# PLANT HORMONES

BY

E. D. C. Baptist

## Introduction

The word hormone from a Greek word meaning to "arouse to activity," was first used in animal physiology by Bayliss and Starling (1) in 1906 and in plant physiology by Fitting (2) in 1910.

Hormones are chemical substances, quite distinct from nutritive materials. They are produced in certain cells or tissues and transferred to other tissues of the same organism where they determine and regulate physiological processes. Other names in common use for plant hormones are phytohormone, growth substance, growth-promoting substance, growth-regulator, "Wuchstoff" and auxin.

The investigations of Charles Darwin marked the beginning of research on plant hormones. Darwin(3) in 1880 showed that when young shoots of seedlings are illuminated from one side some influence perceived by the growing tip is transmitted downwards along the shaded side and the seedlings bend towards the light. It was not until 40 years later that Boysen Jensen (4) and Paal (5), showed conclusively that the influence or stimulus was of chemical nature and was caused by a growth-substance or growth hormone, formed and secreted at the growing point. The curvature observed in Darwin's experiment was caused by the increased growth of the shaded part of the young shoot due to the presence there of a higher concentration of growth hormone. The bending of horizontally-placed roots and shoots has also been shown to be caused by an unequal distribution, under the influence of gravity, of hormones on the two sides of the growing region of these organs (Cholodny (6)).

The famous German botanist Sachs (7) in 1882 postulated the existence of a special root-forming substance which is formed in the leaves and transmitted to the roots. He confirmed results, obtained by Duhamel du Monceau (8) in 1758, that, on a ring-barked stem, callus and, under favourable conditions, roots are formed above the rings where downward movement of the hormone is interrupted. Sachs explained morphological differences in plants as due to the influence of specific organ-forming substances.

Beijerinck (9) in 1888 ascribed the development of galls on leaves to a stimulating material, "growth enzyme," the action of which he likened to that of an enzyme in that it is effective when present in minute amounts. We now know that cell outgrowths on leaves can be artificially produced by local applications of the synthetic auxin, indolyl-acetic acid (La Rue (10)). The initiation and growth of nodules on the roots of leguminous plants are, according to Thimann (11), caused by the auxin produced by the symbiotic bacteria. Errera (12) ascribed apical dominance and inhibition of lateral buds to an internal secretion by the apical bud of the main stem. Loeb (13) found that leafy shoots of Bryophyllum root more vigorously than defoliated shoots and suggested that root formation is stimulated by a hormone.

Van der Lek (14) showed that the presence of a young leaf or developing bud on a woody cutting promotes root formation. Similar results were obtained with Acalypha cuttings by F. W. Went (15) at Buitenzorg in 1929 indicating that root formation was due to hormones produced in leaves and developing buds; these hormones migrate and accumulate at the basal end of the cuttings. Further investigations by Bouillene and Went (16) showed that rooting could be stimulated on some defoliated and debudded cuttings (which form few or no roots) by grafting a leaf or by treating the apical end with a water extract of papaya leaves or with diastase. The first evidence was thus obtained that specific substances formed in the leaf could stimulate root growth.

Went named the active substance rhizocaline, the root-forming hormone, and by the development of a sensitive biological method, the Avena test, was able to determine its chemical nature. These growth substances are formed in young leaves growing in the light and disappear slowly when the plants are placed in the dark (Avery (17)). Older leaves produce little or no hormone (Thimann and Skoog (18)). Gregory (19) had postulated the existence of a substance necessary for leaf growth which is formed in leaves under the influence of light. Growth substances are also present in the seeds of plants and play an important part in germination and early growth of the seedling.

Seubert (20) was the first to show that growth substances occur outside the plant and to demonstrate their presence in human saliva, diastase, and malt extract. They are widely distributed in nature and have been found in rice polishings, in most vegetable oils, in peas, beans, lentils, tomatoes, oranges, and lemons, in foliage leaves and sprouting buds, in the pollen of many species of plants, in yeast, in some algae, in cultures of fungi or bacteria, in human saliva and in urine. Kögl, Haagen Smit, and Erxleben (21), (22) isolated in pure form three hormones or auxins. Two of these, auxin-a and auxin-b occur in the higher plants and the third, heteroauxin, is produced by fungi and bacteria. Heteroauxin when analysed was identified as indolyl-acetic acid, *a product of decomposition of protein, which had been isolated from* human urine by Salkowski (23) in 1885 and prepared synthetically by Majima and Hoshino (24) in 1925. The root-forming properties of synthetic indolyl-acetic acid were first demonstrated by Thimann and Koepfli (25).

Zimmerman and Wilcoxon (26) of the Boyce Thompson Institute U.S.A. have shown that other organic acids, not known to occur in plants, also possess the property of stimulating root formation. They studied the effects of some thirty-two different compounds and found that alpha-naphthalene-acetic and indolyl-butyric acids were the most effective for root formation. Zimmerman, Crocker and Hitchcock (27) showed that ethylene, propylene acetylene and carbon monoxide induce root formation on the stems of many species of plants. The organic acids (synthetic auxins) are now available in pure form and their value for vegetative propagation has been tested at many centres. A number of growth substances placed on the market in England (six) and in the U.S.A. (twelve) and sold under various proprietary names are most probably solutions of indolyl-acetic acid, alpha-naphthalene acetic acid or indolyl-butyric acid or mixtures of these acids.

### Other Factors affecting Rooting

*Sugars* are essential to the successful rooting of cuttings. Cuttings taken from plants grown in the dark and therefore depleted of carbohydrate reserves root feebly or not at all. Treatment with auxin solution to which sugar has been added may result in the formation of many times the number of roots formed as a result of treatment with auxin alone.

*Biotin*, a constituent of the yeast growth-promoting substance, bios, which was isolated in the crystalline state by Kögl and Tönnis (28) in 1936, has been shown by Went and Thimann (29) to cause a large increase in the number of roots formed on cuttings treated with auxin. Yeast extract has been shown by Robbins (30) and White (31) to be essential for the growth of isolated root tips in synthetic media. Kögl and Haagen Smit (32) obtained a large increase in growth of excised pea embryos in a medium containing only inorganic salts and sucrose by the addition of 0.00008 milligram of biotin per 10 c.c. of the culture solution.

*Aneurin* (vitamin $B_1$), the anti-beri-beri vitamin, is another factor necessary for root growth. Aneurin is formed in leaves in the presence of light and translocated to the roots. It is stored in seeds and is needed for the development of the seedling.

Went, Bonner, and Warner (33) showed that, on cuttings treated with auxin, the number of roots which grow out is limited by the available aneurin. Leafy cuttings of Camellia, which had failed to root after repeated auxin treatment, were placed in a solution of aneurin (1 mg per litre) for 24 hours. One week later all the cuttings treated with aneurin had rooted successfully. Since aneurin without auxin treatment produced no rooting, proof was obtained that development of the embryonic roots (primordia) initiated in these cuttings by auxin is brought about by aneurin.

