VEGETATIVE PROPAGATION OF USEFUL PLANTS.

By

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I.

VEGETATIVE VERSUS GENERATIVE PROPAGATION.

The generative or sexual propagation always depends upon the production of flowers and fruits, involving the fusion of special cells, (or cellstructures). This leads in the higher plants to the formation of an embryo, inside a seed, the starting point of a new plant. The completion of the sexual phase of the life cycle always requires months and often years.

Vegetative or a-sexual propagation, in contrast, does not require such seed formation, nor such time; reproductive cells are formed, without a previous fusion, and tissues are built up, without preceding flower-and fruit formation, through stimulation of the cambium or other embryonal tissue. There is evidence, I wrote 15 years ago, (after studying recorded research, the practice in tree surgery, and carrying on some experiments myself), that embryonal tissue is well distributed in plant life; this embryonal tissue will develop to new organs, should conditions be suitable for bringing this about. To emphasize the marvellous function of the cells which form the cambium or the embryonal tissues (thin-walled, undifferentiated, plastic, usually unencumbered by vacuoles, and secretory inclusions) the author is tempted to refer to them as the "Divine Cells." [1]) Surely with their help, so it appears even more obvious to-day, the process of growth can be speeded up and superior strains or varieties can often be more quickly produced and more cheaply maintained.

In a practical way, this vegetative propagation can be brought about as the result of experience gained mainly with plants of horticultural interest: namely, 1) by separation of bulbs, buds and tubers; 2) by division of tubers, roots, root stocks, plants and suckers; 3) by basal and lateral shoots, by layers and runners; 4) by cuttings of tubers, roots, leaves, stems and plants; 5) by pruning and forced removal of leaves or bark; 6) by budding and grafting. In addition, seed development without fertilization,—(apogamy or somatic parthenogenesis), another form of vegetative propagation—is possible, occurring in numerous compositae, e. g., dandelion or Taraxacum. The propagation by cuttings has been used by gardeners as a means of preserving choice varieties, preventing thus the mixing of strains, likely in sexual reproduction, or preventing the reversion into the component species of hybrides with the composite desirable qualities. The art of budding, grafting (and pruning)—by at least as many as 137 different methods— has firmly established the value for improving and maintaining choice varieties, especially of fruits such as apples, oranges, grape-fruits in the old and the new world. Sour orange stock, highly resistant to the disease "gummosis," is now extensively used, at least in British colonies, for citrus propagation. It is even claimed that "topworking" permits the conversion of all but very old citrus trees to any desired variety of citrus.

The following disadvantages have been pointed out: Through continued vegetative reproduction the leaf tissue of young plants of the grape vine (Vitis vulpina) became constantly smaller, as observed in tissues up to 60 years old. For certain plants, as the apple, continued vegetative propagation is believed to cause loss in vigor and fruit bearing. For others as the hollyhock (Althaea rosea) prolonged cultivation from cutting is believed to have resulted finally in increased susceptibility to fungus disease of the rust Puccinia malvacearum. This even threatened to extinguish the species, until seedlings were again grown, which appeared to give more resistent plants. In contrast to these findings, many species and varieties have been kept in cultivation for many generations of plants, exclusively by vegetative propagation (such as bananas), which show no sign of deterioration.

Chromosome-Mechanism of Inheritance.

Each plant cell of lower and higher plants has in its cell nucleus a definite number of granular units, taking up dyes and thus called chromosomes. In nuclear division the chromosomes are split, divided into two, transmitting to the daughter nuclei the same characteristics. Each cell with a nucleus can grow up to a plant of the same kind. In the vegetative propagation the same facts of inheritance prevail as in the ordinary nuclear and cell division; individual changes of the stock are retained in the plants produced.

As an example saffron (Crocus sativus L) may be mentioned, having apparently been propagated for several thousand years exclusively by vegetative reproduction. Although the ovary is developed and mature pollen grains are formed, these do not fertilize another flower, and thus plants are sterile—on account of clone-formation. The sexual propagation is abandoned because of too close a relationship. Vegetative propagation permits the fixation of mutation, a change that is anchored in the chromosomes—and utilized in varieties of fruits, vegetables and ornamental plants with a peculiar growth.

The generative or sexual propagation involves in the fertilization process the division into half the number and then the fusion of the divided nuclear elements (chromosomes) of the pollensperm with those of the egg cell. This combines the characteristics of the male and the female plant and thus tends to equalize individual differences in the new plant, resulting from the fusion.

II.

FUNDAMENTALS AND FACTORS OF GROWTH.

