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The interaction of kinetin and indolebutic acid on the rooting of cuttings

Mathews C. Oommen

To the Graduate Council:

I am submitting herewith a thesis written by Mathews C. Oommen entitled "The interaction of kinetin and indolebutic acid on the rooting of cuttings." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant Sciences.

Bill Pickett, Major Professor

We have read this thesis and recommend its acceptance:

Joe S. Alexandra, Roger B. Thompson

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

August 16, 1960

To the Graduate Council:

I am submitting herewith a thesis written by Mathews C. Oommen entitled "The Interaction of Kinetin and Indolebutric Acid on the Rooting of Cuttings." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Horticulture.

Major Professor

We have read this thesis
and recommend its acceptance:

Joe S. Alexander

Roger B. Thompson

Accepted for the Council:

Dean of the Graduate School

THE INTERACTION OF KINETIN AND INDOLEBUTIC ACID ON THE ROOTING OF CUTTINGS

A Thesis

Presented to

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The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Mathews C. Oommen

August 1960

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M.C.O.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Not only is the growth of plants dependent on environmental circumstances, on the mineral material taken up from the soil, and such primary anabolic processes as photosynthesis, but it is also regulated by the interaction of particular organic substances within cells. The integration of activities of one part with others is achieved in large measure through the synthesis, transport and utilization of specific individual chemical messenger substances--the plant growth regulators and enzymes.

As a concept, the idea of plant hormones appears on the horizon with the publication of The Power of Movement in Plants by Charles Darwin in 1880. Since then, startling advances have been made. The substances involved are called auxins, hormones or growth regulators. Experiments by numerous workers in the field led finally to botanical, agricultural, and horticultural applications of these chemicals (40).*

The group of chemicals best known as growth regulators which have attained popularity at the beginning, as an aid

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*Numbers in parentheses indicate the corresponding number of reference in the Bibliography.

for the rooting of cuttings, are indole compounds such as beta-indoleacetic acid, beta-indolebutyric acid, naphthalene compounds such as alpha-naphthalene acetic acid and its derivatives. These have a function of inducing cell elongation. Another kind of material which affects the growth of plants is kinin, an example of which is kinetin with a defined property of encouraging cell division.

The use of these plant growth regulating substances as an aid in the rooting of cuttings has been explored by many of the leading scientists. The environments under which the cuttings are rooted have become almost stabilized through the efforts of numerous workers. Several species of plants have responded well to treatment with growth promoting substances under controlled environment. There are still many plants which do not respond to these treatments.

The object of this study is to understand what effect the interaction of indolebutyric acid and kinetin would have on the rooting of cuttings. If an interaction should show up it might provide a clue for methods of inducing roots on cuttings which are difficult to root. In some cases, as noticed in tobacco pith tissue, kinetin increases callus formation and rooting from callus tissue is sometimes easier than from more differentiated tissue.

CHAPTER II

LITERATURE REVIEW

Vegetative propagation of desirable plants has been practised by man for countless centuries. Documented evidence to this effect can be found in Enquiry into Plants written by Theophrastus about 300 B.C. The present day knowledge of vegetative propagation has increased considerably with the research of the past two decades and new techniques are now finding their way into general practice. The modern period of studies on vegetative propagation began with attempts to correlate the internal physiological condition of the stem with readiness to root. This led to studies on the effect of chemical substances on rooting. Several substances such as sugars, potassium permanganate and so on have been applied to stem cuttings with the object of aiding rooting, either by providing nutrients or sterile conditions at the base of cuttings, but due to the highly variable results they cannot be used advantageously (3).

I. AUXINS

The different chemicals which control or regulate the phenomenon of growth have been called plant hormones, auxins or growth regulating substances. Of the many different groups studied, one in particular, the auxins, are of special interest

for a variety of reasons. The auxins were not only the first to be discovered, but also the one for which the largest regulatory duties in plants has been discovered.

In keeping with the original intent of the term, auxin will be used to refer to "an organic substance which promotes growth along the longitudinal axis, when applied in low concentrations to shoots of plants freed as far as practicable from their own inherent growth promoting substances" (29). Auxins are involved in the control of stem growth, root growth, lateral bud inhibition, abscission of leaves, fruits, and fruit growth. In all, some twenty different physiological activities in plants are known to be more or less controlled by auxin actions (43). It now appears that auxin "a" should be classified as a hormone. Since hormones are defined as "substances which produced in any one part of an organism, are transferred to other parts and there influence specific physiological process." It is evident from this definition that chemicals produced exclusively in laboratories, or such compounds as sugars and amino acids, are not within the definition of hormones (29).

The search for an actual growth hormone in plants led to the isolation of the first pure active compound, not as the indigenous active hormone, but rather as an ingredient of such miscellaneous biological materials as urine, corn oil, and malt extract. The definite discovery of auxin was made

by Went in 1928, as stated by Bonner and Galston.

He extracted the growth promoting substance present in oat coleoptile tip, and showed that it is a natural auxin capable of diffusing from coleoptile tip to agar block and from agar block back to coleoptile stump (4).