*Ascorbic acid* (vitamin C), which occurs in plants, was found effective in promoting profuse root and shoot development in willow cuttings at a dilution of 1 in 100,000 (Davies, Atkins and Hudson (34)).

The dividing line between a hormone and a vitamin is by no means well defined. A substance may be a vitamin to one organism and a hormone to another. Aneurin and ascorbic acid are true hormones for higher plants, but they are vitamins for most higher animals, which do not produce them.

*Animal hormones.*—A number of these hormones have been found in plants but their function in the plant is still obscure. Went and Thimann (35) have obtained evidence that a pure preparation of the female sex hormone theelin (oestrin) increases the number of roots developed by cuttings treated with auxin. Scharrer and Schrop (36) claim to have obtained increased production and growth in soya beans and other crops as a result of treatment with prescribed amounts of crystalline follicular hormones. Schoeller and Goebel (37) and Georgieff (38) obtained large increases in the weight of the fruit crop by dosing tomato plants with oestrone.

## Root Formation on Cuttings

Tincker (39) has carried out extensive tests at the Royal Horticultural Society's Laboratories, Wisley, England, with a wide range of species of plants which are known to differ markedly in their ability to root from cuttings. He placed the plants into three groups (a) those rooting easily (b) those rooting less easily and (c) those rooting with great difficulty and found that 90 per cent of the species in group (a), 70 per cent of those in group (b) and 17 per cent of those in group (c) showed increased and accelerated rooting as a result of treatment with synthetic growth substances. Tincker obtained the best results with indolyl-acetic-acid, indolyl-butyric acid and alpha-naphthalene-acetic-acid which he found equally effective in causing increased rooting in about 54 per cent of the species tested. A number of proprietary solutions tested by him gave satisfactory results at the dilutions recommended by the makers. Several hundred plants were raised from the

PLATE 1

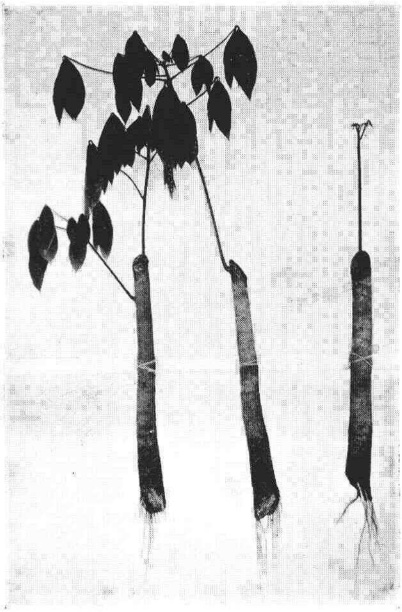


FIG. 1. Treated basal cuttings from two-years-old seedlings showing profuse rooting six weeks after planting.

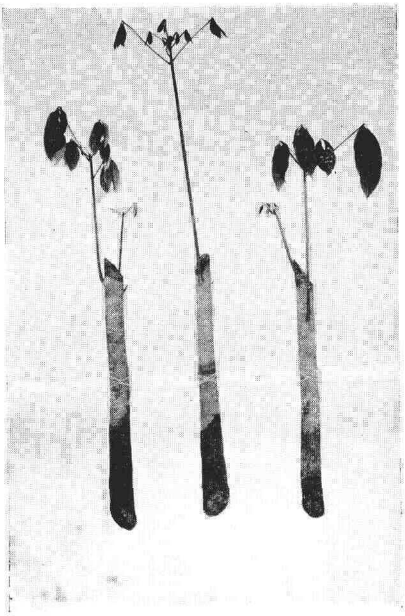


FIG. 2. Untreated basal cuttings from two-years-old seedlings showing callus but no roots six weeks after planting.

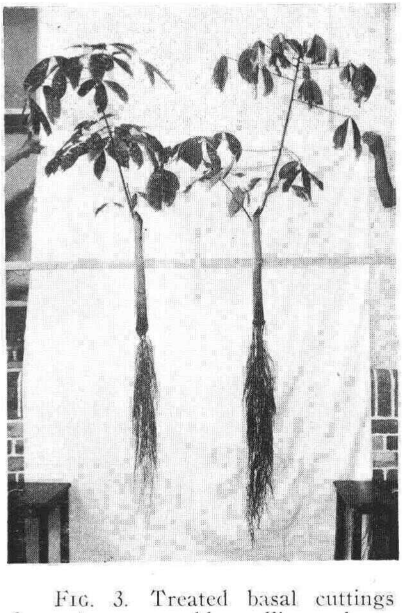


FIG. 3. Treated basal cuttings from two-years-old seedlings showing root formation five months after planting.

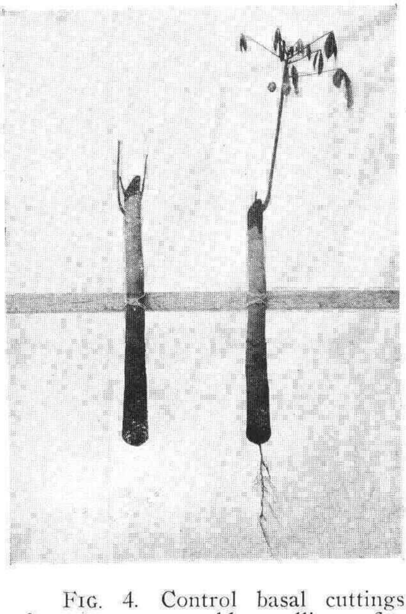


FIG. 4. Control basal cuttings from two-years-old seedlings five months after planting.

rooted cuttings and the initial advantage in growth due to accelerated and increased rooting was maintained in the subsequent growth of the treated cuttings.

Various methods of application of growth substance to plants have been tried: spraying, addition to the rooting medium, injection, local application in a lanolin paste (Laibach's method) but the best results have been obtained by standing cuttings in a very dilute solution of the growth substance in water for periods varying from 24 to 48 hours. Concentrations of one part in 10,000 to one in 20,000 have given good results for woody cuttings but the optimum strength of the solution varies with different species of plants. For herbaceous material a solution of one part in 40,000 has proved very effective. High concentrations damage the tissues.

### Experiments with Hevea brasiliensis

(1) Cuttings from the Main Stem of Young Seedlings

Preliminary investigations carried out at this Institute on the effect of growth substances on the rooting of cuttings of Hevea have demonstrated a marked stimulation of root production.

