of the roots usually appeared between the 10th and 20th days. On treated leafless cuttings there was usually no increase in number of visible roots after 20 days, though histological examinations showed many root primordia. Leafy cuttings, on the other hand, always showed an increase in number of visible roots after 20 days. The significance of these observations will be discussed later in this paper.

C. Practical Applications

1. General Considerations

The fact that high concentrations of indole-3-acetic acid, when applied at the base of cuttings, causes root formation has been used successfully in the propagation of many plants. The writer has found that lemon, Chrysanthemum, holly, geranium, carnation, Camellia, English ivy, rose, Macadamia, Calothamnus, Oenothera, and Mimosa respond favorably to the treatment. Hitchcock and Zimmerman (1936) and Zimmerman (1937) have extended this list considerably. They have found that Taxus, Hibiscus, Pachysandra, Japanese maple, Azalea, blueberry, Clematis, dogwood, hazelnut,

Daphne, Magnolia, elm, and crab apple respond to the treatment with indole-3-acetic acid. They also found that indole-3-butyric acid and naphthelene acetic acid were just as effective as indole-3-acetic acid, or more so in some cases. The writer has found very little difference in the response of lemon cuttings to the different compounds.

In addition to the above, the promotion of root formation by indole-3-acetic acid has been studied on cuttings of Diervilla rosea, Deutzia scabra, and Viburnum carlesii by Tincker (1936); alfalfa by Burton (1936); Carica papaya by Traub and Marshall (1937); tung oil tree by Yin (1937); and Ceanothus, Amorpha occidentalis, Calycanthus occidentalis, Myrica californica, mulberry, liliac, and Gardenia by Warner*. A good response was obtained by the treatment in all cases.

In general, all of these workers reported that the highest non-toxic concentration gave the best results. This concentration varied for the different plants and was lowest for softwood cuttings. A treatment with 0.20 mg. per cc. for 20 hours gave best results on the lemon, holly, Macadamia, and Calothammus--all hardwood cuttings--while softwood Chrysanthemum and geranium cuttings responded best to a 10 to 15-hour treatment with a 0.10 mg. per cc. solution. It is recommended that, before large-scale applications are made, the toxic limit for each species be ascertained.

Although a rather wide variety of species responds to the treatment, there are still a great many plants, such as the commercial varieties of apples, Satsuma orange, pecans, and walnuts, which do not respond. In most cases cuttings of these plants never show any evidence of root formation under any conditions. A possible explanation for this lack of response is given later in this paper under the heading of "The Apple Cutting Problem".

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* Unpublished data obtained in the Plant Physiology Laboratory of the California Institute of Technology 1937.

Some of the plants which have been found to respond favorably to the treatment are ordinarily propagated by cuttings, but the quicker rooting, the greater percentage of plants taking root, and the more extensive root system obtained by indole-3-acetic acid make the treatment valuable in the commercial propagation of these plants.

Many of the plants that respond favorably to the treatment are not ordinarily propagated by cuttings but by budding or grafting onto a seedling rootstock. During the last 15 years, attention has been focused on the great importance of the character of the rootstock used in horticultural propagations. It has become increasingly evident that the genetic variation in seedlings used as stocks is to be considered responsible for much of the variation in tree size and production exhibited in orchards. Therefore, in the propagation of economic plants, particularly of orchard fruits, there is great need for available rootstocks of the same genetic composition which will react similarly under the same environmental conditions with a given scion variety. The favorable response of cuttings of many of these plants to the indole-3-acetic acid treatment suggests that this method may be useful in obtaining the desired uniform rootstocks.

2. Lemon Experiments

The most comprehensive information obtained by the writer on the effects of indole-3-acetic acid on root formation has been on lemon cuttings. It is seen from table II that a large increase in number of roots as compared with controls was obtained on leafy lemon cuttings during every month of the year. The increase was significant in every instance and, for the 15 different experiments, the increase averaged 17.1 roots per cutting. The data should not be used as an exact indication of the monthly variation in

TABLE II

Effect of Water Solutions of Indole-3-Acetic Acid on Root Formation of Leafy Eureka Lemon Cuttings at Different Times of the Year

Date	Grove	Conc. of indole-3-acetic acid solution	Length of treatment	Time in sand	Average number of roots per cutting(1)		
					Treated	Control	Difference
		mg. per cc.	hours	weeks			
Jan 1936	A	0.5	24	6	19.7 ± 2.8	3.7 ± 0.9	16.0 ± 2.9
March 1936	A	0.5	24	3	20.4 ± 3.7	1.5 ± 0.5	18.9 ± 3.7
				6	31.3 ± 3.9	4.6 ± 0.5	26.7 ± 3.9
April 1936	A	0.5	8	3	20.6 ± 2.2	1.1 ± 0.6	19.5 ± 2.3
				6	21.9 ± 2.1	3.9 ± 0.5	18.0 ± 2.2
July 1936	A	0.4	13	5	17.6 ± 2.5	2.3 ± 0.6	15.3 ± 2.5
Aug 1936	A	0.25	20	3	15.0 ± 1.3	2.0 ± 0.6	13.0 ± 1.4
Sept 1936	A	0.25	20	3	28.5 ± 2.1	2.5 ± 0.4	26.0 ± 2.1
Feb 1937(2)	B	0.20	20	4	6.0 ± 1.2	0	6.0 ± 1.2
March 1937	B	0.20	20	3	16.0 ± 2.8	2.7 ± 0.5	13.3 ± 2.8
April 1937	B	0.20	20	3	26.6 ± 3.2	2.7 ± 0.5	23.9 ± 3.2
May 1937	B	0.20	20	3	12.6 ± 1.0	0.9 ± 0.3	11.7 ± 1.0
May 1937	B	0.20	20	3	15.4 ± 2.6	1.9 ± 0.3	13.5 ± 2.6
June 1937	C	0.20	20	3	18.2 ± 1.1	0	18.2 ± 1.1
July 1937	C	0.20	20	3	16.7 ± 1.0	0.7 ± 0.3	16.0 ± 1.0

(1) Ten cuttings in each experiment.

(2) Following a period of cold weather.

root-forming activity because the cuttings of the different experiments were taken from three different groves. Moreover, different strengths of solutions were used in some of the experiments; however, all solutions contained at least 0.20 mg. of indole-3-acetic acid per cc., which is the threshold value for effective root formation. The results are of chief value in showing that the method of basal application of strong indole-3-acetic acid solutions is effective on lemon cuttings at any time of the year.

The results presented in table II show that usually untreated lemon cuttings will initiate 3 or 4 roots. This has long been knows and has been reported by Coit (1917), Swingle, Robinson, and May (1929), and Halma (1931). Experiments conducted by the writer, however, show that subsequent growth on such cuttings is much less than on hormone-treated cuttings, at least during the first eight months after planting in the nursery. In the March, 1936 experiment both the treated and untreated cuttings were transplanted to the nursery after six weeks in the propagating frames. At the time of transplanting (May 7) the control cuttings had an average of 4.6 roots and the treated ones, 31.3 roots per cutting. On July 16 the new shoot growth was 16.5 cm. per cutting on the treated lot as compared to 6.0 for the controls (figure 7). The treated cuttings continued to grow at a slightly faster rate than the controls up to December 27 when the treated cuttings had an average shoot growth of 69 cm. and the controls, 36 cm. Shortly after this date the plants were injured by cold weather, and the measurements were discontinued. A picture of a sample of the treated and control plants on August 14 is shown in figure 8. Figure 9 shows a picture of two of the treated plants on December 27, seven months and 20 days after transplanting from the frames, or nine months and ten days after taking the cuttings from the tree.