The degree of curvature caused by differential growth when an agar block containing auxin is placed unilaterally on the cut surface of the coleoptile tip is used as a measure for the concentration of natural auxin present in the plant (4). Composts, leaf mold or other farm yard organic manures were known to possess the proper growth promoting ability very early. The realization of this fact finally led to the isolation of indoleacetic acid (IAA) from urine. Kogl and Hagen Smit were the pioneer workers in the field. Following the discovery of IAA, several other chemical substances capable of regulating the various plant processes were brought to light. The group of chemicals best known as root inducing substances are indole compounds such as beta-indoleacetic acid, beta-indolebutyric acids, and naphthalene compounds such as alpha-naphthalene acetic acid (43).

II. KININS

The twin aspects of growth, the cell multiplication and cell enlargement, are commonly treated separately as though they are subjected to distinct stimuli and controlling

factors. However, cells divide first and then they enlarge. These two processes merge into one another. Frequently enlarging or highly vacuolated cells divide and growth is by no means as confined to organized growing regions as it is often supposed to be. The more recently emphasized stimuli to cell division have been termed "kinins" (13).

A substance which markedly promotes cell division in various plant tissue cultures, at concentrations as low as one microgram per c.c. of solution, has been isolated in pure form from heated deoxyribonucleic acid preparations. It has been shown to be similar to 6-furfuralamino pruine. The specific name kinetin has been applied for this substance and the generic term kinin is suggested for any substance which similarly stimulates cytokinesis (13).

The liquid endosperm of coconut has been recognized as a potent source of stimuli which effect growth and morphogenesis in the immature embryo. However, the responsible growth promoting substance may accumulate precociously in the endosperm in advance to embryo development. This line of thought led to the isolation of kinetins from coconut milk. From several hundred gallons of coconut milk Shantz and Steward obtained a crystaline compound which they identified as 1-3-di-phenylurea. This substance is active only when applied with Casein hydrolysate. Warm water extracts made from immature corn fruits were also found to be effective as kinetin.

A third substance is an extract from the innermost layer of the fleshy pericarp of the banana fruit (38).

III. MECHANISM OF ACTION OF AUXINS AND KININS

The formation of root meristem constitutes one portion of the development of a plant embryo from a fertilized ovule. The root primordia may also arise at other locations and for the formation of these adventitious roots on leaves, stems, roots, or such unusual locations, it must be preceded by the development of an organized root meristem (18). Application of auxin will result in the formation of root primordia. These primordia first appear as areas of meristematic activity often in the pericycle of herbaceous stems or in the phloom parenchyma of woody stems. Accompanying continued cell divisions, these meristematic regions become organized finally to a recognizable root meristem.

Of the numerous effects known to be produced by auxin, only one class, the promotion of cell elongation, has been at all intensively studied with respect to mechanism of auxin action. But there is a possible multiplicity of auxin action and it is difficult to separate the various effects from cell elongation. The various effects of auxin may be in the form of increased cell wall plasticity, increased water uptake, and altered protoplasmic streaming (20). During the past twenty-five years many extremely interesting facts have been

set forth bearing on the mechanism of auxin action. Some of them will be discussed in the following paragraphs.

One hypothesis about the formation of root primordia, when the auxin is applied on a cutting, is that the auxin interacts with a second material known as "Phizocaline," resulting in the formation of roots. Although auxin brings about growth activity, it is the presence of Phizacoline which directs the activity along the specific pathway of root formation. Evidence for this view is tha fact that auxin application to a stem cutting results in the loss by the cutting of the power to form additional roots in response to further auxin treatment. Thus when the base of a cutting is treated with auxin, the treated base will ultimately form roots. If the treated base is excised from non-treated portion, there will be no rhizocaline left for further root formation (5, 41, and 42). The hypothetical rhizocaline would appear also to be more or less equally distributed through the stems. The stems of many species of plants lack entirely the ability to produce root primordia in response to auxin treatment. According to the rhizocaline hypothesis, we would say that the stems of such plants lack rhizocaline.

Leopold, in his book Auxins and Plant Growth, mentioned several of the theories suggested by different people from time to time. A provocative theory is that auxin brings about dissociation of the protein constituents of the cytoplasm.

The dissociation effect would increase water permeability, increase the osmotic nature of the cytoplasm, and possibly also bring about increased enzymatic activity. Consequently, a great deal of attention has been given to the water uptake mechanism as a possible means of describing growth in its simplest form. The uptake of water could be due to changes in cytoplasm itself, especially changes in osmotic value, or it could be due to changes in properties of the wall and the cell membranes, particularly with respect to extensibility and permeability. Each of these two factors in water uptake has been defended as a possible mechanism through which auxin may bring about growth (30 and 6).

Audus describes the work of Dutch scientist Vanderbeck who made the first extensive investigation about the role of internal factors in the rooting of cuttings. He demonstrated that in currant, willow, and grape the presence of developing buds was a prerequisite for rooting and that the intensity of root production was directly related to the rate of bud development. Cuttings with dormant buds fail to root even under the most favorable conditions; but in spring when the buds renew their activity, rooting occurs. The removal of a ring of bark from a short section of the stem below the buds also prevented rooting. He explained the phenomenon as the formation of a hormone or hormones in the developing buds and its conduction in the bark to the base of the cutting where it

initiates rooting (1). This work was subsequently extended by several others to show that leaves were also a source of this hormone.