*Method of Treatment.*—The basal ends of the cuttings are pared with a sharp knife and the latex allowed to exude. The coagulated latex is then peeled off and the cuttings treated by standing them with their bases in the solution of the growth substance for 24 hours. The basal end of each cutting is rinsed in water and the apical end dipped in just-melted grafting wax before planting.

Cuttings eighteen inches long from the basal part of the main stem of one and two-years-old seedlings all showed root formation within six weeks from planting after treatment for 24 hours with a proprietary growth substance at a dilution of 1 in 150 of water (1 fluid oz. per gallon). The treated cuttings had produced ten to sixteen roots each, the longest roots ranging from six to nine inches in length. (Plate 1, Fig. 1). The control cuttings which were stood in tap water for the same period of time before planting had formed a callus but no roots. (Plate 1, Fig. 2). Four months later the roots on the treated cuttings had attained a length of three feet (Plate 1, Fig. 3). One control cutting had produced one root six inches long in that time; others had formed a callus but no roots. (Plate 1, Fig. 4). The roots on the treated cuttings grew vertically downwards and had numerous lateral branches abundantly supplied with feeding roots which formed a dense network in the top foot of soil. The root distribution of one such cutting, thirteen months after planting in poor sandy soil, is well brought out in the drawing shown in Plate 2. The drawing was made during the excavation of the rooted cutting and is an accurate representation of the roots in their natural position.

Subsequent investigations have shown that the position on the parent plant from which the cuttings are taken is an important factor for successful rooting. Four cuttings about fifteen inches long were made from the main stem of one and two-years-old seedlings at different times of the year and stood in weak solutions of each of several synthetic chemicals and of two proprietary growth stimulants for 24 hours before planting. The cuttings were labelled 1 to 4 according to their origin from the base of the stem upwards. Control cuttings were stood in tap water for the same length of time. Data from a large number of experiments are summarised below.

| | Percentage of rooted Cuttings after three months | |
|---|---|---|
| | Treated | Control |
| Cutting No. 1 ... | 90 | 19 |
| „ No. 2 ... | 28 | 3 |
| „ No. 3 ... | 8 | $<3$ |
| „ No. 4 ... | 0 | 0 |

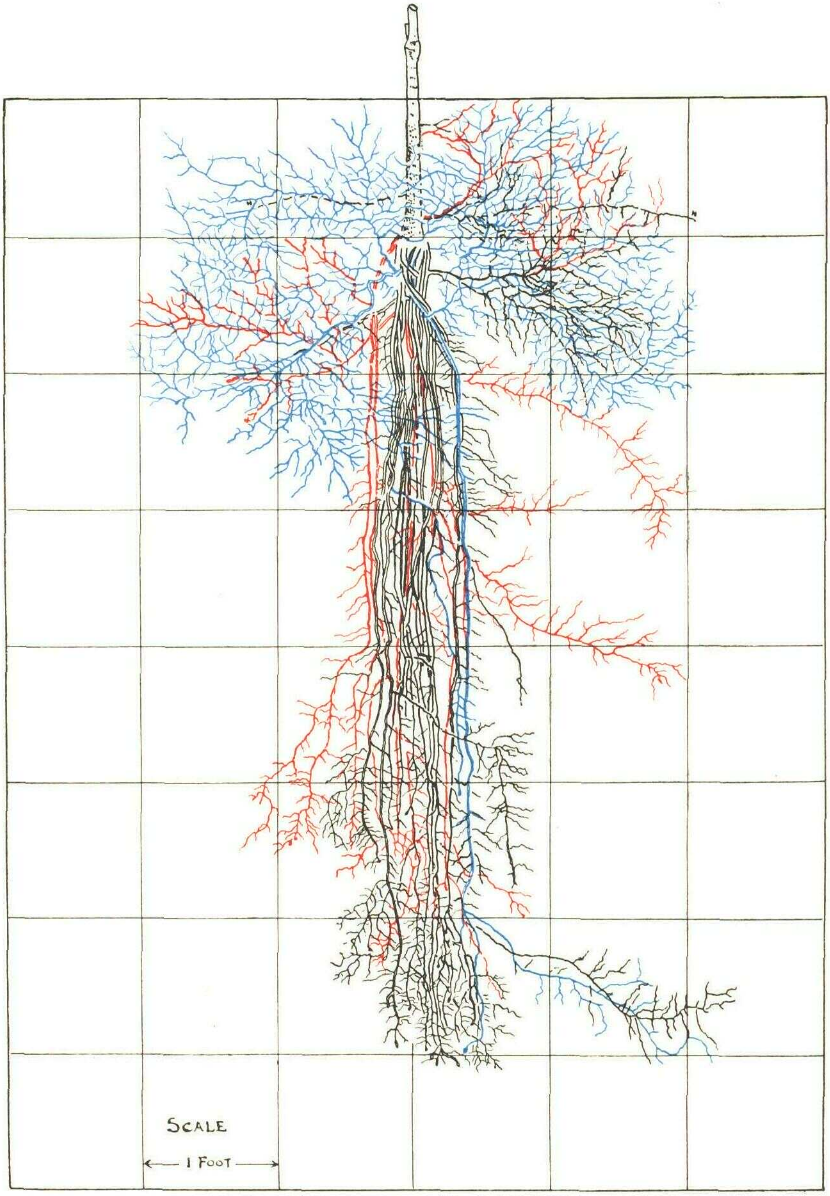
The basal cuttings from the main stems of young seedlings, which the preliminary experiments had shown to be relatively free rooting after treatment with growth substances, have been used in an investigation of some of the factors affecting rooting, such as the composition of the growth substance, the physiological condition of the cutting, the composition of the rooting medium, temperature and humidity.

*Composition and Concentration of Growth Substance.*—Cuttings removed in the late afternoon from the main stem of eighteen-months-old seedlings were treated with a number of different synthetic growth substances for 24 hours before planting.

The number of cuttings which formed roots is given below: the numbers in brackets refer to the number of roots produced on the successful cuttings upon examination eleven weeks after planting.

| | Alpha-naphthalene-acetic acid | | Indolyl-acetic acid | Proprietary Solution A | Tap Water |
|---|---|---|---|---|---|
| | 0.03 g/litre | 0.06 g/litre | 0.03 g/litre | 1 in 300 | (Control) |
| Basal Cutting ... | 3(28,8,5) | 4(19,15,12,9) | 2(5,4) | 3(12,7,6) | 1(6) |
| Cutting No. 2 ... | 3(8,2,1) | 2(1,1) | 0 | 0 | 0 |

# Plate 2




Basal cutting from one-year-old seedling showing root growth 13 months after planting. The roots shown in blue are to the front and those in red to the rear, of the central group of roots shown in black.

The results show that alpha-naphthalene-acetic acid at both concentrations used has been more effective in stimulating rooting than either indolyl-acetic acid or growth substance A.