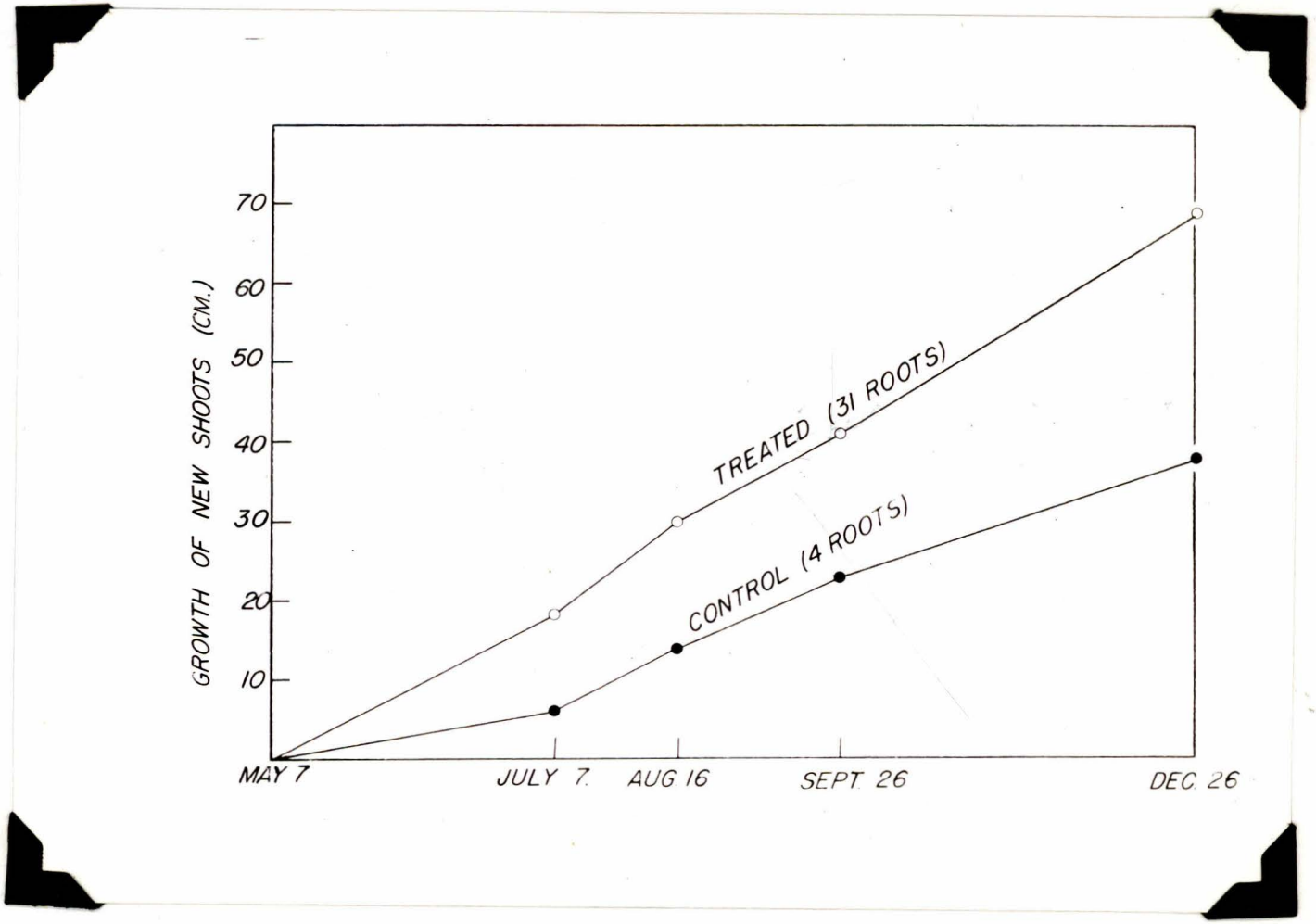

Figure 7. New shoot growth on treated and control lemon cuttings after transplanting to the nursery. See text for details of experiment.

Figure 8. Lemon cuttings 13 weeks after transplanting to nursery. Right side, hormone treated; left, tap water controls.

Figure 9. Hormone treated lemon cuttings 31 weeks after transplanting to nursery.

The usual method of commercial propagation of the lemon is by budding onto a seedling rootstock. It is quite likely, however, that less time will be required by the cutting method to grow a tree of suitable size for planting in the orchard because the cuttings will grow uninterruptedly in the nursery until they are set in the orchard, while the growth of the budded seedling is checked when the top is cut off to force the bud into growth. There is some doubt in the case of the lemon as to whether more uniform trees can be obtained by the cutting method than by the seedling rootstock method because of a high percentage of apogamy occurring in the strains of Citrus used as seedling rootstocks for the lemon. These apogamic seedlings are supposedly of uniform genetic constitution and do not exhibit genetic variations such as are observed in sexually produced embryos. The elimination of the variants from a batch of nursery seedlings usually removes most of the seedlings that have developed from sexually produced embryos.

3. Chrysanthemum Experiments

Chrysanthemums responded well to treatments with indole-3-acetic acid. Out of nearly fifty varieties tested, only one failed to show increased rooting over untreated cuttings. The results for sixteen different varieties are shown in table III. The results on the first four varieties were obtained on leafy cuttings in the propagating frames at the California Institute of Technology. It is seen that in all four cases the response to the hormone treatment was great.

The data for the other varieties shown in table III were obtained from experiments conducted in a commercial greenhouse in Pasadena. The cuttings were treated in the usual commercial manner in every respect

TABLE III

Effect of Water Solutions of Indole-3-Acetic Acid on Root Formation on Cuttings of Different Varieties of Chrysanthemum

(0.10 mg. per cc. solution applied at base in all instances*)

Variety	Date	Number treated	Length of treatment	Time in sand	Average number of roots per cutting		
					Treated	Control	Difference
			hours	weeks			
Mary Pickford	June 1936	10	24	2	87.0 ± 9.9	1.9 ± 0.8	85.1 ± 9.9
Yellow Pocket	Apr 1937	10	15	3	44.0 ± 3.7	0	44.0 ± 3.7
Meta Bergen	Apr 1937	10	15	3	34.3 ± 3.2	8.6 ± 1.4	25.7 ± 3.5
White Rayonandi	Apr 1937	10	15	3	49.0 ± 4.3	10.7 ± 1.4	38.3 ± 4.5
Floyd Gibbons	Apr 1937	24	6	4	9.8 ± 0.6	4.7 ± 0.5	5.1 ± 0.8
Louiseau Rosseau	Apr 1937	29	6	4	9.5 ± 0.6	4.2 ± 0.6	5.3 ± 0.8
La France	Apr 1937	14	6	4	7.7 ± 1.0	3.0 ± 0.6	4.7 ± 1.2
Mrs. Cravens	Apr 1937	28	6	4	8.3 ± 0.9	2.8 ± 0.6	5.5 ± 1.1
Good Gracious	Apr 1937	30	6	4	24.6 ± 2.0	9.0 ± 0.7	15.6 ± 2.1
Autumn Glints	Apr 1937	18	6	4	9.0 ± 0.6	4.9 ± 0.6	4.1 ± 0.8
Juva Nicholson	Apr 1937	41	6	4	5.6 ± 0.6	1.4 ± 0.3	4.2 ± 0.7
Tom Carrington	Apr 1937	18	12	3½	9.0 ± 0.5	3.0 ± 0.5	6.0 ± 0.7
Glenview Improved	Apr 1937	37	12	3½	17.4 ± 0.7	6.0 ± 0.4	11.4 ± 0.8
Chiefton	Apr 1937	36	12	3½	15.9 ± 0.9	7.0 ± 0.5	8.9 ± 1.0
Peggy Ann Hoover	May 1937	10	14	3	14.0 ± 1.6	5.6 ± 0.5	8.4 ± 1.7
Silver Wedding	May 1937	10	14	3	19.0 ± 2.6	4.5 ± 1.3	14.5 ± 2.9

* No bottom heat was used.

except for the hormone treatment. Tip cuttings about three and one-half inches long were used. All leaves were removed except a few extremely small ones surrounding the terminal bud, this procedure being the accepted practice in this region. A significant increase in number of roots on treated cuttings as compared with the controls was obtained for all varieties. Figure 10 shows results obtained with a variety that ordinarily roots with difficulty.

D. The Apple Cutting Problem

During the past fifty years most American horticulturists, at some period in their careers, have attempted to grow commercial varieties of apple trees directly from stem cuttings. A summary of these many attempts was published by Auchter (1930). Several hundred thousand cuttings have been used, nearly every conceivable type of cutting and treatment has been tried, but all have failed except some recent experiments of Gardner (1932 and 1937). The latter's results will be described in some detail after presenting results obtained by the writer on treatments of apple cuttings with indole-3-acetic acid.

Cuttings of several different varieties of apples, including Baldwin, Rhode Island Greening, York Imperial, and Delicious, were treated at the base with various concentrations of indole-3-acetic acid. All shapes and kinds of cuttings taken at every month of the year were used in experiments, and not one root was initiated. The question then arose as to whether apple cuttings failed to take up the hormone or contained some mechanism whereby the hormone was destroyed quite rapidly on being absorbed. In order to solve this problem, apple and lemon cuttings were treated in the usual

Figure 10. Root formation on Juva Nicholson Chrysanthemum cuttings. Right, treated at base with 0.10 mg. per cc. indole-3-acetic acid solution for 6 hours; left, treated at base with tap water for 6 hours. Photographed 25 days after treatment.

manner with a 0.20 mg. per cc. indole-3-acetic acid solution for 20 hours, and three auxin extractions* were made of the bark of both the lemon and apple; immediately after the treatment, the next day, and 3 days after the treatment. The results given in table IV show clearly that there is very little difference in the amounts of auxin recovered from the lemon and apple. Therefore, failure of the apple to respond to indole-3-acetic acid appears to be due not to excessive destruction of the substance or to a failure of the cuttings to take it up, but to causes other than a lack of indole-3-acetic acid.