1. Stimulation of Growth.

A. Medicinal Plants: My first experience in growing useful, and in particular medicinal plants by vegetative propagation goes back over 15 years. [1)] Then I obtained, contrary to the expectations of our experienced gardener in the Experimental Gardens of the Phila. Coll. Pharm. & Science, and for the first time to my knowledge, new plants from leaves and leaf fragments of Digitalis, the heart tonic, well-known in medicine; of Belladonna, the narcotic stimulant; of Artemisia cina, the vermifuge and others. A few years ago—after many failures—I succeeded in regenerating the tissue of various leaves of common house plants. This tissue had been purposely removed in order to follow the subsequent formation, with observation through windows, of new cells and cell groups. Favorable soil conditions, such as texture, reaction, moisture and soil-life, and of atmospheric conditions such as temperature and humidity, were readily recognized as important factors, contributing to the success of propagation and regeneration. A rather recent survey of data concerning the generative and especially the vegetative cultivation of medicinal plants is recorded on Table I in tabulated form for quick orientation. (The review of literature, including esp. American, is being continued.)

TABLE I.

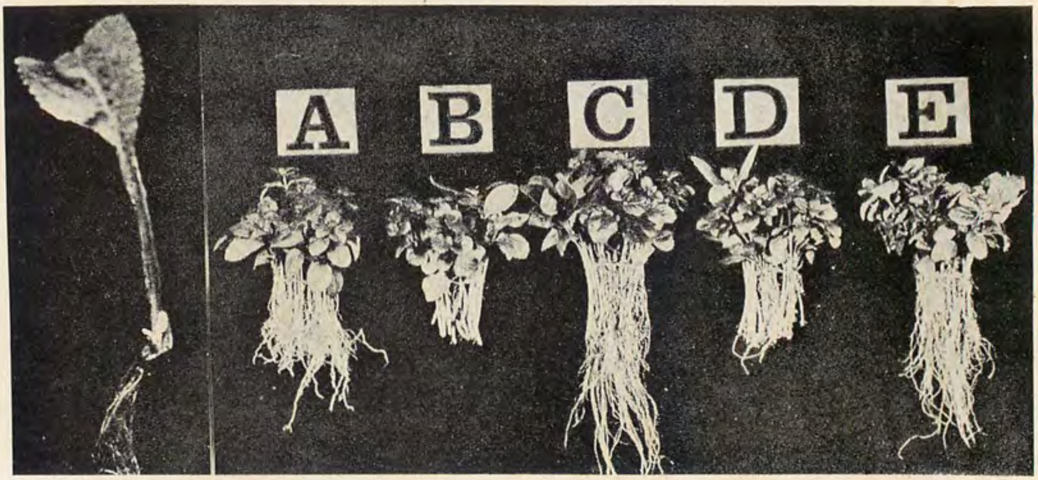
PROPAGATION OF MEDICINAL PLANTS [2]

Scientific Name	Seeds	Separation: Bulbs	Separation: Buds	Division: Tubers	Division: Roots & Root Stocks	Division: Plants & Suckers	Shoots: Runners	Shoots: Shoots	Cuttings Stems	Remarks
Aconitum L.	+ Germinating after frost			+						
Althaea off. L.	+							Young Basal	?	Shoots 1-7 cm long, cut in late fall; planted out in spring.
Althaea rosea L. (C)	+					+			+	Division of plant stock in spring.
Artemisia absinth. L.	+				+					
Datura Stramonium L.	+ 1) Brown stage 2) Black stage Maturity									Treat seeds of black stage 2 hrs. with very dil. nitric acid, wash, dry enough for sowing.
Hydrastis can. L.	+		on roots + root stocks		+					Division of root stock in fall. Buds of roots and old rhizomes.
Iris florentina L.					+ with roots					Rooted root stock.
Iris germanica L.					,,					
Iris pallida Lam.					,,					
Inula helenium L.	+				+					Root stock division in fall.
Lavandula spica L.	+					old plants				Better through division of old plants in fall; cutting back of plants in fall for stronger development.
Levisticum off. K.	+					+				
Melissa off. L.	+				+					
Mentha crispa L.							+			1. Division of older plants. 2. Runners planted in fall.
Mentha piperita L.									+	Cuttings in spring.
Orchis morio L.	+	+								Cultivation of bulbs, spreading mycelium for growth of seeds in soil.
Paeonia off. L.	+	+								Division of roots in fall.
Rhamnus frangula L.									+	Stock division. Plant in moist soil.
Rheum palmatum v. t. M.	+							Lateral		
Rosmarinus off. L.	+								+	10 cm. long cuttings separated from the mother stock in May.
Salvia off. L.	+								+	Division of old stock in fall.
Sambucus nigra L.						+		+		1) Old shrubs are divided. 2) Vigorous shoots (20 cm.) are planted as cuttings.
Thymus vulg. L.	+				+					Division of old stock.
[illegible]	+				+					Division of root stock

I.

Vegetative Propagation.

1.