The results of a comparative test of two proprietary preparations at different dilutions are presented below. The cuttings were lifted nine weeks after planting.

| Treatment | No. of Cuttings | No. of Cuttings rooted | Roots | | Shoots | |
|---|---|---|---|---|---|---|
| | | | Number | Wght. (gm) | Number | Wght (gm) |
| Growth substance A (1 in 640) | 6 | 4 | 22+(14)=36 | 5.8 | 12 | 100.5 |
| Growth substance A (1 in 320) | ,, | 5 | 26+(14)=40 | 4.2 | 12 | 90.0 |
| Growth substance B (1 in 900) | ,, | 3 | 15+( 8)=23 | 5.0 | 14 | 79.0 |
| Growth substance B (1 in 450) | ,, | 3 | 10+( 4)=14 | 4.1 | 10 | 62.0 |
| Growth substance B (1 in 230) | ,, | 4 | 13+(16)=29 | 2.5 | 14 | 123.5 |
| Growth substance B (1 in 115) | ,, | 4 | 14+(10)=24 | 6.5 | 13 | 143.0 |
| Tap Water | ,, | 2 | 2+( 5)= 7 | 0.5 | 10 | 62.5 |

The figures between brackets in column 4 refer to root swellings on the callus.

The percentage of rooted cuttings and the weight of roots produced have been increased by both growth substances at the concentrations used. The best rooting response, as judged by the percentage rooting and the number of roots produced, has been caused by growth substance A at both concentrations, while growth substance B at dilutions of 1 in 230 and 1 in 115 has produced the largest amount of shoot growth. The increased rooting obtained with substance A may have some bearing on the smaller amount of shoot growth as compared with the large increase in shoot growth obtained with substance B at the best concentrations.

*Physiological Condition of Cuttings.*—Cuttings from young seedlings taken at different times of the year, during the hot, dry season and during the wet months (September to November), have not shown any significant differences in rooting response. Preliminary experiments have given indications that the time of the day at which cuttings were taken from the plant might be an important factor in rooting response. Early tests indicated that cuttings made in the afternoon and treated with growth substance A showed significantly better rooting than cuttings taken in the early morning. This is being further investigated.

*Composition of the Rooting Medium.*—Better callusing and rooting has been obtained in a medium of fairly coarse-grained sand than in fine river sand or coir dust or a mixture of sand and peat (2 of sand 1 of peat). Callused cuttings planted six to nine inches deep in the poorly aerated media of fine sand and coir dust showed blackening of the callus. Coir dust had a toxic effect on some cuttings.

*Temperature and Humidity.*—The saturated atmosphere and the smaller daily variation in temperature inside a Trinidad-type propagator had no marked beneficial effect on the rate of callusing and rooting of the leafless, hardwood cuttings used in these experiments. Equally good results have been obtained from cuttings planted in sheltered beds in the open, where the rooting medium consisted of a light sandy soil. The rate of opening of buds was, however, markedly accelerated in the case of cuttings planted in the propagator.

Cuttings used in these experiments were fifteen to eighteen inches in length with an oblique basal cut, not necessarily at a node. Shoot growth invariably preceded root growth; the bases of the cuttings had in most cases formed a thin complete ring of callus ten days after planting and buds began to break about ten days later. Roots usually made their appearance from six to eight weeks after planting but in a few isolated cases root initials have been observed as early as four weeks after treatment with growth substance. No root proliferation up the stem, as has been recorded for some other plants, has been observed with the concentrations of growth substance used and roots were always produced only from the wound callus. Cell outgrowths from the lenticels were almost invariably found near the basal end of the cuttings after a few days in the rooting medium.

## (2) Cuttings from Budded Trees

Leafless hardwood cuttings from the main stem of one and two-years-old buddings and one to three-years-old branches of budded trees of various clones have shown no response to treatment with indolyl-acetic-acid and alpha-naphthalene-acetic acid at dilutions of 1 in 3,300, in 10,000, 1 in 25,000 and 1 in 33,300 of water and two proprietary growth substances at several concentrations. A very small number of cuttings developed a thin incomplete ring of callus but in no case was rooting induced. Shoot and leaf growth observed on many cuttings proceeded until the food reserves became exhausted after which the shoots withered and died.

Attempts were made to induce rooting by increasing the carbohydrate reserves in the cuttings. The main stems of a number of two-years-old buddings were ringed a few inches above the union two months before severance so that the products of photosynthesis accumulated in the stem above the ring, as evidenced by the thick callus produced and the greatly increased starch content of the wood and bast cells. After severance, the stem was made into cuttings, fifteen inches long, which, after treatment with favourable concentrations of growth substance for 24 hours, were planted in a suitable sand medium contained in a propagator. Short leafy shoots were produced on a few cuttings but all cuttings had died without callusing six weeks after treatment.

Attempts to feed cuttings by placing their basal ends in solutions supplying nitrogen and sugars, were also made. The cuttings taken from branches of budded trees were treated for 24 hours with a favourable solution of growth substance to which had been added glucose 2 per cent, sucrose 2 per cent and urea 1 per cent, singly and in combination. Taka-diastase 0.01 per cent was also used as it was thought that the enzyme, by increasing the concentration of soluble sugars near the base of the cutting, might promote rooting. The results were negative, all the cuttings died without forming either root or callus about two months after planting.

In another experiment the cuttings were treated with several dilutions of two proprietary growth substances for 24 hours and planted in the propagator. Five days later half the number of cuttings in each treatment were taken out and the basal end placed in tap water containing one milligram aneurin (vitamin $B_1$) per litre for 24 hours after which they were returned to the rooting medium. Negative results were again obtained; all treated cuttings and the controls (treated with tap water) were dead three weeks after treatment.

Softwood, semi-hardwood, and hardwood cuttings taken from branches of budded trees during the early stages of refoliation after " wintering " (when the supply of food reserves and growth hormones are optimal) and treated with growth substance have also failed to show any sign of root development.

*Treatment of ringed shoots with growth substance before severance for preparation of cuttings.*

Main stems of vigorous young buddings two years of age were suitably ringed and the rings were treated with alpha-naphthalene-acetic acid dissolved in lanolin at concentrations of 1 in 100, 1 in 300 and 1 in 500. At the lowest concentration there was a marked stimulation of callus growth; at the highest concentration callus growth appeared to be inhibited.

Six weeks after treatment the plants were cut down, cuttings were prepared from the ringed sections of the stem and planted in a propagator. The cuttings prepared from the ringed stems treated with the lowest concentration of growth substance and the cuttings treated with lanolin only showed healthy shoots at seven weeks from planting. The cuttings treated with the highest concentration of the growth substance died without shooting. None of the cuttings produced roots.