It is known to horticulturists that the presence of leaves on cuttings greatly increases the chances of root formation. Experiments with hibiscus cuttings have shown that without auxin treatment and without leaves, no roots were formed. Without auxin treatment an increase of the number of leaves left on the cuttings is also ineffective for root formation. When auxin treatment was given to leafy cuttings roots were formed in proportion to the number of leaves present on the stem. Leaves, then, provide some factors which together with auxin cause root formation. In order to investigate the co-factors contributed by leaves, several substances like sugar and ammonium sulphate, were tried and it was found that a combination of the above substances will act like one of the leaf hormones. Since ammonium sulphate cannot be present in the leaves, the search for a more likely substance was launched. It was found that arginine met these requirements. Since both arginine and sugar are natural constituents of plants, it seems reasonable to assume that one of the functions of the leaf is to provide cuttings with these compounds, which then together with auxin cause the root formation. Cuttings of many kinds have responded to auxin treatment, yet many have not. The reason for the failure is not known. It

would seem that auxin will be active only when co-factor requirements are satisfied (40).

Linsor and Kaindle conducted a series of experiments with alcoholic extracts of different plants to study the effect of auxins in the regulation of growth. They postulated that the auxins are being absorbed by molecules of the living substance. It is assumed that there are spaces that can be occupied by the molecules of the growth regulator. This means that the molecule of the growth regulator has a certain affinity for a certain "space." The hypothesis postulates two types of spaces. The first type filled by growth promoting substances, resulting in increased growth, and the second one filled by growth inhibiting substances, resulting in the inhibition of growth (32).

Terry, while studying the effect of indoleacetic acid on pea stem for inducing the adventitious roots, explicitly assumed the existence of an unknown substance in the plant organism which interacts with the applied auxin to induce roots. He states that normally this unknown substance is transported acropetally and consumed. At the time of root formation it will be accumulated (7 and 39).

In conclusion, it may be said that the mechanism of auxin action remains unsolved, but a variety of promising lines of evidence seem to be emerging. Auxin has been found to react with some proteinaceous material in a manner which

is suggestive of a constructive step in growth. A picture of how auxin may react with its receptor compound has come from kinetic studies, as well as a picture of the means by which other compounds may alter auxin action. The means by which auxin may finally bring about cell elongation is being hotly debated between those who feel that the action is principally based upon the cytoplasmic uptake of water and those who feel that it is principally based upon the growth of the cell wall. The clarification of this important problem will not only be extremely interesting, but no doubt will greatly accelerate the advance of practical and theoretical knowledge of auxins and growth (20 and 30).

Kinetin at all levels was tested for the promotion of cell division and this has been verified by anatomical sections. The effects were particularly striking with tobacco pith tissue. The control cultures without kinetin showed no cell division. Promotion of cell multiplication was observed with as little as 1 microgram per c.c. of this substance.

The sunflower hypocotyl was tested in suitable media for the multiple auxin activity wuch as root initiation and cell elongation with auxin and kinetin. In the absence of auxins, kinetin produced a significant increase in the fresh and dry weight of the fragments under study, but it was found that kinetin did not induce the initiation of adventitious roots in these fragments (34 and 36).

IV. METHODS OF AUXIN APPLICATION

Lanolin paste method. Lanolin is a soft fat which will dissolve auxins. It has been used by many experimenters for application to various plant organs. The paste when applied to the base of the cutting sticks firmly to it. It does not dry out immediately and merely maintains a reasonable constant concentration of the auxin in contact with the treated portion. Lanolin is without effect as a root inducing substance. IBA and NAA usually dissolved in it are at concentrations ranging from 0.01 to 0.05 per cent (1).

Dilute solution method. This is one of the earliest methods of application. Concentrated stock solutions of the auxin are first made up in 95 per cent alcohol and then diluted to the appropriate strength in water. Concentrations ranging from 0.005 to 0.01 per cent are usually employed, depending on the species chosen. The basal end of the cutting is immersed for about twenty to twenty-four hours in order that the required amount of auxin should be taken up. This period of immersion will naturally vary with the type of cutting and more praticularly with the environmental condition during the period of soaking. In the case of leafy cuttings, the amount of auxin taken up will depend upon the amount of water absorbed. This in turn will be controlled by the amount of water lost by transpiration, a process which

increases with the dryness of the atmosphere. Thus, under warm dry conditions excess auxin may be absorbed which will eventually result in toxicity. Hence, during the soaking period it is safest to keep these cuttings in a humid atmosphere. For leafy cuttings of woody species, one to two hours of soaking with a 0.01 per cent solution or ten to twenty-four hours of soaking with a 0.005 per cent solution represents this type of treatment (15 and 1).

Concentrated solution dip method. This method of application is rapidly gaining the favor of propagators. The auxin solution is made up in 50 per cent ethyl alcohol. The concentration usually employed for woody plants is about 1 per cent. This may vary under several conditions which will be mentioned in the following pages. Cuttings, preferably tied in small bundles, may be dipped to a depth of about one inch for one to five seconds. In such a short time only a very thin layer of auxin will be covering the base. The cuttings can be planted immediately (8).

Dust method. Dust method essentially consists of applying to the base of the cutting a fine inert powder containing a small admixture of the solid auxins. Talc, clay, or finely powdered charcoal are most commonly used, and the order of concentration is 0.02 to 0.1 per cent for herbaceous cuttings and five times higher for hardwood cuttings. Dust

mixtures are made by two methods. One is to grind the auxin crystals to a very fine powder and then mix them thoroughly with the carrier. This requires great care to insure a uniform mixture. A better way is to soak the carrier thoroughly in an alcoholic solution of the active substance in suitable concentration. The alcohol is then dried off and the carrier is reground if necessary.