In view of evidence presented in section V, one possible explanation for the failure of apple cuttings to respond to the indole-3-acetic acid is the absence of another substance necessary for root formation. It was concluded that in lemon cuttings indole-3-acetic acid functioned in mobilizing a naturally occurring root-forming substance, and that without the presence of this substance indole-3-acetic acid was ineffective. It may be that this other substance is lacking in apple cuttings.

The failure of apple cuttings to respond to the hormone treatment may also be due to some peculiar anatomical feature of the mature stem that makes root formation impossible. Results of Gardner's (1937) etiolation experiments support this view. He wrapped apple shoots with black insulating tape for two inches near the growing tip in the spring after terminal growth had started. The tape allowed normal elongation and radial growth, but provided a thorough etiolation of a short section of the stem. During the fall months cuttings were made of these shoots, using the etiolated portion of the shoot as the basal end of the cutting. Usually root primordia were

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* By the chloroform extraction technique described in section IV.

TABLE IV

Comparison of Amount of Auxin Recovered from Bark of Leafless Lemon and Apple Cuttings at Different Times after Treatment at Base with 0.20 mg. per cc. Water Solution in Indole-3-Acetic Acid for 20 Hours

Month treated	Time after treatment	Lemon		Apple	
		Auxin in units per gram bark		Auxin in units per gram bark	
		Tip	Base	Tip	Base
January	7 hrs	12	88	18	92
March	immediately	4	680	4	800
	1 day	1	20	1	23
	3 days	0	4	0	7

quite evident in the etiolated portion when the shoots were taken from the tree and the tape removed. Roots usually developed on most varieties within one or two weeks. It was further found that if young shoots were taped to within one inch of the growing tip instead of as close as possible to the tip, subsequent rooting decreased markedly. This indicated the necessity of etiolating early in the differentiation of the tissue of the growing tip, and suggests that the initial stages of root formation take place before much differentiation of the primary tissues has occurred. It appears that etiolation of the young growing tip may alter the normal anatomical development of the stem in such a manner that root formation is possible. This coupled with the absence of light might cause an accumulation of root-forming hormones.

A final attempt to root apple cuttings with indole-3-acetic acid is now in process. Instead of using a single initial treatment of the hormone, the cuttings are being treated at monthly intervals. After two such treatments the cuttings have developed large calluses, but as yet no roots. This procedure of repeated applications is based on results obtained with a single Camellia cutting of a "hard-rooting" variety. After three treatments at monthly intervals the cutting developed a large callus from which numerous roots developed (figure 11). It is hoped that other hard-rooting species such as the apple will behave similarly.

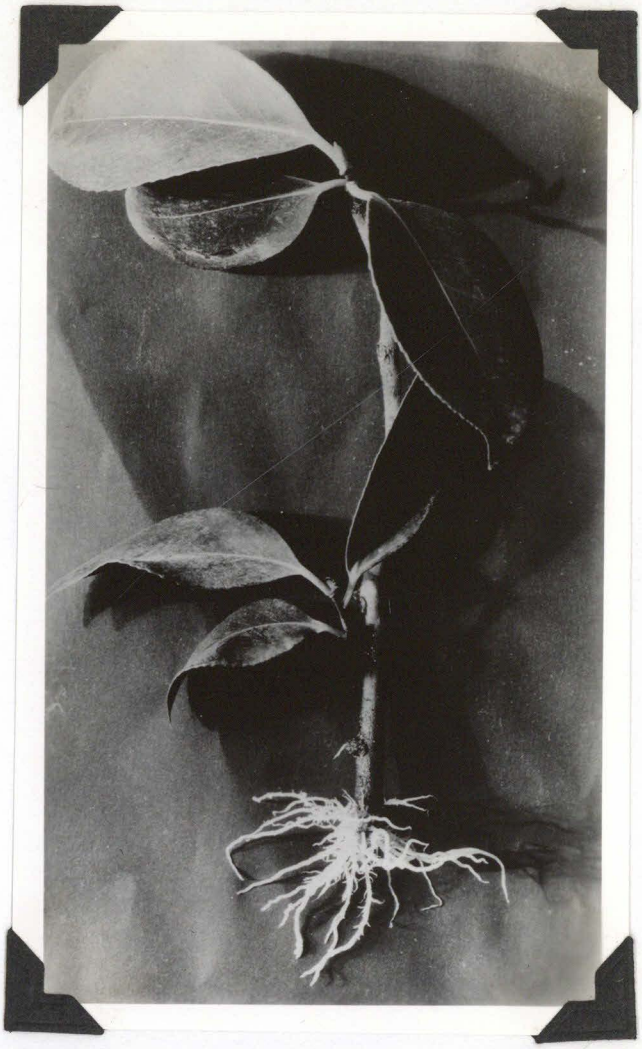

Figure 11. Root formation on a Camellia cutting treated at the base with 0.20 mg. per cc. indole-3-acetic acid solution three different times at monthly intervals. Photographed 3 months after initial treatment.

IV. TRANSPORT OF INDOLE-3-ACETIC ACID IN CUTTINGS

A. Polar Transport Concept

Many conflicting views are prevalent regarding the polar transport of auxin. Extensive study by Went (1928) and van der Weij (1932, 1934) has shown that there is a strict polar basal transport of auxin in Avena and that this polarity in transport is determined by some property inherent in the living cells. The same polarity of transport appears also to exist in other plants, such as Raphanus hypocotyls (van Overbeek, 1933), Vicia faba stems (Thimann and Skoog, 1934), Nicotiana leaf-veins (Avery, 1935), and Pisum stems (Went and Thimann, 1937).

In contrast to these results showing polar transport of hormones as a basis of the well-known morphological polarity in plants, Hitchcock and Zimmerman (1935, 1936) have denied that the transport of auxin is polar. They found that when very high concentrations of various sunthetic growth substances, including indole-3-acetic acid, were applied to the roots of intact tomato plants, some of it was absorbed and moved upward, presumably in the transpiration stream; and that, when water solutions were admitted through the cut surface of a stem or leaf, there was a longitudinal movement in either direction through dead stem tissue, this movement being influenced by transpiration. Thus they concluded that living cells were not essential for the transport of these substances, and that the main channel of transport is in the transpiration stream.

Although these results of Hitchcock and Zimmerman do show that the transpiration stream may possibly provide a means for the upward transport of hormones absorbed from the soil by the roots, they can hardly be considered as proving that the normal channel of transport of hormones in the plant is in the transpiration stream. It is generally known that any substance, even though toxic, when once in the vessels will be carried in the transpiration stream. The auxins would not be expected to be exceptions to this. Recent experiments conducted in this laboratory have shown that auxin transport in Avena is completely polar so long as auxin is applied in concentrations of the same order of magnitude as those normally occurring in the plant, but when concentrations 100 to 1000 times the normal are used, such as were used by Hitchcock and Zimmerman, some inverse transport may take place. Conclusions can hardly be drawn in regard to normal auxin transport in the plant by the use of concentrations of auxin so much higher than normally occur in the plant.

The following experiments show that auxin transport is also polar in lemon cuttings.

B. Girdling Experiments

It has already been shown that an application of a lanoline mixture of indole-3-acetic acid (0.50 mg. per gr. lanoline) to the apex of lemon cuttings induced greater root formation at the base than occurred on untreated cuttings. This is evidence that indole-3-acetic acid, or some root-forming substance present in the cutting and activated by indole-3-acetic acid, is transported downward in lemon stems.

TABLE V

Root Formation on Girdled Lemon Cuttings Treated at the Apex with

Indole-3-Acetic Acid

Expt.	Date set in sand	Leaves	Treatment	Time after set in sand weeks	Average number of roots per cutting			
					Not girdled	Girdle A	Girdle B	Girdle C
A	4/30/35	+	Pure lanoline	4	1.9 ± 0.6	0.2 ± 0.1	---	---
			Indole-3-acetic acid-lanoline mixture	4	6.0 ± 0.6	0	---	---
B	9/30/35	+	Pure lanoline	6	2.5 ± 0.3	0	---	---
			Indole-3-acetic acid-lanoline mixture	6	4.8 ± 0.5	0	3.4 ± 0.6	0
C	4/6/36	-	Tap water for 18 hours	3	0.2 ± 0.1	0	0.3 ± 0.3	0
			0.5 mg.* per cc. water for 18 hours	3	4.2 ± 1.0	0	1.0 ± 0.5	0.4 ± 0.2
D	5/3/36	-	Tap water for 15 hours	3	1.7 ± 0.6	0	0	---
			0.17 mg.* per cc. water for 18 hours	3	2.0 ± 0.5	0	1.1 ± 0.5	---

* indole-3-acetic acid.