The failure to stimulate rooting in these experiments indicates that some factor or combination of factors, other than the presence of auxin, essential for the development of roots, is lacking in cuttings made from branches of Hevea or from scion shoots from plants propagated by bud-grafting. Repeated attempts with cuttings made from shoots of different ages with a wide range of concentrations of the more effective growth substances have met with no success in the stimulation of root growth from cuttings made from branches of buddings of Hevea.

### Stimulation of Rooting on Transplanting

New root formation on an older root has been shown by several investigators (Bouillene and Went (16)) to be controlled by the same factors as root formation on a stem cutting. Zimmerman and Hitchcock (40) showed that growth substances also stimulate the formation of new roots on older roots.

These findings have been confirmed in experiments on the stimulation of rooting of Hevea "stumps." A number of eighteen-months-old seedling stumps were placed with the cut ends of the tap root in a solution of a proprietary growth substance in water at a dilution of 1 in 320 for 24 hours. Control stumps were stood in water for the same length of time. The stumps were planted in beds in the open and were carefully excavated after periods of one and two months and the results summarised below were recorded.

| | One month after planting | | Two months after planting | |
|---|---|---|---|---|
| | Treated | Control | Treated | Control |
| Total No. of Stumps ... | 11 | 11 | 15 | 15 |
| No. of rooted Stumps ... | 7 | 3 | 15 | 10 |
| Total No. of Roots ... | 67 | 4 | 98 | 36 |
| Fresh Weight of Roots (gm.) | — | — | 90 | 30.5 |

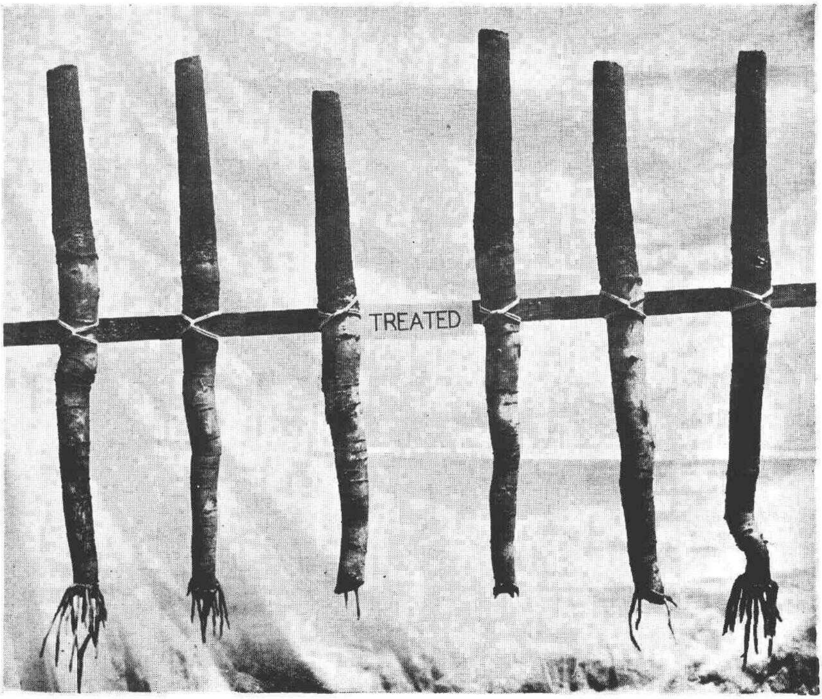



FIG. 1. Seedling stumps treated with growth substance and dug up one month after planting. Root formation is accelerated and the number of roots formed is considerably increased, compared with untreated stumps of the same age shown in Fig. 2.

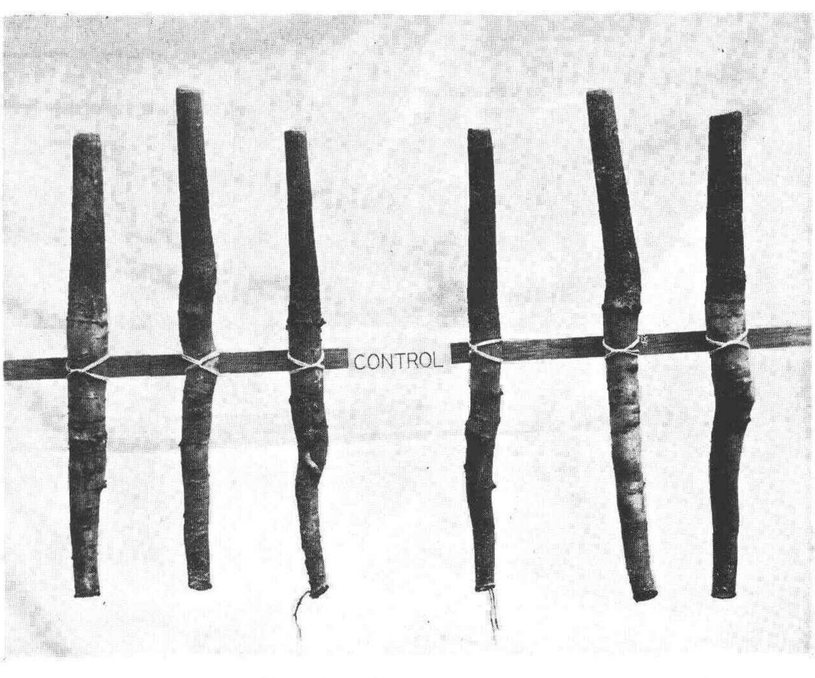
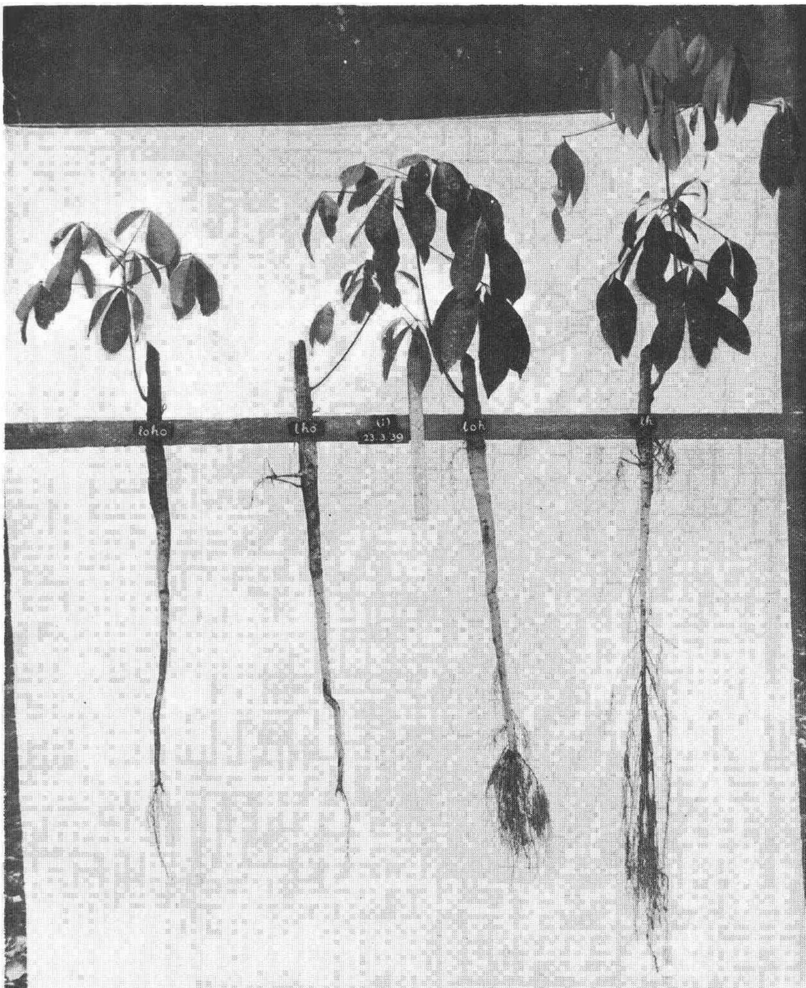