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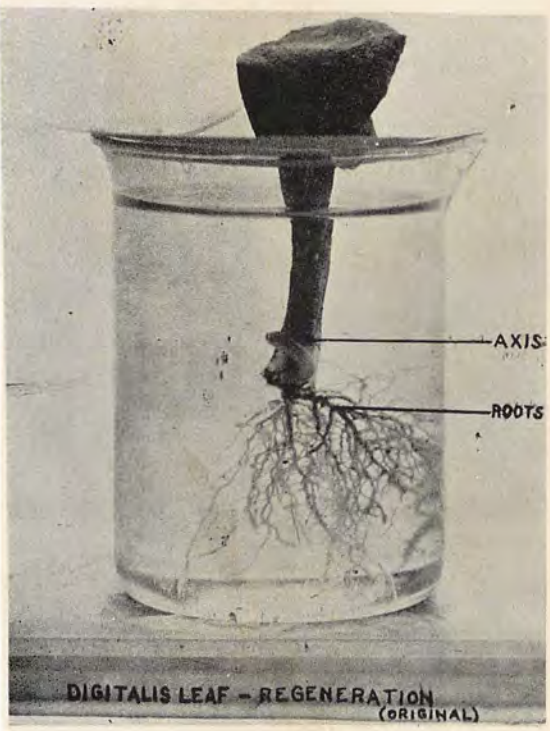

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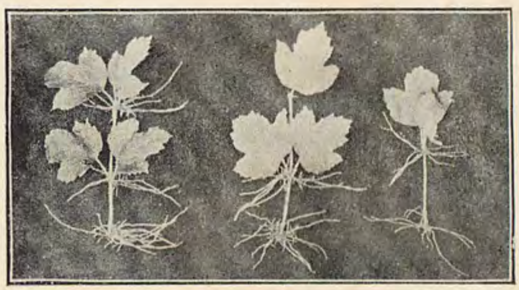


4.

5.

1—2. Digitalis (Foxglove) growth on leaf base, detached from plant rooted in Hyper Humus p H

3. Mentha piperita (Peppermint). Cuttings rooted in different media: A, sand medium (p H 7.0 +); B, mixture of peatmoss and sand (p H 4.1-4.4); C, peatmoss medium (p H 3.6); D, neutral peatmoss (p H 7.0-7.3) for ten days, then transferred to peatmoss (p H 3.6); E, peatmoss (p H 3.6) for ten days, then transferred to neutral peatmoss (p H 7.0-73), (after Hitchcock).

4. Viburnum opulus (Snowball); Roots arise uniformly at nodes of cuttings. (after Zimmerman).

5. Lobelia inflata. Regeneration and rejuvenation after complete removal of leaves (after Molisch).

The list, we are confident, can be much extended. Of special significance is the success of vegetative propagation of peppermint (Mentha piperita), rooted in different media in experiments by Hitchcock on rooting response. [3] (see Plate I Fig 3.) The success is all-important for the maintenance of the superior strains, giving high yields in peppermint oil and menthol. Propagation by seed would immediately cause a split of "piperita" into ancestral forms of the triple bastard —with 3 kinds of parents: Mentha aquatica, a pure species, crossed with a bastard of Mentha spicata (Mentha sylvestris) and Mentha rotundifolia. The vegetative propagation is to be added to the list of snow ball (Viburnum opulus), showing a uniform root growth at the nodes of the cuttings, and of Lobelia, the latter greatly rejuvenated by the complete removal of all leaves from the full-grown plant. (see Plate I, Figs. 4, 5.)

Tschirch, writing in 1930 on the subject of medicinal plants, concludes surprisingly that vegetative propagation, in general, is only possible with perennial plants. [4] Propagation by cuttings, often possible in woody tissues, is scarcely used in European medicinal plants.

Cinchona, the source for quinine, deserves special attention. 1. The grafting of the high quinine yielding C. ledgeriana upon the sturdier and more disease-resistant C. succirubra, has resulted in a vigorous hybrid. 2. The possibility of controlling the chromosome numbers by chemical means, in order to obtain a further improvement, has also been considered. 3. The forcing of new shoots from the root stubs, after coppicing (felling) of the tree, has led to new vegetative growth. The Jesuit monks in South America required from the cascarilleros the planting of 5 cuttings for each cinchona tree felled. 4. The removal of the bark in strips led to the formation of "Renewed Bark" from cambium, richer in alkaloid inasmuch as the cortical parenchyma contains the alkaloid.

Cork. New "female" cork from the corkoak is vegetatively produced (regenerated) after the removal of the "male" cork by the phellogen or corkmeristen every 8 or 9 years.

B. Economic Plants: The problems of vegetative propagation and of regeneration of economic plants have received much consideration, especially from horticulturists and plant physiologists. Much progress has been recorded in connection with propagation of soft, and hard wood cuttings, leaf and root cuttings, with root development of transplanted seedlings, with budding and graftings, especially in connection with the administration of special growth substances. Thus it appears more success has been possible with stem cuttings than with root cuttings, although new root growth has definitely been stimulated by the treatment, preceding or following transplantation, with various chemicals, referred to below. The propagation of leaf buds (leaves with axillary buds and smaller portions of stem tissue attached) has been more successful than of the leaf cuttings. The vegetative propagation of important tropical plants as the Hevea rubber plants, of coffee and cocoa, and thus the cheap and rapid production of high yielding strains has been undertaken; also the exploratory application of growth substances. However much further work appears necessary.[5)]