TABLE V

Root Formation on Girdled Lemon Cuttings Treated at the Apex with Indole-3-Acetic Acid

Expt.	Date set in sand	Leaves	Treatment	Time after set in sand	Average number of roots per cutting			
					Not girdled	Girdle A	Girdle B	Girdle C
				weeks				
A	4/30/35	+	Pure lanolin	4	1.9 ± 0.6	0.2 ± 0.1	---	---
			Lanolin mixture *	4	6.0 ± 0.6	0	---	---
B	9/30/35	+	Pure lanolin	6	2.5 ± 0.3	0	---	---
			Lanolin mixture *	6	4.8 ± 0.5	0	3.4 ± 0.6	0
C	4/6/36	-	Tap water for 18 hrs.	3	0.2 ± 0.1	0	0.3 ± 0.3	0
			0.5 mg.** per cc. for 18 hrs.	3	4.2 ± 1.0	0	1.0 ± 0.5	0.4 ± 0.2
D	5/3/36	-	Tap water for 15 hrs.	3	1.7 ± 0.6	0	0	---
			0.17 mg.** per cc. for 15 hrs.	3	2.0 ± 0.5	0	1.1 ± 0.5	---

* 0.50 mg. indole - 3 - acetic acid per gr. lanoline

** Indole - 3 - acetic acid.

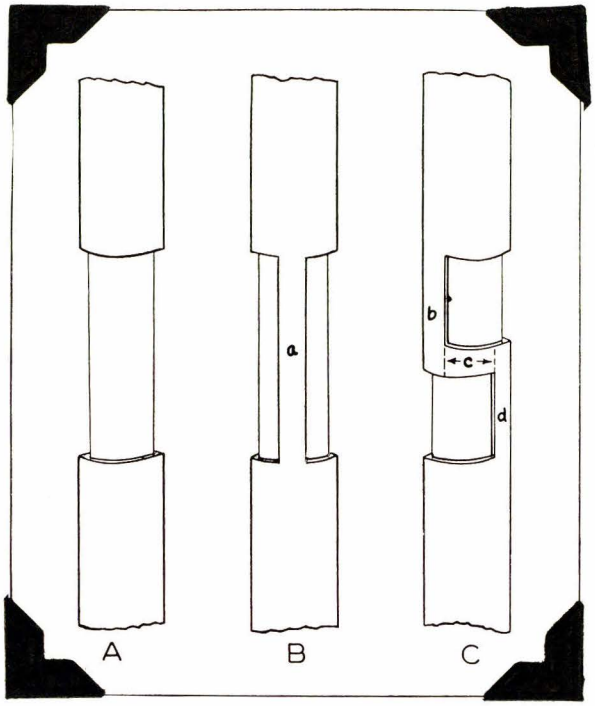

Figure 12. Diagrams of different types of girdles used. A, complete ring of phloem removed. B, partly ringed with straight vertical bridge of phloem (a) left across the girdle. C, partly ringed with two-right-angled bridge of phloem (b,c,d) left across the girdle; (b) upper vertical arm, (c) horizontal arm, (d) lower vertical arm.

Root formation at the base of the cuttings with a two-right-angled bridge of phloem across the ring (figure 12-C) was found to be influenced by the horizontal distance (c) between the side of the upper vertical arm (b) and that of the lower arm (d). When this distance was greater than 2 mm., there was usually a strong callus development at the sides and base of the upper vertical arm, but very little on the horizontal arm, and none on the lower vertical arm. In some instances roots would appear at the base of the upper vertical arm, but none on the horizontal arm or at the base of the cutting. Although in cuttings where the horizontal arm (c) was less than 2 mm. long an occasional root would form at the base of the cuttings; at no time was root formation as great as on cuttings with a straight vertical bridge across the girdle.

In another experiment with two lots of partly girdled cuttings (type B), hormone solution was applied in one lot to the entire cut end of the apex and in the other lot only to the side of the apex opposite the phloem bridge across the ring. No roots were formed on the cuttings to which hormone was applied only on the side opposite the bridge; but in the other lot, roots formed at the base of the cuttings. These results, along with those obtained with the two-right-angled type girdle, indicate clearly that the hormone moves downward in the phloem, mainly in straight lines parallel to the phloem elements.

MacDaniels and Curtis (1930) report that when lateral transfer of food in apple tree trunks was forced by spiral ringing, more rapid lateral conduction was provided for by structural changes in the phloem, beginning soon after ringing and resulting in the re-orientation of the cambium so as to be parallel with the spiral ring. They state that partial accommodation to the changed condition of conduction probably occurred immediately after

ringing, by the first formed elements being connected through their radial walls. Perhaps this would account for the slight lateral movement of the root-forming hormone observed with the two-right-angled type of girdle on lemon cuttings.

C. Local Chilling Experiment

Chilling at 33° to 40°F. and at 38° to 46°F. of about 50 mm. of the stems near the mid-portion of leafless lemon cuttings was accomplished by inserting the cuttings through special insulated chilling units. These were set up in the propagating frame so that bases of the cuttings were in the sand at a temperature near 80°F., the middle of the cutting in the chilling device, and the tips, treated with indole-3-acetic acid-lanolin paste, in the air at a temperature near 70°F. The chilling unit consisted of small water-tight tin cans into which were soldered 10 copper tubes in a vertical position and parallel to each other. This arrangement of copper tubes permitted insertion of cuttings through the device so that when cold water was circulated through the can, the portions of the cuttings in the tubes were chilled without actually coming in contact with the water. Two such chilling units, insulated with one-half inch of celotex, were connected with rubber tubing. Water at about 32°F. was circulated at a slow rate through the two-unit system for two weeks. Cuttings in the first unit were chilled locally to 33° to 40°F., and in the second unit to 38° to 46°F.

After two weeks of chilling, the indole-3-acetic acid-lanolin paste was removed from the tips, and the cuttings were taken out of the device and reset in the sand. There was no sign of callus formation at the base of

either of the chilled lots, while a strong callus had formed on the cuttings which had been treated with hormone but not locally chilled. One week later, or three weeks from time of setting in sand, roots appeared on the non-chilled cuttings, while roots did not appear on the chilled cuttings until three weeks after chilling, or five weeks after setting in sand, and then largely on the 38° to 46° lot (table VI). Thus we see that root formation on the locally chilled cuttings was delayed by two weeks--the length of the chilling period--and that root formation on chilled cuttings never did approach that of the non-chilled lot. Local chilling, then, must have acted to prevent the transport of indole-3-acetic acid downward, because the few roots which formed during the five weeks after chilling can be accounted for by naturally-occurring root-forming hormones in the stems. This seems obvious, since the untreated cuttings showed more roots than were formed on the chilled cuttings.

This evidence that local chilling actually does prevent the downward transport of indole-3-acetic acid indicates that living cells take an active part in the transport of indole-3-acetic acid or of some substance in the stem activated by indole-3-acetic acid. It therefore seems well established from the combined results of the girdling and local chilling experiments that the hormone is transported downward in the phloem.

D. Distribution of Auxin in Treated Cuttings

Some time after having completed the girdling and local chilling experiments it was found that by certain modifications in Thimann's (1934) chloroform extraction technique it was possible to extract and measure

TABLE VI

Influence of Local Chilling of Lemon Cuttings on Downward Movement of Root-forming Hormone*

Indole-3-acetic acid	Chilling treatment	Average number of roots per cutting			
		End of 2 weeks chilling	One week after chilling	Three weeks after chilling	Five weeks after chilling
-	Not chilled	0	0.4 ± 0.1	---	1.8 ± 0.3
+	Not chilled	0	0.7 ± 0.2	2.4 ± 0.3	2.8 ± 0.4
+	Chilled 33-40°F	0	0.0	0.1 ± 0.1	0.4 ± 0.2
+	Chilled 38-46°F	0	0.0	0.7 ± 0.2	0.9 ± 0.2

* Indole-3-acetic acid-lanolin paste applied at top of cutting above chilled area.

quantitatively the auxin in treated lemon cuttings. Accordingly the distribution of indole-3-acetic acid in treated cuttings was measured directly and the question of auxin transport has been elucidated more than was possible from root counts only.

From the first few trials at extracting auxin from treated cuttings with chloroform it became apparent that such large amounts of auxin were involved that it would be necessary to modify the procedure developed by Thimann (1934) for minute amounts of auxin. The procedure finally adopted which was found to give good results was as follows: Two and one-half grams of freshly scraped bark were immersed in a mixture of 45 cc. Merck's reagent chloroform and 5 cc. IN.HCL. After 20 hours the bark was filtered off and the chloroform layer separated from the filtrate. Next the chloroform was evaporated down to about 5 cc., transferred to a small test tube, and evaporated to dryness. Two and one-half cc. of water was added to the residue at the bottom of the test tube and was heated over a water bath at about 75°C. for one hour. The water was poured off and cooled to room temperature, and several dilutions were made for the auxin test. An agar plate (8 x 10.7 x 1.5 mm.) was added to each dilution and was left for one hour. Finally the agar plate was cut into 12 equal blocks and tested for auxin by the standard Avena technique (Went and Thimann, 1936).