FIG. 2. Untreated stumps dug up one month after planting.

PLATE 4




Budded stumps dug up three months after planting. The two stumps on the right were treated with growth substance; the two on the left were not treated.

The results show a marked acceleration in rooting and a large increase in the number and weight of roots formed as a result of treatment with the growth substance (Plate 3).

The stumps used for this experiment were trimmed shorter than would be the case in normal planting practice but this does not affect the results obtained.

Similar experiments were carried out with budded stumps with the results shown below.

*Budded stumps planted in the field under favourable weather conditions.*

| | No. of Months after Planting | | | | | |
|---|---|---|---|---|---|---|
| | 2 Months | | 4 Months | | 6 Months | |
| | Treated | Control | Treated | Control | Treated | Control |
| No. dead ... | 0 | 6 | 2 | 8 | 3 | 8 |
| No. with bud shot | 58 | 46 | 69 | 63 | 69 | 63 |
| No. alive, bud still dormant ... | 14 | 20 | 1 | 1 | 0 | 1 |
| Average height (inches) ... | 14.6 | 12.8 | 18.2 | 14.2 | 25.0 | 22.7 |

A number of stumps in each treatment were excavated three months after planting and quantitative data of root and shoot growth are given below.

| | Roots per Stump | | Average weight of Shoot and Leaves in gm. |
|---|---|---|---|
| | Number | Weight in gm. | |
| Control ... ... | 21 | 28.2 | 51.0 |
| Treated ... ... | 12 | 14.6 | 34.9 |

The results show that treatment with growth substance has accelerated both root and shoot growth and has reduced the number of losses on transplanting. (Plate 4).

*Budded stumps planted in the field during a dry period*

| | Time after Planting | | | |
|---|---|---|---|---|
| | 1 month | | 2 months | |
| | Treated | Control | Treated | Control |
| No. dead ... ... | 1 | 3 | 3 | 36 |
| No. with bud shot ... ... | 112 | 51 | 181 | 128 |
| No. alive, bud still dormant ... | 87 | 146 | 16 | 36 |
| Average height of those shot (inches) ... ... | 9.9 | 5.5 | 17.3 | 15.1 |

These results confirm those of the first experiment and illustrate the marked influence of the treatment in speeding up the rate of successful establishment after transplanting.

The beneficial effects of treatment of stumps with growth substance are; reduction in the number of deaths, hastening of the appearance of both roots and shoots, increase in length and weight of the shoot, and increase in the number and weight of roots.*

The initial advantages of early establishment have been maintained in the subsequent growth of the stumps during a period of six months after treatment.

### Activation of Cambium

Jost (41) in 1891 discovered the stimulating influence of developing leaves on the growth of the cambium of the stem; the stimulus passing from the leaves to the stem and travelling in a morphologically downward direction. Keeble (42) ascribed this cambial activation to "chemical stimulators" and Kastens (43) suggested that it might be due to a hormone. Experimental evidence of the hormone nature of the stimulus was first produced by Snow (44) in 1933. This last investigator (45) obtained a good activation of the cambium by applying low concentrations (1 to 2 parts per million) of pure auxin-a and indolyl-acetic acid to the upper ends of decapitated Helianthus seedlings.

Van der Weij (46) first demonstrated the presence of hormone in leaves of trees. Hormone has also been extracted from sprouting buds of poplar, willow, oak and horse-chestnut by Czaja (47) and from buds of many woody plants by Söding (48). The presence of hormones in sprouting buds and young leaves and the observed effects of these substances on the activation of the cambium provide indirect evidence that cambial activity in stems of dicotyledons is governed by hormones formed in buds and

**Note*—The method of treatment has been described in detail in the R.R.I. *Planters' Bulletin*, No. 4, 1st June, 1939.

leaves. Moreover, it is known that cambial activity in the branches and stem which causes growth in thickness begins about the time of the sprouting of buds in spring when hormone production is maximum. (Koning (49); Avery, Burkholder and Creighton (50)).

### Stimulation of Bark Renewal in Hevea

Indolyl-acetic acid dissolved in lanolin at concentrations of 1 in 1,000, 1 in 5,000, 1 in 50,000 and 1 in 500,000 applied to the tapping panel and directly on to the cambium after stripping the 'bark' has failed to stimulate renewal; the thickness of renewed bark after one year was not significantly different from that of the controls.

The negative results may be due to the high concentrations used or to the physical nature of the auxin 'solvent', lanolin, limiting the access of oxygen to the actively respiring meristematic cells. This explanation would seem to be borne out by the fact that highly significant increases in the amount of bark renewal were obtained by panel treatment with palm oil which, like other vegetable oils, such as maize, sunflower, peanut, linseed and mustard oils, contains free auxin in physiological concentrations.

In another investigation* on the effect of palm oil, and mixtures of palm oil and fungicides, applied to the tapped panel of Hevea trees, significant increases in the rate of growth of renewed bark have been demonstrated during the early stages of renewal.

A common practice on native holdings, which consists in smearing the tapped panel with mixtures of clay (or earth from ant-hills and buffalo pens) and cowdung or mixtures of clay and coconut or other vegetable oil, for the stimulation of bark renewal, would appear to have a sounder scientific basis than was at first supposed. Recent research which has greatly increased our knowledge in the field of plant hormones has shown that plant growth substances are produced by numerous fungi and bacteria and that they occur in animal excrements and in vegetable oils.