With tobacco, for instance, topping is practiced by the Philippine growers, (e.g. the removal of the flower head by pinching); and suckering (the removal of all suckers or buds at leaf axils). In weak plants, thus topped, the plant food helps the development of the standard leaves. In vigorous plants, however, topping should be deferred until 1) the standard leaves are harvested, which would otherwise become coarse and thick, 2) the plants for seed production have been selected. The suckers, chiefly formed after topping, are removed, in "suckering" when they appear, in order not to deprive the plant of much food. A few are permitted to develop, if the main plant has been damaged, or after harvesting of the standard leaves, as basal suckers for the production of filler and binder. [6)]

In tea and cinnamon topping and pruning is practised, thus forcing the treelets to assume bush form. In cocoa the typical shoot is cut in order to keep the tree lower and broader. The two or three equally developed basal shoots are forced to grow, causing the formation of 2-3 fork-like little trees.

When trees, such as oaks and elms, die, they may often be rejuvenated by lowering the whole head below the dead part.

2. Auxins—Growth Stimulating Substances.

The first growth-promoting substance, called "rhizopin" was isolated by Nielsen in 1930 from the common breadmold Rhizopus and another fungus.[7)] Rhizopin was later identified by Koegl as almost certainly identical with 3-indole acetic acid.[8)] The first phytohormones or "Auxins," as substances with growth stimulating effect, have been isolated by Koegl and coworkers[9] from natural vegetable and animal sources. The most important ar: 1. auxin-a., auxentriolic acid, a monocyclic trihydroxy-monocarboxylic acid, 2. auxin-b, auxenolonic acid, with like properties, with one hydroxyl group replaced by a carboxyl oxygen, another by hydrogen. Malt and oils of maize, peanut, mustard, sunflower, corngerm, flax, as well as human urine are more or less rich sources. In addition 3. heteroauxin: b-indole acetic acid in yeast and also in urine, has marked auxin properties.

IMPORTANT AUXINS.[9, 10]

Characteristics	Auxentriolic acid (auxin a)	Auxenolonic acid (auxin b)	3-Indole acetic acid (heteroauxin)
Empirical formula	$C_{18}H_{32}O_5$ (V)*	$C_{18}H_{30}O_4$ (IX)	$C_{10}H_9O_2N$ (XI)
Molecular weight	328 (V)	310 (IX)	175 (XI)
Crystal characteristics and melting point.	Crystals 196° (V)	183°	164-165°
Dissociation constant	$K=1.10^{-5}$ (XVI)		
Specific gravity ..	=1.292 at 19° (IV)	=1.269 at 20° (IX)	
Specific rotation ..	$(a)^{20}_{D}=3.19$ (V)	$(a)^{20}_{D}=2.79°$ (IX)	$(a)^{20}_{D}=3.8°$ (XI)
Solubilities	Readily soluble at low temperatures in methanol, ethanol, and ethyl acetate; not readily soluble in ether; ca. 1% soluble in cold water; practically insoluble in petroleum, ether, ligroin, benzene (V)		
Other characteristic	Thermostable; not decomposed by light; becomes physiologically inactive as a growth promoter after a few months, by isomerization, even if kept under vacuum in the dark (V)	Isomer of auxin a lactone; light- and heat-stable; crystals lose activity in a few months by isomerization; easily oxidized; first prepared from corn-germ oil (IX)	Probably produced by micro-organisms (XIII)
Acid and alkali sensitivity.	Stable to acid; sensitive to alkali (XII)	Sensitive to acid; sensitive to alkali (XII)	Sensitive to acid; stable to alkali (XII)

*Roman Numerals refer to Number of published contributions (Mitteilungen) of Koegl and Co-workers.