The average curvature obtained for the 12 blocks was multiplied by the amount of dilution of the 2-1/2 cc. water extract (containing auxin from 2-1/2 grams bark), and this value was divided by 2-1/2 to give "units auxin per gram bark". These units are equivalent to about 160 "Avena-Einheit" (AE) of Kögl and Haagen Smit (1931). A control 10-unit stock solution of indole-3-acetic acid was tested on each day of the experiment in order to

determine the variation in the sensitivity of the Avena test plant. The experimental determination for auxin in the samples under test was corrected accordingly.

In all probability the growth substance extracted from cuttings treated with indole-3-acetic acid is largely indole-3-acetic acid. In this paper, however, the more general name, auxin, is used because a small amount of the native growth hormone, probably auxin a, was extracted from untreated cuttings.

In several instances a second extraction of the bark was tested. The results were as follows:

First Extraction	Second Extraction
560 units	42 units
664 "	58 "
110 "	6 "

Since the amount of auxin in the second extraction was in all instances less than 10 percent of that of the first, the procedure of only one extraction was adopted as it was desirable to have as simple a procedure as possible. The results should be considered as only relative and not as representing the absolute amount of auxin in the sample.

Table VII shows the amount of auxin obtainable from the bark of cuttings, some of which had been treated at the apex and some at the base with various concentrations of indole-3-acetic acid solution. Ten leafless lemon cuttings exactly 12 cm. long were used in each extraction. Tip extractions were made from 3 cm. of bark at the extreme apex, base extractions from 3 cm. of bark at the base, and middle extractions from 3 cm. of bark midway between the base and tip. Any excess bark over 2-1/2 grams fresh weight was discarded.

TABLE VII

Distribution of Auxin in Leafless Lemon Cuttings at Different Times after Treatment for 20 Hours: Some treated at Tip, Others at Base, with Water Solutions of Indole-3-Acetic Acid of Various Concentrations

Conc. of Indole-3-acetic acid solution	Time with respect to 20-hour treatment	Auxin in units per gram bark								
		Treated at tip March			Treated at tip April			Treated at base May		
		Tip	Middle	Base	Tip	Middle	Base	Tip	Middle	Base
mg per cc										
0 (tap water)	Just before	-	-	2	4	3	3	1	-	1
	Just after	1	-	1	1	1	1	0	-	0
	1 day after	-	-	-	1	0	0	0	-	0
	3 days after	-	-	-	1	1	1	0	-	0
	10 days after	-	-	-	0	0	0	0	-	0
	20 days after	-	-	-	0	0	0	0	-	0
0.01	Just before	-	-	2	4	3	3	1	-	1
	Just after	12	-	1	8	2	4	3	-	8
	1 day after	3	-	5	2	12	2	0	-	1
	3 days after	1	-	1	0	0	0	0	-	0
	10 days after	2	-	0	0	0	0	0	-	0
	20 days after	-	-	-	0	0	0	0	-	0
0.05	Just before	-	-	2	4	3	3	1	-	1
	Just after	41	-	6	72	8	10	0	-	34
	1 day after	8	-	2	8	16	6	0	-	18
	3 days after	2	-	-	2	0	0	0	-	-
	10 days after	1	-	1	-	0	0	0	-	1
	20 days after	-	-	-	0	0	0	0	-	0
0.20	Just before	-	-	2	4	3	3	1	-	1
	Just after	800	-	8	1280	*	3	3	-	680
	1 day after	72	-	20	60	3	3	1	-	30
	3 days after	17	-	3	11	0	1	0	-	4
	10 days after	2	-	0	9	0	0	0	-	0
	20 days after	-	-	-	5	0	0	0	-	4

- Indicates no determinations made.

* Below 20 units.

The treated portions of the cuttings, whether apex or base, showed large amounts of auxin immediately after treatment, indicating that solutions of the strengths tested were actually taken in by the cutting. However, the amount taken up was by no means proportional to the concentration of the solution. The stronger solutions were taken in much more readily than the weaker ones. The 0.20 solution was only four times as strong as the 0.05 solution, yet over ten times as much auxin was recovered from the former. Likewise, 100 times as much was extracted from the 0.20 treated cuttings as from the 0.01 cuttings, while the difference in concentration of solution was of the order of 1 to 20. It is also noted that in apical treatments, the yields were greater than in basal treatments; however, since the determinations were made a month apart and since very few data are given, conclusions as to differences in amount of uptake for apical and basal treatments are not justified.

The relatively large amounts of auxin occurring in the treated portions of the cuttings did not exist long. The auxin disappeared rapidly, around 90 percent disappearing during the first day in some cases. When the cuttings were treated at the apex with the 0.01 and 0.05 mg. per cc. solutions, there was apparently a downward transport of the auxin as indicated by the recovery of fair amounts of auxin in the base samples in both the March and April experiments, this accumulation of auxin in the base being greater for the 0.05 than for the 0.01 mg. per cc. solution. In the apical treatments with the 0.20 mg. per cc. solution, a fair amount of auxin was recovered from the base samples in the March experiment (more than for the 0.05 solution) but very little in the April experiment (less than the 0.05 solution). The amounts recovered in both cases were extremely small as compared to the amount occurring in the apex. Apparently applying high

concentrations to the apex must bring about some abnormal condition in the tissue at the point of application, for after the high level of auxin is quickly reduced, the remaining small amount are retained in the apex for a period of 20 days even when other regions of the cutting are devoid of auxin; i.e., in measurable amounts. Since this result was not observed in connection with 0.05 and 0.01 solutions, it is suspected that the normal polar transport is upset by the use of high concentrations and that, in this case, transport downward is controlled somewhat by the condition of the cutting at the time of the treatment.

Root counts on cuttings treated at the apex with these same solutions are given in table VIII. It is seen that there was no significant difference in the number of roots initiated at the base of cuttings treated at the apex with the 0.05 mg. per cc., o.01 mg. per cc., and tap water solutions. There was a slight increase in number of roots for cuttings treated with a 0.20 mg. per cc. solution but the difference was barely significant. A 0.50 mg. per cc. solution applied at the apex did show a significant increase in number of roots at the base as compared with controls, but this increase was not very large. In some instances treatment at the apex with the 0.50 solution caused root formation both at the apex and base as shown in figure 13. This condition, however, occurred only occasionally. There probably is a slightly greater accumulation of auxin at the base for cuttings treated with 0.20 and 0.50 mg. per cc. solutions than for 0.01 and 0.05 mg. per cc. solutions, and this accounts for the slightly greater root formation at the base. Nevertheless, the important observation is the high auxin content maintained in the apex of cuttings treated with the stronger solutions. This accounts for roots forming at the apex as well as at the base. Although on the basis of relative auxin content, one would expect a much greater

TABLE VIII

Root Formation on Leafless Lemon Cuttings Treated in Some Cases at the Apex and in Others at the Base with Water Solutions of Indole-3-Acetic Acid

Expt.	Date treated	Conc. of solution mg. per cc.	Duration treatment hours	Time set in sand weeks	Average number of roots per cutting: Treated at base	Treated at apex	Control	Increase basal treatment over control	Increase apical treatment over control	Increase basal treatment over apical treatment
A	4/6/36	0.50	18	3	Injured(2)	4.2 ± 1.0	0.2 ± 0.1	--	4.0 ± 1.0	--
B	5/3/36	0.17	15	3	4.1 ± 0.5(3)	2.0 ± 0.5	1.7 ± 0.6	2.4 ± 0.8	0.3 ± 0.8	2.1 ± 0.7(3)
C	5/1/37	0.01	20	3	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.2	0.1 ± 0.2	0	0.1 ± 0.1
		0.05	20	3	1.4 ± 0.3	0.6 ± 0.2	0.5 ± 0.2	0.9 ± 0.4	0.1 ± 0.3	0.8 ± 0.4
		0.20	20	3	11.0 ± 1.0	1.8 ± 0.5	0.5 ± 0.2	10.5 ± 1.0	1.3 ± 0.5	9.2 ± 1.1
		0.50	20	3	Injured(2)	2.1 ± 0.5	0.5 ± 0.2	--	1.6 ± 0.5	--

(1) Includes roots plus root primordia initiated at the base of cuttings. Ten cuttings used in each of experiments A and B, and 20 cuttings in each concentration tested in experiment C.

(2) Bases of cuttings injured by treatment, thus making reliable root count impossible.

(3) Root primordia not counted, thus accounting for the low value as compared with the 0.20 treatment of experiment C.