### Stimulation of Callus Growth

Weisner (51) in 1892 ascribed the regeneration of callus tissue at pruned surfaces (wound-healing) to the action of substances produced by the injured cells which pass into the uninjured cells and stimulate cell division. Haberlandt (52) obtained experimental evidence that these substances are wound-hormones. He showed that the juice from crushed leaves markedly stimulated cell division in uninjured cells. These results have since been confirmed by other investigators.

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* This Journal p. 40 *et seq.*

The presence of wound-hormones has been demonstrated in leaves, fruits and tubers. A plant wound-hormone has been isolated from crushed beans by English and Bonner (53) who named it traumatin. Laibach and Fischnich (54), Rogenhofer (55) and others have observed increased callus growth after the application of a lanolin paste containing indolyl-acetic acid.

Sharp (56), investigating the effect of a number of wound dressings on the rate of healing of snags on large stocks of Hevea, obtained the best results with a mixture of cowdung and clay. Shear (57) obtained good results with lanolin (wool fat) as a wound dressing for pruning cuts on trees and attributed the beneficial effect to its protective action in preventing the drying of the cambium and newly-formed callus. Indolyl-acetic-acid dissolved in lanolin at a concentration of 1 part to 200 parts of lanolin failed to stimulate the growth of callus, presumably because of the high concentration used.

Experiments are now in progress in which pastes of indolyl-acetic acid in lanolin at concentrations of 1 in 5,000, 1 in 10,000, and 1 in 50,000 and lanolin alone are being tested as wound dressings when smeared over the exposed surface after the final pruning of stocks.

### Auxin Treatment of Seedlings

The effect of addition of growth substances to the rooting medium has been studied by numerous investigators and somewhat conflicting results have been obtained.

Kögl, Haagen Smit and Erxleben (58) showed that growth in length of roots of oat seedlings immersed in a solution of auxin is inhibited by as low a concentration as 0.1 milligram per litre, while the normal growth of the coleoptile is unaffected. Hitchcock and Zimmerman (59) reported on the effect of indolyl-acetic, indolyl-butyric, indolyl-propionic, naphthalene-acetic, phenyl-acetic and phenyl-propionic acids added to soil in which tomato and tobacco plants were growing. All acids when added at the rate of 10 milligram per pot caused bending of leaves and stems. Higher concentrations, 10 to 27 milligram per pot, caused roots to appear on the main stem, and caused considerable retardation in growth.

Loewing and Bauguess (60) grew stock seedlings in pots and found that addition of 15 c.c. of a 1 in 15,000 aqueous solution of indolyl-acetic acid stimulated the growth in length of the stem. This initial advantage was not maintained and no difference in length of stem between the treated and control plants was apparent ten days later. Greenfield (61) applied indolyl-acetic acid to stock seedlings growing in soil. Concentrations of 0.75 to 3.0 milligram had no effect, 6 to 12 mg. accelerated growth and 48 to 96 mg. caused bud inhibition, leaf bending and thickening and blanching of stems. Meesters (62) showed that the growth of roots may be strongly inhibited by indolyl-acetic acid at a concentration of 0.5 mg. per litre. Marmer (63) found that addition of the synthetic auxins, indolyl-acetic, indolyl-butyric, and indolyl-propionic acids, to water cultures in which wheat seedlings were growing had a stimulating effect on the growth of the primary foliage leaves. Growth in length of the primary root was decreased and the number of secondary roots increased. A concentration of 0.12 mg. per litre caused a 50 per cent decrease in root growth. Pearse (64) added 1 c.c. of a 0.01 per cent solution of indolyl-acetic acid daily for one week to 500 c.c. of the culture solution in which seedlings of broad bean were growing and obtained a marked retardation in growth in length of the roots, although the total root weight remained practically unaltered. The same amount of the growth substances sprayed on to the shoots caused swelling of the stem, bending of the leaves and inhibited the growth of the terminal bud, and root growth was slightly decreased. Shoot growth was retarded by both treatments. Solacolu and Constantinesco (65) found that addition of indolyl-acetic acid to bean and castor oil seedlings growing in solution culture inhibited the growth of rootlets and caused tumours to appear at the collar. Roots were formed from the tumours 24 to 26 hours after transference to an auxin-free culture solution. The same investigators (66) reported a marked stimulation of root growth in pea and bean seedlings in water after a previous immersion in a 0.2 per cent solution of indolyl-acetic acid for 15 minutes. Macht and Grumbein (67) observed a stimulation of root growth of lupin seedlings after a 15-minutes' immersion in a weak solution (1 part in 5 billion) of indolyl-acetic, indolyl-butyric and alpha-naphthalene-acetic acids. Root growth was inhibited by longer exposures or shorter exposures to higher concentrations.

Gautheret (68) studied the effect of indolyl-acetic acid in concentrations of 0.1 per cent to 0.000001 per cent on the growth of seedlings in culture solution. He observed the formation of tumours on roots and a retardation of growth with concentrations of 0.001 per cent and above. Lower concentrations caused extensive branching of the roots. Castan and Chouard (69) found that indolyl-acetic acid at concentrations of 5 to 20 milligram per litre retarded the growth of the main roots of melon seedlings.

Grace (70) reported on the effect of alpha-naphthalene acetic acid on nasturtium plants growing in sand culture. The best activation was obtained with a concentration of 1 in 10 millions applied at the rate of 50 cubic centimetres daily for 23 days. The weight of lettuce tops was increased threefold by addition of the growth substance at a dilution of 0.01 part per million of water at intervals of three days. Smidrkal (71) treated seedlings of celery with 0.01 per cent solutions of alpha-naphthalene-acetic acid, indolyl-acetic acid and indolyl-propionic acid. He obtained increases in root weight of 59 per cent, 64 per cent and 47 per cent respectively. Templeman (72) applied solutions of indolyl-acetic acid, alpha-naphthalene-acetic acid and ascorbic acid (vitamin C) at concentrations ranging from 0.002 per cent to 0.04 per cent to white mustard and barley plants by watering on to the sand in which the plants were growing and by spraying the foliage. He obtained no increases in growth.

The inferences to be drawn from these experiments are that excessively high concentrations of auxin added to the rooting medium result in retardation of growth of both shoot and roots and cause physiological responses such as bud inhibition, bending of leaves and stems, loss of chlorophyll, and formation of tumours on roots. Extremely dilute auxin solutions accelerate the growth of roots.

The effect of pre-treatment of seedlings with auxin is seen in a marked stimulation of growth when the seedlings are transferred to a medium free from auxin. It would seem therefore, that synthetic auxins stimulate the production of roots but retard their growth when in direct contact. Soil treatment is moreover costly, as large volumes of the active solution are required. Evidence has been obtained of the inactivation of auxins in the soil.