Treatment itself consists of moistening the basal half to one inch end of the cuttings with water and then rolling it in the powder. The excess material sticking on the cutting should be removed before planting by shaking it gently. The most important disadvantage of the use of dust method is that the physical properties of the carrier can greatly influence the effectiveness of the active compound and different carriers may cause widely different responses in the same plant even with the same concentrations (33 and 37).

Spray technique. Some success has been obtained by spraying leaves with dilute aqueous solution of auxins. This treatment has been applied both to the mother plant before the removal of the cutting and to the cutting itself after insertion in the rooting medium. Considerable success with certain evergreens was obtained by spraying parent plants with ten to one hundred parts per million of 2, 4, 5 trichlorophenoxy acetic acid. Cuttings taken nine to forty days

after this spraying showed essentially the same rooting response as cuttings treated by the more usual methods outlined above (1).

Other methods. Direct insertion of auxin crystals under the bark or into the base of shoots was effective, but was more prone to give serious toxic effects. The insertion of toothpicks soaked in auxin was also found to be successful. Attempts have also been made to improve the response by a second treatment after a period in the rooting medium. Success has been claimed for a number of species that are normally difficult to root.

The concentrated solution dip method seems to be easier to practise. Cuttings which otherwise show great difficulty in rooting, such as apple, rhododendron, hemlock, were found to be more responsive to this method. The alcoholic stock solution can be prepared easily; large numbers of cuttings can be treated easily and the time required for the treatment is quite short. Solution dip further enables a more uniform distribution, whereas the uniform concentration of a talc mixture may not be prepared easily. Therefore, a given growth substance may bring about varying results (1). Experiments with elliptice cuttings showed that a concentration dip method of 5.0 mgs/c.c., soaking for five seconds and a dilute solution dip method consisting of soaking for twenty-four hours

were equally effective for the production of the same number of roots within the same period (9). Several experiments conducted by numerous workers have proved neither beneficial nor harmful effects in the use of alcohol for preparing the solution. Furthermore, the dipping was done with one, five, ten, twenty, and thirty seconds. There was slight improvement with a thirty second treatment as compared with a one second dip. Maximum production of roots on cuttings was with a dip having a five second limit. Apparently little precaution is required while dipping in solutions (37).

V. CUTTING AND THE CHOICE OF SHOOT

Nearly any plant organ is capable of forming roots. Stems, leaves, stolons, and roots will do so. Stems are usually considered as the ideal structure since they have undifferentiated tissues to permit easy differentiation of root primordia. Auxin treatments do not increase the number of buds or enhance the growth of buds. In general it is desirable to have buds already formed on propagation pieces (31).

A cutting is a detached part of the plant intended to be placed in the soil or some other medium for the purpose of developing roots. The essentials for the rooting of cuttings are the same as those for the germination of seeds, such as warmth, moisture, and oxygen. These essentials bring about a

chemical change in the stored plant food of the tissue of the cuttings such that the development of roots may take place.

The development of adventitious roots may take place at nodes, internodes, on roots, tubers, and even on leaves. They may arise in connection with buds or independently of buds and may form in both older or younger tissues. However, they are often initiated in the vicinity of the differentiating vascular tissues of the plant part. If the plant part is young, the adventitious primordium is initiated by a group of cells near the periphery of the vascular system. If it is older, the seat of this organ is located deeper near the cambium. In young stems the cells forming the root primordia are commonly formed from the interfascicular parenchyma, and in older stems they will arise from the vascular rays. Often the seat of the first divisions forming the root primordia in stems is identified as the pericycle. Such an origin places the young root close to the vascular bundle of the parent axis and facilitates the establishment of vascular connection between the two organs. When a cutting is planted in the soil having proper conditions for the production of adventitious roots, the rooting takes place at the proximal-end and the buds may arise on any parts of the shoots, with respect to leaf axils. In some cases it was found that the buds arising at the distal end of the cuttings were in direct connection through the internodes with the roots arising at the

proximal end (18).

While attempting to grow apple trees by stem cuttings, it was observed by Gardner and others, that if the cuttings were taken from one-year old seedlings very little difficulty was encountered in getting them rooted. Cutting taken from two to three-year old seedlings were more difficult. Experiments were conducted to test the ability of rooting, with cuttings of different ages from plants of the same species which had grown side by side, or at least under similar conditions. The cuttings within a species were made at the same time and placed in the same media under similar treatments. The results indicate that every species worked with has shown better rooting for the cuttings taken from very young seedlings than from older trees. The time required for rooting was also much shorter for those taken from young seedlings. The cuttings from one-year old apple trees have rooted within ten days. But one-year old cuttings taken from a growing branch of an old tree failed to root completely. An important observation to be considered is that it is the age of the tree from the seed and not from the bud which has much influence in initiating the roots. The marked morphological differences which often exist between one-year old and older seedlings may be expressions of just as pronounced nutritional or anatomical differences within the plant, which in turn might be responsible for the differences in the rooting

ability (19).