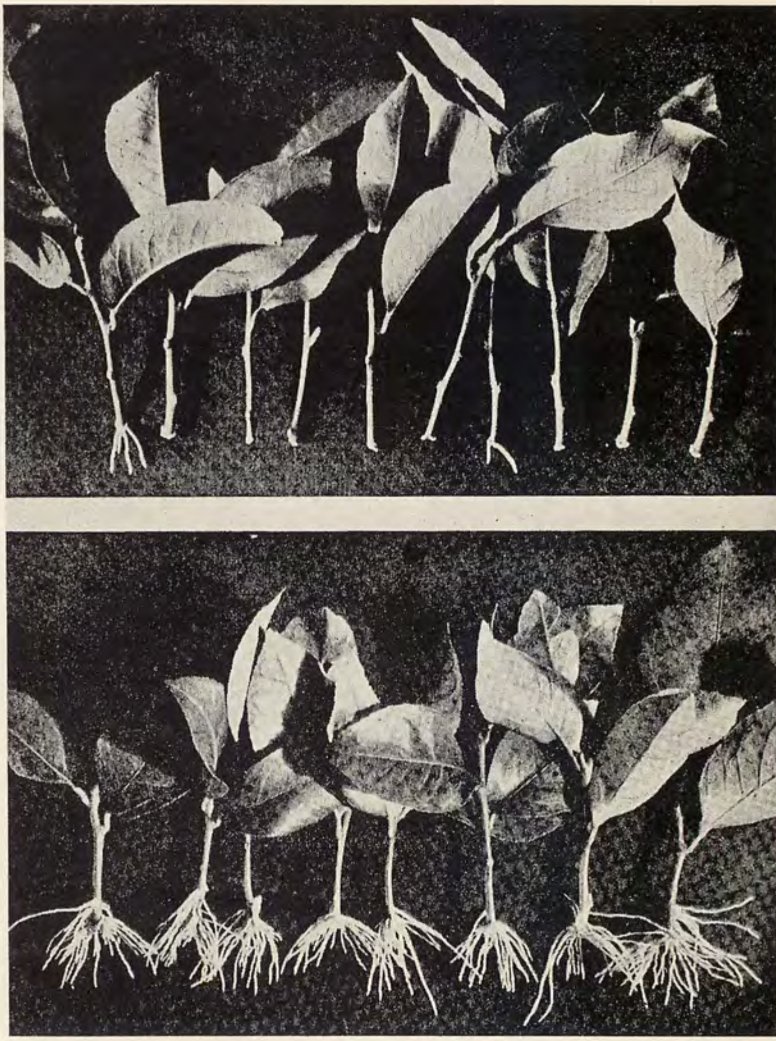
Many additional substances, often called growth hormones, have been studied for possible growth-promoting effects, especially by workers (Hitchcock, Zimmerman and others) of the Boyce Thompson Institute for Plant Research in Yonkers, New York.[11] Many of these substances used are,. however, foreign to plant-and animal life, and thus hardly represent hormones, (which have originally been defined as substances, produced in one part and transferred into another part of the same organism, where they exert specific physiological effects). Many experimenters have agreed that auxin increases 1) the percentage of cuttings rooted, 2) the number of roots produced per cutting, and 3) shortens the time for rooting. Indole-acetic acid, indole-butyric-acid, and naphtalene acetic acids are now believed to be the most active. They are usually applied in amounts of 0.01— to 0.5 mg. to 1 cc.

Cooper (1935) obtained excellent root formation on cuttings of lemon, Acalypha, Lantana, and fig by apical application of auxin in lanoline.[12] Subsequently the fact, that high concentrations of auxin cause root formation when applied at the base has been utilized successfully by Hitchcock and Zimmerman (1936) and Cooper (1936) for cuttings of Ilex, Taxus, Hibiscus, Pachysandra, lemon, Chrysanthemum, and some other plants (see Plate II).[13] Private reports from, a number of horticulturists have already extended this list considerably. In general the highest non-toxic concentration of indole-acetic acid, dissolved in water, will give best results. This concentration varies for different plants, and is lowest for green cuttings. A treatment with 0.2 mg. per cc. for 12 to 24 hours has been recommended. Before large-scale applications can be made the toxic limit for such species to be treated should be ascertained. For treatment by the lanoline method a concentration of about 1 mg. indole-acetic acid per gram of lanoline is considered satisfactory,[10] state the authors in their comprehensive survey, from which some of the preceding and subsequent information is here quoted.

Production and Movement of Auxin.

Some of the methods used by gardeners to induce root formation bring about production and movement of auxin (see Bouillene and West, 1933).[14] An example practised in parts of Holland and Scotland is the insertion into the apical slit end of cuttings, of a germinal wheat seed. Generally in non-deciduous plants leafy cuttings are used,

II.
Vegetative Propagation.



Citrus vulgaris (Lemon).

Root cuttings. Upper row, 8 hours in tap water; lower row, 8 hours in indole-3-acetic acid (highest non-toxic concentration, 500 mg. per liter). Photographed 17 days after treatment. (After Cooper).

probably because no auxin is stored in their stems. In deciduous plants leafless cuttings are preferred because of the difficulty with water supply to the leaf, but here the bud acts as auxin supply. As one of the numerous examples might be mentioned the holly cuttings of Zimmerman & Hitchcock (1929); leafless cuttings of the deciduous Ilex verticillata will root, but those of the evergreen varieties will not.[15]

Layering probably depends for its success on the retarding influence on auxin transport exerted by high humidities together with the geotropic accumulation of auxin on the lower side of the stem. Rooting takes place usually at nodes probably because there the transport of auxin is interfered with. (Clematis is an exception, rooting better at internodes). The practice of ringing branches, either by cutting the cortex or by tying a tight wire round during growths obviously operates in the same way. The optimum time of the year to take cuttings varies from plant to plant (Graham, 1935) and depends upon a number of factors such as auxin production, storage, and destruction, as well as water supply and ease of wilting.[16]

Among the causes of failure of cuttings to root, when treated with auxin, one of the most important is doubtless furnished by those plants in which not auxin, but one of the other factors is limiting. Another cause is the loss of the applied auxin or other factors by exudation from a cut surface; cuttings which root with difficulty are frequently those from which much exudation takes place. Lastly, the inactivation of auxin, at the cut surface, by enzymes, freed in wounds, doubtless also plays a part. Of particular interest is the destruction of auxin by ultra violet light and the consequent auxin deficiency in the dwarf plants of mountain tops.