Preliminary experiments have been carried out to test the effect of growth substance on the growth of young seedlings of Hevea. The seedlings were growing in baskets and the solution of growth substance was applied to the surface of the soil in the baskets. Twenty-five two-months-old seedlings were treated each with 500 c.c. of a solution of a proprietary preparation at a dilution of 1 in 300 of water. The control consisted of the same number of seedlings treated with 500 c.c. of tap water.

| Treatment | Mean Height at Time of Treatment | Mean Height after 3 months | Increase |
|---|---|---|---|
| Growth substance ... | 13.1 inches | 17.9 inches | 4.8 inches |
| Tap water ... | 13.9 ,, | 18.7 ,, | 4.8 ,, |

Twenty-five one-month-old seedlings were treated with 50 c.c. of the solution of growth substance at a dilution of 1 in 150 of water.

| Treatment | Mean Height at Time of Treatment | Mean Height after 2 months | Increase |
|---|---|---|---|
| Growth substance ... | 10.6 inches | 16.7 inches | 6.1 inches |
| Tap water ... | 9.5 „ | 16.0 „ | 6.5 „ |

The results of these experiments show no indication that the growth substance has stimulated growth.

### Auxin Treatment of Seeds

The practical disadvantages of seedling treatment are not encountered with seed treatment, which consists in soaking the seeds for a specified time in solutions of growth substance prior to planting in the field.

Cholodny (73) has reported large increases in growth and yield as a result of soaking oat seeds in a solution of indolyl-acetic acid (0.01 to 0.02 per cent) for 24 to 48 hours before planting out. Davis, Atkins and Hudson (74) found that pretreatment of oat, mustard and cress seeds with indolyl-acetic acid or indolyl-propionic acid at concentrations of 0.1 per cent and 0.001 per cent retarded germination. Ascorbic acid (Vitamin C) at these same concentrations stimulated germination. Grace (75) observed a stimulation of both root and shoot growth in wheat and barley as a result of seed treatment with indolyl-acetic acid or indolyl-propionic acid or indolyl-butyric acid or naphthalene-acetic acid incorporated in absorbent dust (talc) in concentration of five parts per million. In a further communication (76) Grace reported that the decreased germination and retarded growth of the seedlings after seed disinfection with formaldehyde is largely overcome by treatment of seeds with the disinfecting solution containing 0.01 to 5.0 parts per million of indolyl-acetic acid or naphthalene-acetic acid. Solacolu and Constantinesco (77) found that pretreatment with 0.01 to 0.04 per cent solutions of indolyl-acetic acid retard the germination of bean, cucumber, sunflower, castor oil and pea seed and cause roots to appear on the aerial parts of the plants.

Cajlachjan and Zhdanova (78) reported that soaking seeds of wheat, oat, millet, vetch, hemp, white mustard, perilla, flax and peas for 24 hours in 0.01 per cent, 0.025 per cent and 0.05 per cent solutions of indolyl-acetic acid in water hastened the maturation of the plants produced from them. The growth of oats was accelerated slightly and that of flax and vetch partially or totally inhibited. Thimann and Lane (79) treated seeds of oat and wheat with high concentrations of indolyl-acetic acid (0.01 per cent) before planting in soil and obtained a marked increase in the percentage germination, in growth in length of the stem, in yield of grain, in dry weight of straw and roots, and in the number of roots formed. Shibuya (80) broke the dormancy in ground nut seed by applying growth substance to the wounded radicle. Amlong and Naundorf (81) claimed that soaking sugar beet seed in 0.175 per cent indolyl-acetic acid for 24 hours prior to sowing resulted in 157 per cent increase in root weight, 129 per cent increase in leaf weight and 123 per cent increase in the total yield of sugar. The percentage germination of twelve poorly germinating seed types was increased by soaking for 24 hours in solutions of each of the following growth substances: indolyl-acetic acid (0.0175 per cent), alpha-naphthalene-acetic acid (0.16 per cent), and indolyl-butycric acid (0.02 per cent). The increases were 85 per cent, 89 per cent and 106 per cent respectively. Templeman (82) in a series of carefully controlled experiments obtained no growth stimulation after soaking white mustard seed in solution of indolyl-acetic acid (0.005 to 0.05 per cent) or alpha-naphthalene-acetic acid (0.0005 to 0.005 per cent) for 24 hours. The dry weight of the tops was decreased in all cases.

Results obtained by different investigators on the effect of growth substance on the germination of seeds and subsequent growth of the seedlings are conflicting. Incomplete information as to the conditions of the experiment renders judgment difficult. Standardisation of technique, and controlled experimentation by workers on the subject are pre-requisites for a full understanding of the factors involved.

Work on auxin treatment of Hevea seeds to improve germination and the growth of the seedlings is now in progress. Increased germination of seed produced late in the season when the percentage germination is generally low may be of considerable economic importance. Further work on the treatment of seeds of cover plants will also be undertaken.

## Other Sources of Growth Substances

The occurrence of plant-growth substances in soil organic matter was first demonstrated by Bottomley (83) who showed that "bacterised peat," peat inoculated with certain aerobic soil bacteria which decompose it rapidly, is a source of substances which stimulate the growth of higher plants. These substances, which are active in very minute quantities, were named by Bottomley "auximones" (Greek *auximos*=promoting growth).

More recent research has shown that such growth substances are in fact produced by numerous bacteria and fungi in soil, in farmyard manure, and in culture. It has been shown that tryptophane, an amino-acid present in plant residues and in excretions of animals, is acted on by bacteria and fungi to produce an auxin, indolyl-acetic-acid, and that certain fungi in culture produce, besides auxin, other growth-promoting substances such as bios and aneurin (vitamin $B_1$). The important part played by fungi and bacteria in the decomposition of plant residues may account for the relatively high production of growth substances in farmyard manure and compost heaps. Fungi and bacteria may therefore influence the development of higher plants by producing plant growth-promoting substances.

Certain proprietary preparations consisting of pure cultures of bacteria such a *Azotobacter chroococcum* or *Pseudomonas fluorescens* in a medium of unsterilised peat have been shown to stimulate markedly the growth and yield of plants.

## Summary

A brief survey of the literature tracing the development of research on plant hormones is given.

The first isolation of a plant hormone by Went in 1926 and the synthesis of three auxins by Kögl and his co-workers (1931 to 1933) was followed by important work done at the Boyce Thompson Institute in America. This resulted in the discovery that a number of organic acids and their esters not known to occur in plants, and of different chemical constitution from the auxins, are active in stimulating plant growth, and thus led to the practical application in horticulture of the results of academic research in plant physiology. The numerous proprietary "synthetic auxins" now on the market have been developed as a result of this work.

The functions of hormones in plants, their rôle in photo-tropism (response to light), geo-tropism (response to gravity), cambial growth, callus formation, root formation and growth are described and the results of preliminary experiments on Hevea are recorded.

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