The type of cutting and the seasons of making the cuttings depend mostly on the nature of the plant, whether herbaceous, deciduous tree or shrub, evergreen or conifers, etc. As a general rule, leaves should be retained on basal portions of the cuttings since they are an important source of natural auxin and other rooting factors. Having taken the cuttings, they should be subjected to ideal rooting conditions. These are adequate water supply to the whole cutting and adequate aeration for the basal portions. The development and growth of young roots are highly susceptible to oxygen concentration. Suitable diffused light and optimum temperature have to be maintained (17).

Experimental work by Kirkpatric, Jr., has showed that heeled cuttings of conifers and rhododendron responded much better to auxin treatment than other types of cuttings. Little or no effect was observed with cuttings taken in spring after the new growth had started or on very young material taken in summer. The cuttings taken in winter generally failed (27).

Besides seasonal and anatomical factors there are still many other factors which influence the rooting ability of cuttings. Some may be more important than others, but because of variations within plants it is impossible to state that any one factor is the most important in all cases. The most

favorable time to take soft wood cuttings is when the leaf of the current year's wood has begun to ripen, which denotes a favorable carbohydrate nitrogen relationship. Many investigators have realized the importance of carbohydrates in the development of roots. Reid has pointed out that a high carbohydrate content plus a reasonable amount of nitrogenous compounds in the tissues was best for rooting and for the production of buds (35). Recent investigations in the United States of America with interrupted mist systems and high humidity in the greenhouse indicates that much softer wood can be successfully rooted than previously supposed. Such cuttings are comparatively low in carbohydrates but the lack of carbohydrates will be apparently compensated by the greater photosynthetic activity of the younger leaves. A large number of leaves should be retained on the cuttings in order to facilitate this (21).

It was formerly a common practice by nurserymen in America to establish stock or stool blocks of common shrubs to furnish cutting wood. Plants in these blocks were cut to the ground during the spring season. When the new growth was forced, it was used for propagation. Such cuttings rooted uniformly and readily, developing into high quality plants. The ease of rooting of these stool block cuttings suggests that such shoots may be somewhat juvenile in character. The factor of juvenility influences the rooting ability of

cuttings. Juvenility is a phenomenon associated with the physiological age of the plant and it is apparent in several ornamental and fruit plants (21).

VI. AUXINS--WHEN USED

Cooper and Knowlton reported that cuttings of about sixteen varieties of grapefruit and sweet and sour oranges were successfully rooted by the standard treatment of soaking the basal ends of the cuttings in aqueous solutions of IAA.

Dip, dust, and soak methods of applications were followed. For dipping a solution of .02 per cent was used. For dust dipping one mgm of NAA/gram of talc manufactured by a commercial firm was used. The treated lot showed an increased rooting per cutting. But the dust containing NAA was not found quite effective for lime and lemon cuttings (9).

Further investigations by Cooper reveal that retreatments with growth substances will be quite effective in inducing roots for species which are difficult to root. On cuttings of sweet orange and grapefruit an initial treatment of auxins was made with .01 to .05 per cent aqueous solutions for twenty-four hours. There was no initial response to such an initial treatment except the formation of a callus within three to four weeks time. It has been found, however, by retreating these cuttings at varying periods after the initial treatment, roots were formed. In another experiment, with

Hamlin sweet orange cuttings, the untreated lots produced no roots even after six weeks in a propagation frame. Cuttings initially treated with .02 per cent of IAA produced one to two roots and cuttings retreated about three weeks after the initial treatment produced three to six roots (12). The use of IRA in the propostion of one in two thousand parts of pure lanolin was found to be quite effective for inducing roots on the cuttings of Eureka lemon. The hormone treatment not only caused production of roots on cuttings with leaves, but also induced roots on cuttings without leaves; while on controls without leaves, no roots were formed (11).

Chadwich and Kiplinger describe the effect of IBA on the cuttings of Cornus florida variety ruba, the red flowering dogwood. The treatment consists of soaking the basal portions of the cuttings in an aqueous solution of 1 mgm/100 c.c. for twenty-four hours. The temperature was maintained at about 70° to 74°F. and the relative humidity in the greenhouse was about 60 to 80 per cent. The treated cuttings showed up to 90 per cent of rooting, within six weeks, and the untreated ones rooted little (14).

Cuttings of different types of roses responded well to treatment with IBA. Larger root systems, uniform stand, and a higher percentage of rooted cuttings were induced by the application of auxin. Two mgm/gram of powder worked well on cuttings of most varieties of roses. Solution treatments of

1.25 mgm to 5 mgm per liter for twenty-four hours were equally effective. Another observation in this experiment was that NAA and IAA were not as effective as IBA. Temperatures below 60°F. decreased the effectiveness of IBA. The treated cuttings performed normally in every respect when grown and compared with grafted plants or with the plants grown from cuttings not treated (28).

Hitchcock and Simmerman undertook an extensive study to find out the effect of auxin mixtures when applied to the cuttings. Their observations and results will be dealt with in the following discussion.

In one of the principal experiments three talc mixtures of two different substances (IBA-NAA, IBA-IAA, and IAA-NAA) were used in five different proportions, viz., 0:100, 25:75, 50:50, 75:0, and 100:0. Three different concentrations, 2, 5, and 12 mg/gram, were mixed in the above proportions for the experiment. These preparations were applied on the same day to cuttings of Chrysanthemum, Euyonymus, Hibiscus, and Ligustrum. NAA alone induced about twenty-seven roots. The mixtures of IAA and NAA (25:75, 50:50, and 75:25) gave values intermediate between four and twenty-seven and approximately proportional to the relative activities of the materials tested. Similar results were obtained with IBA-NAA mixtures. The same mixtures of IBA-IAA show the two substances to be equally active in Ligustrum. Although IBA was slightly more effective

than IAA, the quantitative difference is small and hence mixtures of these two substances in any proportion are of equal activity.