3. SPECIFIC GROWTH FACTORS.

From a comprehensive survey of literature, including both original contributions and critical reviews, it is obvious that we must accept the existence of specific growth factors, as well as of hormone-like agents, which initiate or stimulate the growth of roots, shoots, stems, leaves, flowers and fruits.[5,10,17-22]

In such an enumeration we may list

1. The "Auxins" effecting mainly the initiation of root formation. Much information has been collected on their formation,

transportation, mechanism of action, inactivation and chemical relationship. Tryptophane is the most effective amino acid, stimulating root formation, likely due to its conversion by the plant into indoleacetic acid. [23] Boron is possibly an essential element for the formation of auxin. [24]

2. Carotin, the pro-vitamin, recently referred to as a growth factor. [25]

3. Thiamin (B_1) or its pyrimidinic constituent, thiazole, a growth stimulant. In its presence even leafless hardwood cuttings of plants could be successfully rooted, which otherwise would not root at all. Seasonal influences or spontaneous rooting are now believed to be caused by differences in amount of these growth factors, 1 and 3, in the cuttings when they are separated from the plant. With the timely application of thiamin even the increased growth of shoots may be expected. [18]

4–5. Vitamin B_2, B_6 and nicotinic acid, biologically active substances, having a proven stimulating effect on growth. [18, 26]

6. Ascorbic acid (Vitamin C), an efficient growth factor, especially tested on isolated plant-embryos. [18, 27]

7. Pantothenic acid (one of the group of biosfactors necessary for growth of yeast), with apparent favorable effect on the growth of seedling shoots. [28]

8. Bios, biotin and auxin, promoting yeast growth and increasing root growth. [10]

9-10. Folliculin, and theelin (oestrin), animal sex hormones, improving vegetable growth and especially the latter root formation. [10, 25]

11. Rhizocaline, effecting rooting response of cuttings, under influence of leaves (6–8 optimum).[29.30]

12. Caulocaline, moving into the apex of the stem, causing its growth.[31]

13:. Phyllocaline, believed to be essential for leaf growth, the concentration of the calines, controlling the development. [31]

14. Purins, especially adenine, tested on Raphanus leaves, were active as leaf growth factors. [32]

15. Vernalin, the flowering hormone, found early in annual plants, and in biennial plants, after exposure of growing points to low temperature.[33]

16. Florigen, another flowering hormone, formed under right light (photo periodic) conditions.[33]

17. Caprocaline, presumably moving into the ovary of the flower, there causing growth of a fruit.[34]

18. Traumatin or traumatic acid,1-decene,-1.10 dicarboxylic acid, possibly identical with Haberlandt's wound hormone.[35]

Conclusions are drawn particularly by Bonner that:—

1) Dormancy can likely be controlled and removed, so that dormant buds and twigs can be stimulated by these internal growth factors to resume growth.

2) The formation and growth of roots can now be rather exactly regulated.

3) Stem growth can be regulated to a considerable degree.

4) Initiation of growth of buds, growth in thickness and leaf growth is now possible.

5) Stem growth is not yet under control, and that the initiation of flower buds and growth of flowers, and fruit development are problems yet to be solved, though special growth factors are here also involved.

In this connection it is of interest that the Plant Hormone Committee, organized 1939 in Great Britain and including scientists, nurserymen, horticulturists, has under consideration a scheme for compiling a propagation index. It is proposed to include for each species or variety particulars of the locality, time of year, as well as of the nature, concentration and mode of application of any plant hormone which had induced a beneficial stimulus on the rooting of cuttings. It is hoped that the index, which will be on cards in the first instance, will enable more specific directions to be given to those who want to know how to propagate any particular species. Information for inclusion in the index will be taken from published records, as well as from any reliable unpublished data which may be submitted to the Committee. The Secretary of the Plant Hormone Committee solicits particulars for inclusion in the index, at the Royal Botanical Gardens, Kew, Surrey, England.

4. CONTROL SUBSTANCES.

Chloral hydrate,[36] the synthetic hypnotic, and colchicine, the toxic ingredient in the so-called autumn lilly (Colchicum autumnale)—

useful in the treatment of rheumatic afflictions—have been found to stimulate nuclear divisions in all tissues, where divisions normally occur. Colchicine is also said to accelerate plant growth. It has been found to inhibit spindle formation and thus to double the chromosome number in 41 different species, 24 genera, 14 families. Among these are the Cucurbita pepo, Cannabis sativa, Plantago, Nicotiana tobaccum and Datura stramonium.[5, 36-38]

Even spraying of chickweed with colchicine solution caused doubling of chromosomes, characteristically broader leaves, thicker stems and leaf stalks. "We have now," stated Blakeslee, "the opportunity to make new species to order." Acenaphtene (C_{12} H_{14}) and anethole, the main constituent of the volatile oil of anise and star anise, had a colchicine like action.[39-41, 42]