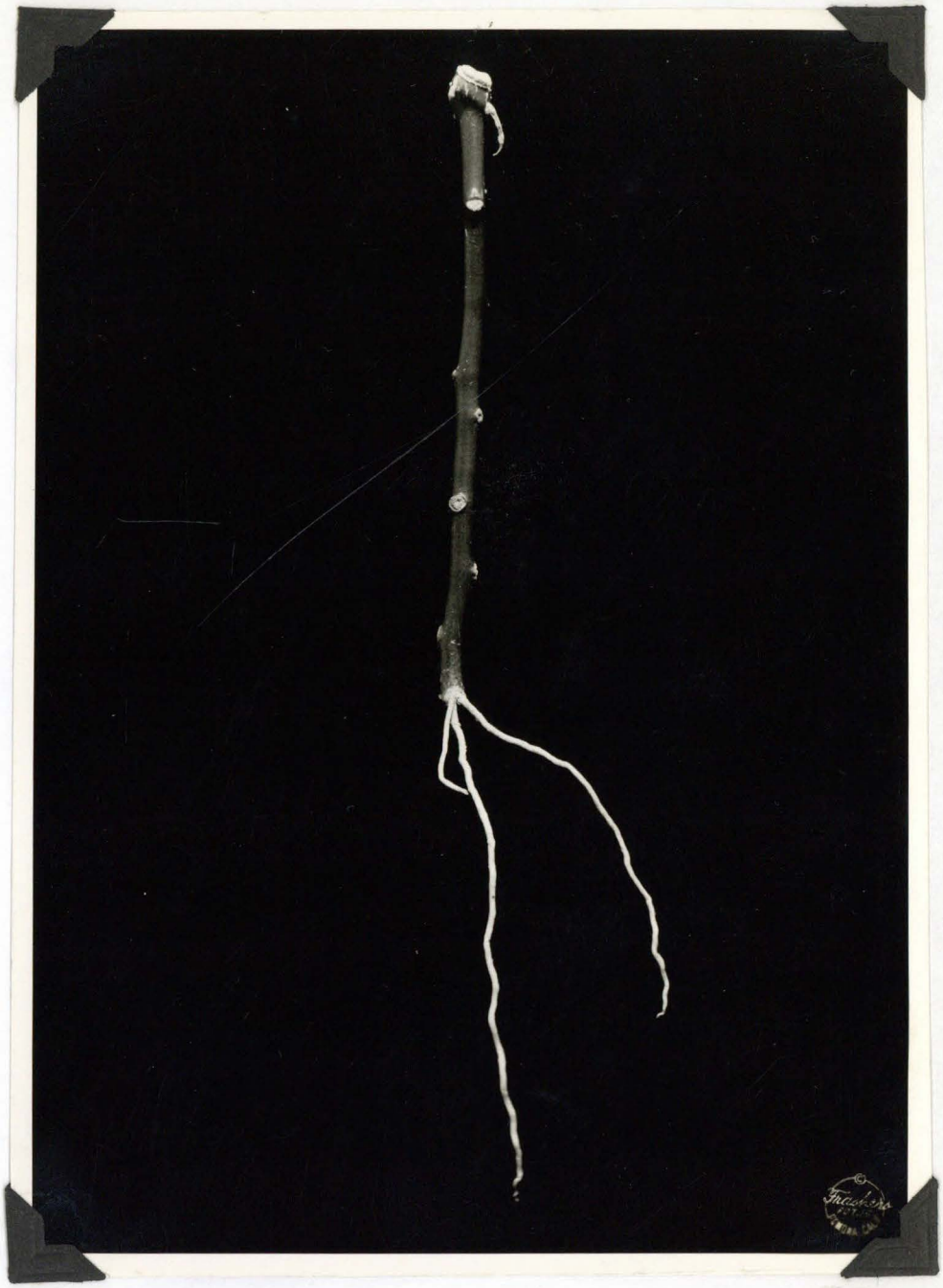

Figure 13. Root formation at both apex and base of a leafless lemon cutting treated at the apex with a 0.50 mg. per cc. indole-3-acetic acid solution for 18 hours. Base placed in sand, leaving apex in air. Photographed 3 weeks after treatment.

number of roots to appear at the apex than at the base, the more favorable temperature and moisture conditions at the base probably account for the results.

When the cuttings were treated at the base, large amounts of auxin were taken up into the bark at the base; but, from the absence of auxin in the apex samples, it is concluded that there was no upward movement of the auxin to the apex. Extractions should have been made of the bark just above the treated base to determine if there were any upward movement part way up the stem. The failure of cuttings to root as a result of excising the treated base (table IX in section V) suggests that there is no upward movement of the indole-3-acetic acid beyond the treated base. However, the role of a second factor in root formation complicates any interpretation of auxin transport which is based entirely on root counts.

A few extractions were made of the wood which showed very little auxin in any part of the cutting other than that immersed in the solution. This has not, however, been investigated very thoroughly, and it should be kept in mind that the results presented in table VII refer only to auxin recovered from the bark. The fact that all the extractions were made on leafless cuttings almost completely eliminates transpiration as a factor of upward transport in the xylem.

Root counts made on cuttings treated at the base showed that only the 0.20 mg. per cc. solution was effective in root formation, the 0.01 and 0.05 mg. per cc. solutions being no more effective than tap water. Since more auxin was found in the base of cuttings treated with the 0.01 and 0.05 mg. per cc. solutions than in cuttings treated with tap water, it appears that some factor other than auxin was limiting root formation. In the case

of treatments with the 0.20 mg. per cc. solution this other factor was apparently no longer limiting since a large number of roots were initiated. One possible explanation for this response is that the strong indole-3-acetic acid solution mobilized the other factor. Evidence that the other factor does exist is presented in part V of this paper.

V. FACTORS OTHER THAN INDOLE-3-ACETIC ACID AFFECTING ROOT FORMATION

A. Mobilization of Rhizocaline by Indole-3-Acetic Acid

The possible role of strong solutions of indole-3-acetic acid in causing root formation is seen from a study of the results given in table IX. Cuttings treated at the base with 0.20 mg. per cc. for 20 hours produced 11.7 ± 1.0 more roots per cutting than did the tap water controls. Cutting off the treated base destroyed the effect of the treatment, and treating the new base for 20 hours with 0.20 mg. per cc. caused only 5.8 ± 1.2 more roots than on the controls or about half as many as found on treated cuttings without subsequent treatment. Excising the treated base likewise destroyed the effect of a 40-hour treatment with 0.20 mg. per cc. solution, but treating the new base for 20 hours with 0.20 mg. per cc. gave no more roots than when not treated. Thus we see that the number of roots obtained after cutting off a treated base and treating the new base depends on the length of the pre-treating period. Cuttings pre-treated 20 hours gave 5.8 ± 1.2 more roots than the controls, while those pre-treated 40 hours gave no more roots than the controls.

TABLE IX

Effect of Strong Solutions of Indole-3-Acetic Acid (0.20 mg. per cc.) in Mobilizing Root-Forming Substances in Base of Leafy Lemon Cuttings. May 1-22, 1937

Time base treated	Subsequent treatment	Number of roots on individual cuttings after 20 days	Average value
hours			
20	None	5 8 9 10 11 11/12 12 13 13 14 14 14 14 15 16 17 21 23	12.6 ± 1.0
20	Treated base cut off	0 0 0 0 0 0 0 0 0 0 0 1 1 1 1 2 2 2 3/4 4	1.1 ± 0.3
20	Treated base cut off, new base treated 20 hours	0 2 3 6 6 6 6 7 7 7 7 7 8 8 9 9 12 12 13	6.8 ± 0.7
20 (tap water control)	None	0 0 0 0 0 0 0 0 0 0 0 1 1 1 1 2 2 3 4 4	0.9 ± 0.3
40	None	1 7 10 10 11 12 13 14 16 17 17 20 21 21 23 23 24 24 25 41	17.5 ± 1.9
40	Treated base cut off	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 2 2	0.4 ± 1.5
40	Treated base cut off, new base treated 20 hours	0 0 0 0 0 0 0 0 0 1 1 1 1 1 1 2 2 3 4 5	1.1 ± 0.3

These results are explained by assuming that the indole-3-acetic acid applied at the base in strong solution causes the downward transport of a substance called rhizocaline, which is necessary for root formation. This rhizocaline accumulates in the base of the cutting under the influence of indole-3-acetic acid, and cutting off the treated base removes the rhizocaline from the cutting. The fact that there were 12.6 roots per cutting for a regular 20-hour treatment and 6.8 roots when the 20-hour-treated base was cut off and the cutting re-treated 20 hours suggests that about twice as much rhizocaline moved to the base during the first 20 hours of treatment as during the second 20 hours. Also the failure to obtain roots by re-treating 40-hour-treated cuttings indicates that the rhizocaline movement to the base in this particular experiment was complete after 40 hours.

Indole-3-acetic acid alone is not effective in initiating roots; otherwise, treating the new base should have given nearly the same response as the original treatment. In order to insure equal amounts of stored foods and other substances, the final length and diameter of the cuttings in all experiments were made the same. Cuttings, the treated bases of which were to be cut off, were made about 2 cm. longer in the beginning to allow for the removal of 2 cm. of the stem at the base after treatment.