Root counts for Euonymus, Hibiscus and Chrysanthemum show essentially the same trends as those described for Ligustrum, with one exception. IBA was considerably more effective than IAA. Consequently, in all three of the mixtures the activity varied according to the proportions of the two substances so that increasing the proportion of the most active substance caused an increase in the effectiveness of the mixture.

Mixtures of equal parts of IAA, IBA, and NAA (50:25:25), making a total of 5 mg/gram of root inducing substances were applied to Euonymus, Hibiscus, Ligustrum, and Taxus cuttings. In all cases the mixtures were used at the same concentration. With Taxus NAA proved most effective. Mixture of IBA and IAA with NAA produced root counts proportionate to the amount of NAA in the mixture.

When successive treatments of Hibiscus cuttings were made with NAA and IBA there was a definite predominating influence of the preparation first applied. Each og the two root inducing substances was used in concentration of 0, 2, and 5, and 12 mgm/gram of talc. When IBA was applied first and NAA second, the root counts were ten, twenty-six, sixty-one, and seventy-eight for each of the above concentrations.

The corresponding values for the IBA as a second application was 2, 12, 42, and 58, respectively. However, the two preparations applied successively did not act the same as mixtures since the order of application actually influenced the total number of roots produced. All these observations support the basic idea that the principal action of applied root inducing substance occurs within a relatively short time, that is, from a few seconds up to about twenty-four hours, depending on the method of application.

Another experiment was with dilute solution mixtures of the Potassin salts of IBA and IAA, indole propionic acid, and phenylenediamine. The mixtures were more effective in inducing roots than any one substance used alone at the same concentration. In the five substance mixture KIBA constituted one-third of the total and in a four substance mixture, one-fourth of the total. It was noted that these concentrations of KIBA were more effective in the presence of other substances than KIBA alone in concentration of 1, 3.2, and 10 mgm/c.c., respectively.

In addition, another experiment varying the concentration of one substance while maintaining the concentration of a second substance at a constant level was set up. No synergistic effects were noted (23).

The effectiveness of the application of auxin varies from species to species. Above a certain concentration, toxic

effects may be found. The variations may well be due to the secondary properties such as ease of penetration of the molecules and the speed of conduction within the plant.

IAA, in contrast to what might be expected of it as a natural auxin, is not the most suitable compound to use in practice. Two other substances of equal rooting activity (IBA and NAA) are superior because of their greater chemical stability and low mobility when applied on the cuttings. The former has prolonged action giving better chances of rooting and the latter the property of being retained near its point of application. Different compounds react differently, not only with respect to the quantity but also the quality of the roots they induce. IBA normally produces few roots which soon become long and establish a strong fibrous root system. The phenoxyacetic acids on the other hand produce dense thick roots with a bushy root system. Much progress has been achieved in regulating vigor and general structure of the root system by using appropriate mixtures of these compounds since such mixtures will induce roots with intermediate characteristics.

On reviewing the several experiments discussed above the use of root growth promoting substances on cuttings as relatively dilute solutions (1 to 80 mg/l), as concentrated solutions (1 to 20 mg/c.c.), and as powders (0.5 to 50 mg/g) produced essentially the same rooting response. Concentration

requirements for optimum rooting varied according to the kind and form of substance, the kind of carrier or solvent, and the species and age of plant (24). Talc controls exhibited better results than non-treated controls or the treatment with tap water. In many, not in all, cases the mixtures were more effective than any of the individual substances. All species of cuttings did not respond equally well to given mixtures. Certain mixtures of IBA and NAA were effective on either IBA sensitive or NAA sensitive cuttings. Lastly, a mixture composed of equal parts of two substances were proved to be as effective or more effective than three or four substance mixtures (23).

CHAPTER III

MATERIALS AND METHOD OF EXPERIMENT

I. TEST PLANTS

For experimental purpose the plants selected were of two types. One belongs to the genus Impatiens and species Sultani, the common name of which is Sultana. The other one was of the genus Chrysanthemum, species morifolium. The common names of these plants will be used in the following discussion.

These plants vary considerably in their morphological and anatomical characters. The former one is a quick rooting plant with softer tissue than the latter one.

The Sultana cuttings were taken from the lateral branches and not from the central leader. All cuttings were more or less equal in length and diameter. Three or four leaves on the top were retained at the top of each cutting. The Chrysanthemum cuttings were taken from the intermediate portion of branches so as to avoid the top as well as the bottom of the portions of the stem. In other words, semi-hardwood cuttings were selected in this case. These cuttings were also of uniform length and retained two or three leaves. The plants from which the cuttings were taken were under similar environmental conditions.

II. GROWTH REGULATING SUBSTANCE X

STOCK SOLUTIONS

The two growth regulating substances used for this particular study were indolebutyric acid (IBA) and kinetin. The former is an auxin with the function of regulating cell elongation and the latter has a function in cell division. Both were obtained in the crystalline form from commercial firms.