In addition to the relationship of the number of chromosomes with the vigor of plant species, Bonisteel considers it possible to increase the toxicity of aconite: diploids were found non-toxic, while the triploids and tetraploids contained the toxic alkaloids.[43]

5. Food Factors.

In addition to the specific growth factors (and 11, well-known food elements and trace elements) we must account for the effect of some important food factors. Their interaction may be different in higher—and lower plants.[44]

Sugar is a major component of the food factor complex, as emphasized by Went.[21, 31.] The beneficial effects of auxins and sugar on growth are interdependent; light brown sugar was more favorable for the growth of excised tomato roots, than pure cane sugar in a solution containing sugar, minerals and thiamin. Sugar must be added to etiolated plant cuttings.[10]

Alcohol: Pretreatment of sugar cane with dilute alcohol, up to 10%, caused a marked increase of root production, due to its being readily available as a high energy containing food.[45]

Vitamin B_6 (adermin) increased growth, when added to solutions of sugar, minerals, thiamine, with reduced brown sugar content.

6. Protoplasma.

Finally we must recognize (states the well-known plant physiologist Jost, as quoted by Swingle,[5] that there are a large number of substances which work similar to auxin, and which have various additional effects on other processes. Cell elongation, which was supposed to proceed only in proportion to the amount of auxin present, has been demonstrated to proceed in the root entirely without this material. The protoplasm, which for a time seemed to be "shelved", has again been returned to its old importance, ... ". Thimann recently concluded: Not the age of the cutting, but the age of the tree, from which it is taken, is important."[22)]

III. EXPERIMENTAL WORK (PRELIMINARY RESULTS).

1. Growing Medium: Lacking auxins or the other effective natural and synthetic growth-promoting substances, I have experimented with a biological solution, using 1) water, as taken from the river Me Nam Chao Phya, flowing through Bangkok, and 2) the same liquid, after filtering, and change into a biological solution, containing calcium carbonate (marble) in excess, and blood, daily administered in amounts of one drop to the litre. The liquid contained a culture of protozoa and bacteria, which in turn served to nourish the daphnia and mosquito larvae, placed into the solution. From experience, daphnia will live, grow and multiply quickly in a balanced solution with inorganic and organic essentials for life, and thus may serve as an indicator of a suitable culture medium.[46] Furthermore, since urine and saliva are known to contain auxins, it is very likely that blood also contains these growth stimulating substances. Growth-promoting substances have been found in the crustacean Palaemonetes, or prawn.[47] It is reasonable to expect them also in related forms, like Daphnia; in their secretions and excretions,[48] as well as in the micro-organisms in the culture medium;[17] the vitamin B_1, (an effective component of manure), the blood vitamins, blood sugar, "Bios," and lastly the so-called wound-hormone of Haberlandt, released from injured plant tissue, should be present in the culture medium.[35]

2. Plant Material: The plants selected for propagation in these liquids were the Thai, Chinese, and Spanish oleander, (Nerium odorum, Soland, and Nerium oleander Linn.)

Propagation of oleander (Yī Toh) gains significance, because of its heart-stimulating properties; it is one of 2 plants, now growing in Thailand, from which we can expect to isolate substances like, or related to oleandrin, restoring force and regular rhythm to an enfeebled, irregular beating heart.[49] Vegetative propagation becomes a practical necessity for mass production, inasmuch as the formation of fruits and seeds is very rare in oleander; no fruits, thus far, could be collected from Chinese and Spanish oleander, the latter growing in the Chiangmai gardens of Nai Kee Nimmanakaeminda. The plant tissues, leaves, and stems without, and with leaves and buds, were transferred into the controlled culture solution, before the absorptive tissue, (laid bare by the fracture or the cut below the nodes), could dry out, In spite of these precautions, experiments of propagation in the untreated river water alone led to failure, pollution set in and the plant-tissues soon decayed and dried out. In the controlled culture medium, however, new roots were observed within 1-2 weeks on the isolated leaves and new roots as well as shoots on stems of soft and hard wood, with and without buds and leaves. (Plate III, 5. 1-6).

Similar experiments of vegetative propagation with other domestic plants are either under way or provided for in our plans, and include such medicinal plants as Aloe, Cassia alata, Datura, and insecticidal plants as Nicotiana, and Stemona. Leaf cuttings of the latter may be readily rooted under our experimental conditions.

IV.

SUMMARY.