A similar conclusion regarding the role of an internal factor in root formation was reached by Went (1937) from experiments with pea cuttings. He found that if a cutting were divided into a number of sections and each treated with a high concentration of auxin the sum of the numbers of root primordia formed was about the same as on an intact cutting so treated. This suggests that the number of primordia is determined by an internal substance which becomes limiting when auxin is in excess. The bulk of the

primordia were on sections some distance from the apex; therefore, they must have contained more of the internal substance. When auxin was applied to the apex of the uppermost one-eighth of a cutting, only about 7 primordia were formed at the top, but, when it was applied to the apex of the intact cutting, 30 primordia were formed at the top. The auxin, therefore, appears to have mobilized some of the other substance from the lower parts of the cutting.

Data from other experiments by the writer in which the treated base of lemon cuttings was excised and the new base treated are shown in table X. The results are somewhat variable. In experiment A, subsequent treatment* of leafless cuttings pre-treated for 12 hours with a 0.50 solution caused no more roots than the tap water controls. Subsequent treatment to leafy cuttings (experiment B) receiving a preliminary treatment of 0.25 mg. per cc. for 20 hours gave about half as many roots as the preliminary treatment without subsequent treatment. This result compares favorably with the results presented in table IX for a 20-hour treatment. On the other hand, the results of experiments C, D, and E (table X) with a 40-hour pre-treating period do not compare favorably with the 40-hour data in table IX. In the latter case there was no response to subsequent treatment of 40-hour pre-treated cuttings, while in experiments C, D, and E there was some response to subsequent treatment of 40-hour pre-treated cuttings. Different material may account for these variations in response; but it seems probable that in most instances 40-hour treatment will not mobilize all of the rhizocaline in leafy cuttings.

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* Meaning excising treated base and treating new base.

TABLE X

Results of Five Experiments with Lemon Cuttings Showing Effect on Root Formation of Excising the Treated Base and Treating the New Base

Expt. No.	Date treated	Leaves	Conc. of indole-3-acetic acid mg.per cc.	Duration of treatment hours	Average number of roots per cutting after 3 weeks			
					Tap water control	Treated	Treated base excised	Treated base excised; new base treated
A	4/17/36	-	0.50	12	1.1 ± 0.4	4.2 ± 0.3	0.9 ± 0.5	0.8 ± 0.2(1)
B	9/9/36	+	0.25	20	2.5 ± 0.4	28.5 ± 2.1	2.6 ± 0.5	12.1 ± 1.1(2)
C	6/30/37	+	0.20	20	0	18.2 ± 1.1	---	4.9 ± 0.5(3)
D	7/15/37	+	0.20	20	0.7 ± 0.3	16.7 ± 1.0	---	9.4 ± 0.9(3)
E	5/14/37	+	0.20	20	1.9 ± 0.3	15.4 ± 2.6	1.7 ± 0.4	9.2 ± 0.5(3)

(1) Treated 12 hours; excise base; treat new base 12 hours.

(2) Treated 20 hours; excise base; treat new base 20 hours.

(3) Treated 40 hours; excise base; treat new base 20 hours.

Although the results of lemon experiments indicate that an important function of indole-3-acetic acid in root formation is to control the transport of rhizocaline, it does not preclude the possibility that indole-3-acetic acid may, for instance, also react with, or activate rhizocaline to cause the phenomenon of root formation. Went* has found that y-phenyl-acetic acid is nearly as effective in mobilizing rhizocaline as indole-3-acetic acid, but is much less effective in initiating roots. It is hoped that further investigations with this and other substances may lead to a better understanding of root formation.

B. Influence of Leaves on Root Formation and Root Development

Since the results presented above indicate that indole-3-acetic acid caused the downward transport of rhizocaline, it was thought that possibly this substance came primarily from the leaves. As has been pointed out repeatedly by investigators since the time of Agricola in 1716, roots in some plants are formed by the action of leaves. If the indole-3-acetic acid treatment causes the rapid transport of this factor from the leaves to the base of the cutting, removing the leaves shortly after treatment should have no effect on the number of roots formed as the rhizocaline will already have moved to the base. The light intensity in the propagating frames was so small that it is doubtful if much assimilation above the compensation point occurred in leafy cuttings after being placed in the frame.

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* Unpublished data.

With these considerations in mind, an experiment was conducted whereby leaves were removed from lemon cuttings before treatment and at various times after treatment. The results are given in table XI. It is noted that the number of developed roots increased with the time that the leaves were left on the cuttings. When root primordia were included, however, the total number of roots initiated for all lots which had the leaves on at time of treatment (B to E, inclusive) was found to be roughly the same.

It is true that there is a wide range in the values for these four lots, which is no doubt due to the difficulties involved in making an accurate determination of the root primordia; but they show no definite trend in relation to the time the leaves were removed and all show a significantly greater number of roots initiated than lot A which had no leaves at the time of treatment.

From these results, it seems probable that in lemon cuttings we are concerned with both root-forming and root-developing substances coming from the leaves. The root-forming substance, rhizocaline, is transported rapidly to the base of the cutting under the influence of indole-3-acetic acid and initiates roots. The number of roots initiated is not influenced further by the presence of leaves. Even if more of this substance is formed in the leaves after treatment, there is no means to take care of its transport to the base. It has already been shown that the high level of auxin in the cutting disappears rapidly after removing the cuttings from the indole-3-acetic acid solution, and the number of roots formed without this high auxin content at the base is for the most part very small.

The existance of a root-developing substance is indicated by the fact that the number of visible roots increased with the time the leaves were

TABLE XI

Effect of Leaves on Root Formation on Lemon Cuttings

(treated at base 20 hours with 0.20 mg. per cc. indole-3-acetic acid solution. May 1-22, 1937)

Lot No.	Time leaves removed	Average number roots per cutting after 3 weeks		
		Roots	Root primordia	Roots plus primordia
A	Before treatment	4.3 ± 0.9	6.8 ± 1.2	11.1 ± 1.4
B	Immediately after treatment	5.3 ± 1.3	23.3 ± 3.9	28.6 ± 3.8
C	20 hours after treatment	7.1 ± 1.2	17.0 ± 2.1	24.1 ± 2.2
D	1 week after treatment	11.3 ± 1.0	22.5 ± 3.6	33.8 ± 3.5
E	Leaves not removed	13.0 ± 0.6	10.8 ± 1.9	23.8 ± 2.1

TABLE XII

Effect of Leaves on Root Formation on Untreated and Treated Chrysanthemum Cuttings(1). March 27 to April 18, 1937

Variety	Indole-3-acetic acid treatment	Time leaves removed	Time in sand	Average No. roots per cutting(2)
			weeks	
Meta Bergen	none	1st day after treatment	2	0
		3rd day after treatment	2	4.5 ± 1.0
		not removed	2	11.3 ± 1.6
		1st day after treatment	3	6.3 ± 1.3
		3rd day after treatment	3	6.9 ± 0.8
		not removed	3	10.2 ± 1.5
Willard's Bronze	0.10 mg. per cc. for 18 hours	before treatment	3	2.2 ± 0.9(3)
		3 days after treatment	3	15.5 ± 1.2
		not removed	3	26.3 ± 1.6
	none but tap water for 18 hours	before treatment	3	0.5 ± 0.3
		not removed	3	0.3 ± 0.05

(1) Cuttings placed in sash covered propagating frame without bottom heat.

(2) Ten cuttings were used in each experiment.

(3) Treated base injured.

C. Extraction of Rhizocaline from Leaves

By the chloroform extraction technique, it was found that normally the bark and leaves of many plants contained small amounts of auxin. This naturally-occurring growth hormone, however, apparently is not rhizocaline because Delicious apple leaves and bark were found to contain as much of it as the lemon. Also, root formation on the lemon is in no way correlated with the auxin content of the twigs. In one experiment, cuttings taken at 6 a.m. contained 7.3 auxin units, at 1 p.m., 55.0 units, and at 6 p.m., 7.5 units, while the number of roots occurring on cuttings taken at the same times were 6 a.m., 4.4; 1 p.m. 4.1; and 6 p.m., 6.0 roots. Thus it seems rather certain that factors other than auxin are involved.

At present, efforts to extract rhizocaline are based on results presented in tables IX and X which indicate that a strong indole-3-acetic acid treatment at the base of lemon cuttings causes the rapid downward transport of rhizocaline from the leaves to the base of the cutting. If such actually is the case, placing the petioles of the cut-off leaves in a strong solution of indole-3-acetic acid might cause the rhizocaline to diffuse out of the leaves.

The results of one experiment of this type are shown in table XIII. Diffusates of Lantana, fig, and lemon leaves were obtained by placing the petioles of 30 leaves of each species in 7 cc. of tap water and in 7 cc. of 0.50 mg. per cc. indole-3-acetic acid solution for a 12-hour period. The experiment was carried out in a saturated atmosphere to prevent the uptake of the small quantities of solution by the leaves. Leafless lemon cuttings were used to test the root-forming activity of the diffusates.