Stock solutions were prepared in 50 per cent alcohol. The different concentrations made up for IBA were 200, 318, 400, 505, and 800 parts per million. In the case of kinetin, the concentrations prepared were 1.0, 1.44, 2.08, and 3.00 micrograms per milliliter. Against both the chemicals 50 per cent alcohol alone was used for the control cuttings.

III. TREATMENT

Two sets of mixtures were used for treating the cuttings. The first consisted of 200, 400, and 800 ppm of IBA and one, two, and three micrograms of kinetin other than 50 per cent alcohol alone. The second set of mixtures contained 200, 318, 505, and 800 ppm of IBA and 1.0, 1.44, 2.08, and 3.0 micrograms of kinetin respectively.

The quick dip method of treatment was followed by immersing about an inch of the basal portion of the cuttings

in the desired concentration. All cuttings were treated uniformly for about four to five seconds before planting them on the bed.

IV. PROPAGATION BENCH AND MIST SYSTEM

The cuttings were set in a clean, coarse, sterilized medium of Perlite. Care was taken not to force the cuttings into the medium so as to cause injury to the basal tissues.

The watering was accomplished by an automatic interrupted mist system. This is a mechanically controlled dispersion of water as very fine particles into the atmosphere above the propagation bench. This helps not only to increase the humidity in the greenhouse, but also to keep the surface of leaves and stems constantly covered with a thin film of moisture. Another advantage of the mist system is that only light shading is required. Even when cuttings were exposed to higher light intensity of the summer season, the tissue temperature seldom rises enough to cause injury. The transpiration and respiration rates are also reduced and at the same time the exposure to sunlight permits normal photosynthesis to function. Food reserves are accumulated and the rooting potential is increased.

Constant misting is not necessary to keep the leaves covered with a moisture film. The intermittent application of mist with the spray being turned on at intervals just often

enough to keep the leaves constantly moist is enough for this purpose (26 and 16). The interval used was a spray of six seconds every three minutes and this continues during the daylight hours. The timer mechanism with an electric clock controls the intermittent automatic mist system.

The corrugated bottom of the propagation bench facilitated adequate drainage. The relative humidity was about 82 to 85 per cent during the period. The observations on Sultana were taken twelve days after planting and on Chrysanthemum twenty-five days from planting.

CHAPTER IV

RESULTS AND DISCUSSION

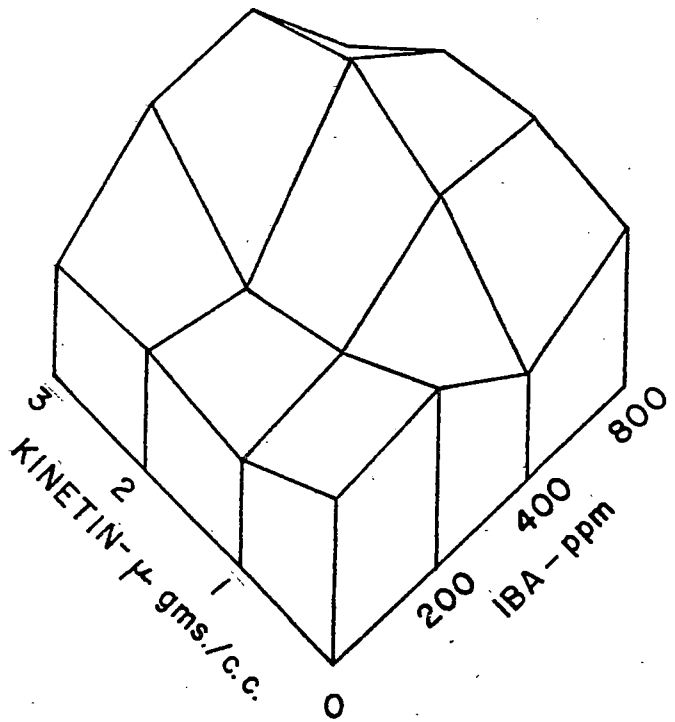
The experiment is designed to use three different mixtures of two different growth regulating substances in one series and four different combinations of the same substances in the other series. These preparations were applied on the cuttings of Sultana and Chrysanthemum on the same day.

I. RESULTS

Figures 1 to 4 show the total number of roots produced by each set of cuttings treated alike. Figures 5 to 8 represent the corresponding weight in grams of the roots produced. The data for the root counts and root weight appear in Tables I to VIII of the Appendix. The curves for the root counts in Figures 1 and 2 are based on the total root count taken for four cuttings treated alike. With Sultana it was found that the minimum number of roots appeared on cuttings treated with a mixture of 800 ppm of IBA and three micrograms of kinetin. Kinetin alone did not have any effect on the number of roots. IBA had some influence on the number of roots produced. Concentrations above 200 and 400 ppm of IBA did not further increase the number of roots. The highest datum point, according to Figure 1, is found when the application of kinetin was two micrograms and IBA was at 400 ppm levels.