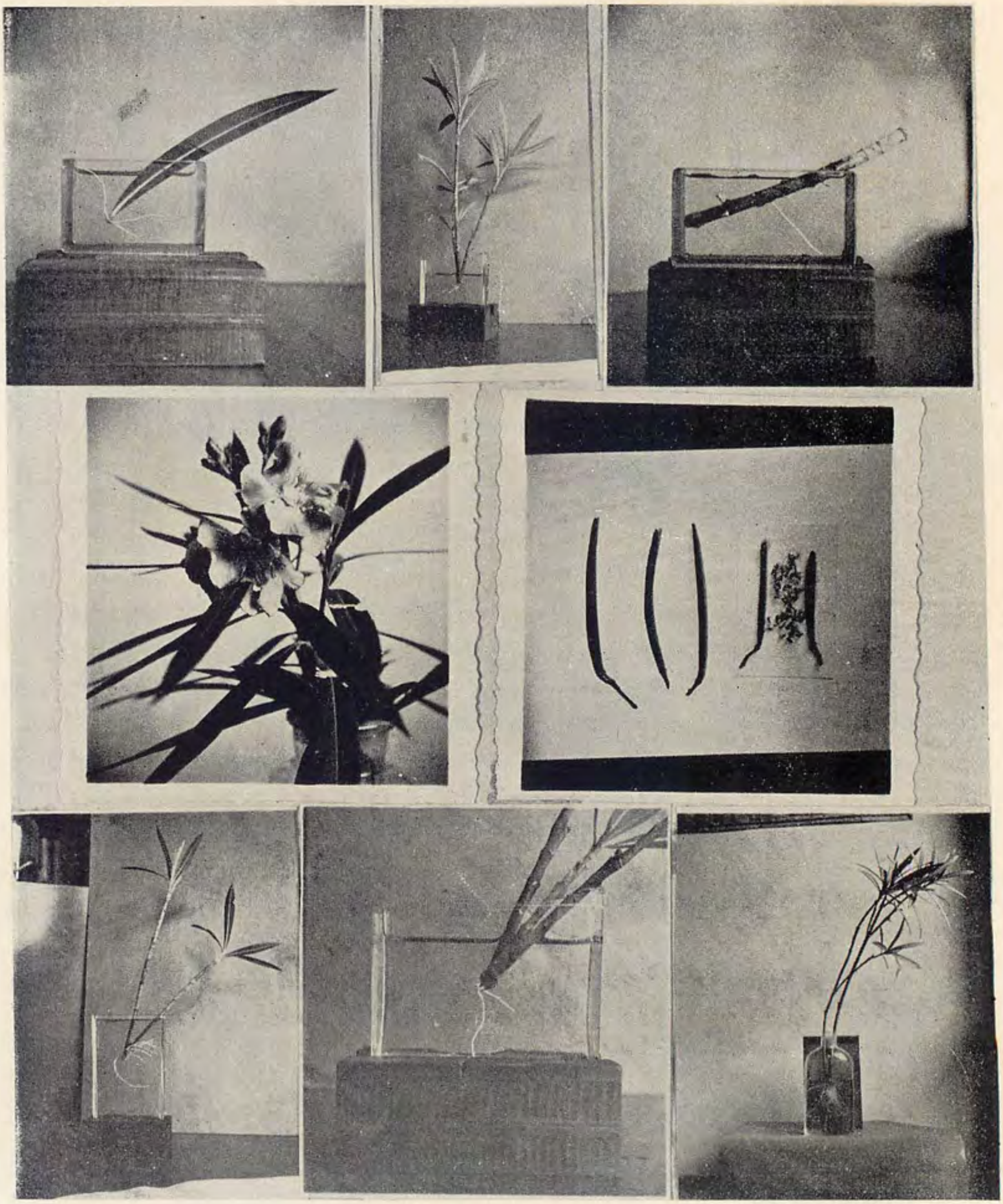
1. Vegetative Propagation of growth, when controlled, is a speedy process of growth, which is, undoubtedly, applicable to all useful plants, medicinal and economical.
2. Many factors, physical—as moisture, air, temperature, and hydrogen-ion and mineral concentration; chemical—as auxins, vitamins, hormones, etc.; nutritional—as sugar; and physiological—vigorous stock, govern the success of vegetative propagation.
3. The significance of colchicine, acenaphtene (chloral hydrate) and anethole, all affecting the chromosome mechanism, as builders of varieties, species, of physiologically active and physiologically inactive forms is indicated from a survey of most recent investigations.
4. The possibility of using animal (pig) blood, as a cheaply available source of growths-and food-factors for vegetative propagation, is suggested.
5. Soft-and hard wood cuttings of stems, as well as leaves, isolated from the plants of Sweet Oleander (Nerium odorum, Soland), a promising heart stimulant, showed new satisfactory vegetative growth within 1–2 weeks. Equally leaves, isolated from the plants of Stemona tuberosa (an effective insecticidal plant), could be rooted under our experimental conditions within a week.

III.

Vegetative Propagation.

1. 2. 3.

7.



8.

4. 5. 6. R. S.

Nerium odorum, Soland (Sweet Oleander).

1. New Root growth on Leaf.
2. New Root growth on Branch (Soft wood cutting).
3. New Root growth (descending) and Leaf shoot (ascending) on Stem.
4. New Root growth on Branch.
5. New Root growth on Branch (enlarged).
6. New Root growth on Branch (hard wood cutting).
7. Top Branch with Flowers.
8. Fruits and Seeds (approx. $\frac{1}{5}$th natural size).

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