TABLE XIII

Root Formation on Leafless Lemon Cuttings Treated with Diffusates from *Lantana*, Fig, and Lemon Leaves in Tap Water in Indole-3-Acetic Acid (0.50 mg per cc) August 8, 1936

Eight-hour treatment	Number of roots on individual cuttings after six weeks	Average value
Tap water	0 0 1 2 2 3 4 4 5 5	2.6 ± 0.6
Tap water *Lantana* diffusate	0 0 0 0 0 0 0 0 1 2	0.3 ± 0.2
Tap water lemon diffusate	0 0 0 0 1 1 1 3 4 4	1.4 ± 0.9
Indole-3-acetic acid	4 6 6 6 7 7 8 8 8 10	7.0 ± 0.5
Indole-3-acetic acid *Lantana* diffusate	0 0 0 0 0 1 1 1 1 3	0.7 ± 0.3
Indole-3-acetic acid fig diffusate	4 4 2 5 2 3 3 5 4 3	3.5 ± 0.3
Indole-3-acetic acid lemon diffusate	6 6 7 7 9 11 11 11 12 14	9.4 ± 0.9

Lantana diffusate into indole-3-acetic acid definitely destroyed the effectiveness of the indole-3-acetic acid solution, while the fig diffusate reduced the activity of the indole-3-acetic acid to half its normal value. Enzymes set free from the cells at the cut surface of the petiole may have destroyed the indole-3-acetic acid in a manner similar to that observed by van Overbeek (1936) for sections of corn mesocotyl bases. Diffusates of lemon leaves into indole-3-acetic acid, on the other hand, appeared slightly to increase the root-forming activity of the solution. The difference in number of roots 2.4 ± 1.0 is, however, barely significant and more experiments will have to be conducted to determine whether the difference is real. The tap water diffusate of lemon leaves showed no increase in activity as compared with tap water alone, a fact which suggests that no measurable amount of rhizocaline had diffused into the water.

D. Experiments with Sucrose and Vitamin B_1

Hellinga (1937) has objected to the rhizocaline hypothesis and has proposed that nutritive substances are the limiting factors involved. He cited the fact that Coleus cuttings kept in light formed more roots than similar cuttings kept in darkness and explained the difference in response as caused by the synthesis of nutritive substances in the leaves in the light. He failed to consider, however, that other materials besides nutritive substances may be synthesized in leaves in the light. Nutritive substances may be a limiting factor in root formation on Coleus cuttings, but that does not preclude the possibility that other substances, such as rhizocaline, may also be involved.

Results presented in table XIV show that in the case of lemon cuttings sugar is not a limiting factor either in root formation or root development. After treating at the base with indole-3-acetic acid and excising the treated base, subsequent treatment with an indole-3-acetic acid in two percent sucrose solution gave practically the same response as a pure indole-3-acetic acid solution. One criticism of this experiment is that perhaps the treating period was of too short a duration for an appreciable amount of sucrose to be absorbed. The experiment should be repeated using a longer period for treatment with sucrose.

Recent results by Mitchell and Martin (1937) show that the application of indole-3-acetic acid to the first internode of etiolated bean plants retarded the transport of nutrient materials from the cotyledons. Their conclusions are based on chemical analysis for total nitrogen, soluble sugars, and starch in the cotyledons and internodes of treated and untreated plants at the beginning and end of a four-day period following treatment. The results are, therefore, good evidence that nutritive substances are not mobilized by indole-3-acetic acid. Since the lemon cutting experiments and Went's experiments with pea cuttings show that a root-forming substance is mobilized by indole-3-acetic acid, it seems likely that this substance is not of nutritive character.

Bonner and Addicott (1937) have shown that vitamin B_1 is necessary in minute amounts for the growth of isolated pea roots. Accordingly it was suspected that the root-developing substance coming from lemon leaves might be vitamin B_1. Application of this substance in conjunction with the indole-3-acetic acid to leafy lemon cuttings resulted in a small increase in number of developed roots in one experiment conducted in January following a period of cold weather, but further tests in April and May failed to give this response. Perhaps vitamin B_1 is a root-developing substance and

TABLE XIV

Effect of Sucrose on Root Formation and Root Development in Lemon Cuttings. July 18- Aug. 19, 1937

Subsequent treatment of leafy cuttings treated 40 Hrs. with 0.20 mg. Indole-3-acetic acid per cc. water.	Average No. of roots per cutting after 4 weeks	
	Initiated	Developed
Cut off base; re-treat 20 hours with 0.20 mg. indole-3-acetic acid per cc. water.	12.6 ± 1.0	10.0 ± 1.0
Cut off base; re-treat 20 hours with 0.20 mg. indole-3-acetic acid per cc. 2% sucrose solution.	14.0 ± 1.1	11.4 ± 1.0
Control 0.20 mg. per cc. indole-3-acetic acid 20-hour treatment with no subsequent treatment.	23.9 ± 1.1	19.4 ± 1.0

normally occurs in the leaves in sufficient quantities to cause root development, but following cold weather its production in the leaf stops and becomes limiting. The response of this and other substances in root development needs to be studied further on leafless cuttings which contain less of the naturally-occurring root-developing substance than do leafy cuttings. Furthermore, cuttings should probably be treated for about one week with root-developing test colutions in order to insure that the substances are available in sufficient amounts at the proper time for the "growing out" of the roots.

E. Grafting Experiments

Earlier in this paper in the discussion of the apple cutting problem, the hypothesis was presented that plants which do not respond to indole-3-acetic acid are lacking in rhizocaline. If this is the case,one might expect that this rhizocaline could be supplied to plants which are hard to root by grafting onto them a part of a plant containing rhizocaline. It was thought that the rhizocaline in the scion might be induced to move across the graft union into the stock if the latter were treated with a strong indole-3-acetic acid solution. Went* had previously found that in pea cuttings the rhizocaline would not move across a wound; therefore, it was felt that, if a union of living tissue could be established between the scion and stock, the rhizocaline might be induced to move into the stock.

As yet no positive results have been obtained from experiments of this nature. The first experiments were made with Chrysanthemum cuttings in which shoots of the Meta Bergen variety were grafted onto cuttings of the Dr. Inglis variety. The former was known to root readily while the latter

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* Unpublished data.

was supposed to be hard to root. The grafted cuttings were placed in the propagating frames with only the basal portion of the Dr. Inglis variety inserted in the sand. After ten days and before a good graft union was established between the two varieties, numerous roots began to appear on the scion just above the graft union. These roots were removed and the cuttings were treated at the base with indole-3-acetic acid. A few roots appeared at the base within a week but no more than occurred on treated non-grafted cuttings of the same variety. It seems likely from these results that the supply of rhizocaline in the scion variety was exhausted in initiating roots on the scion before a graft union could be established .

In other experiments lemon shoots were grafted onto Satsuma orange cuttings which were known not to root even when treated with indole-3-acetic acid. A union of callus tissue was established between the two varieties after six weeks. At this time the cuttings were treated with indole-3-acetic acid, but no roots were initiated. It may be that the graft union was not sufficiently complete to permit movement of the substances across the graft. Besides, the rhizocaline of the scion may have been dissipated by various causes during the long interval required to establish the graft union. Perhaps the best procedure for this problem would be to graft leafy shoots of lemon onto shoots of the Satsuma orange on the tree, rather than onto cuttings of the orange. By this procedure one would know that, when the lemon shoot begins to put out new growth, a good graft union has been established. Moreover, new growth on the lemon might insure a good supply of rhizocaline. Cuttings of such Satsuma orange-lemon graphs would make good material for testing the hypothesis regarding rhizocaline.

SUMMARY AND CONCLUSIONS

1. The application of indole-3-acetic acid in lanoline to the apex of cuttings was effective in initiating roots on lemon, Lantana, Coleus, and Chrysanthemum.
2. High concentrations of indole-3-acetic acid in water solution caused root formation when applied at either the tip or base of cuttings, but was considerably more effective when applied at the base. Dilute solutions of indole-3-acetic acid were no more effective in root formation than tap water.
3. The basal application of high concentrations of indole-3-acetic acid in water solution was used successfully in causing root formation on cuttings of a great number of commercially important plants. Apple and Satsuma orange cuttings, however, failed to root by this treatment.
4. The transport of indole-3-acetic in woody cuttings is polar and takes place mainly in the phloem. High concentrations, however, appeared not to be transported as readily as low concentrations.
5. Experiments with cutting-off the treated base and treating the new base indicate that the action of indole-3-acetic acid in root formation is primarily the mobilization of the naturally-occurring root-forming substances.
6. Evidence is presented which indicates that leaves of lemon cuttings supply both root-forming and root-developing substances. The root-forming substance, rhizocaline, is transported rapidly to the base under the influence of indole-3-acetic acid and, perhaps in conjunction with indole-3-acetic acid, causes the differentiation of cells into root primordia. The root-developing substance is transported slowly to the base and causes the further growth of the root primordia.
7. The hypothesis is presented that most plants which do not respond to indole-3-acetic acid are lacking in rhizocaline.

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