NO: OF ROOTS / 5

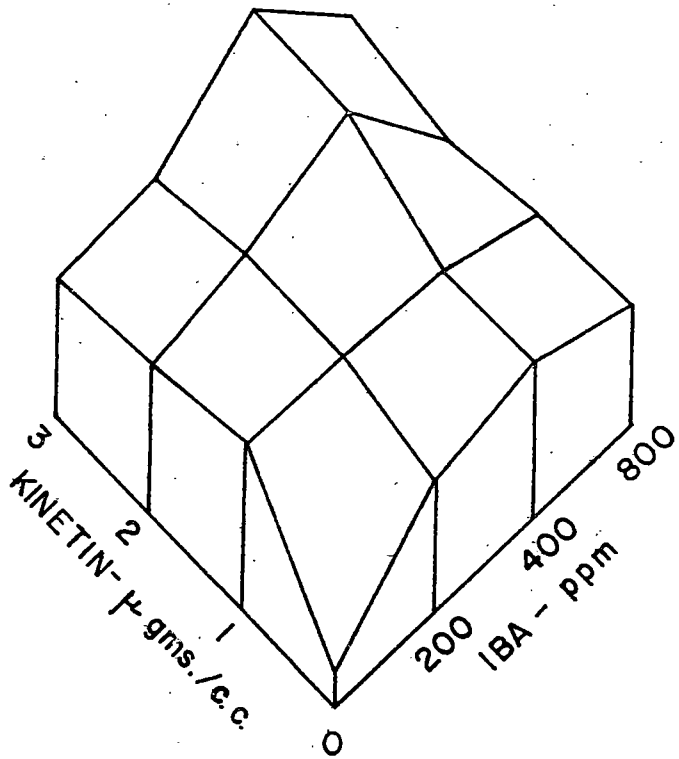
FIG.1



SULTANA

ROOT COUNTS

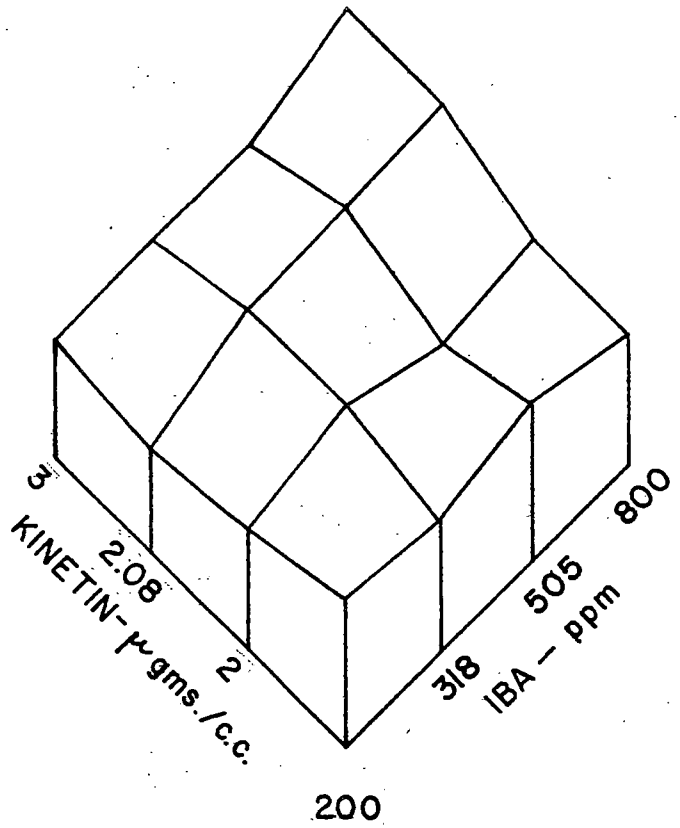
FIG.2



CHRYSANTHEMUM

NO: OF ROOTS / 5

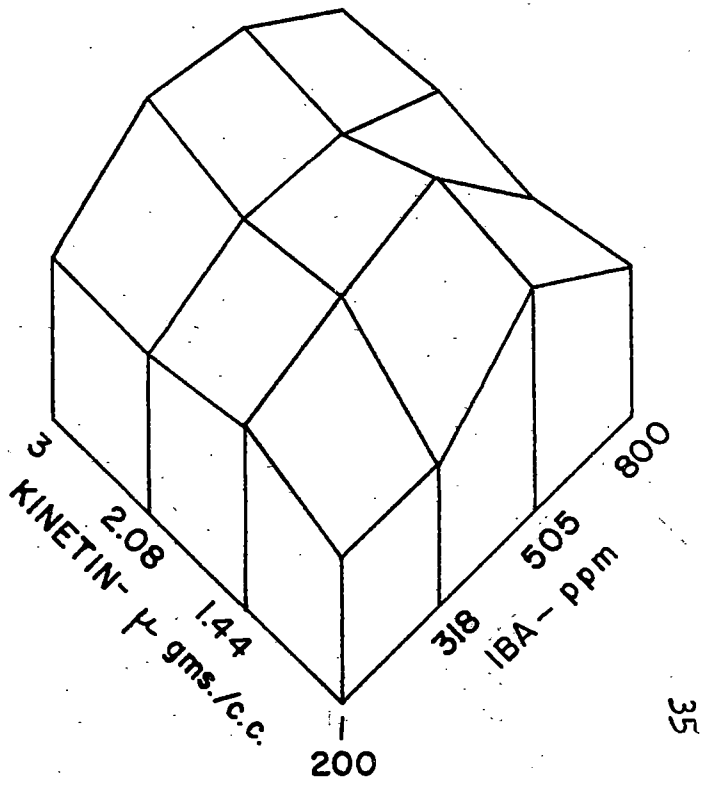
FIG.3



SULTANA

ROOT COUNTS

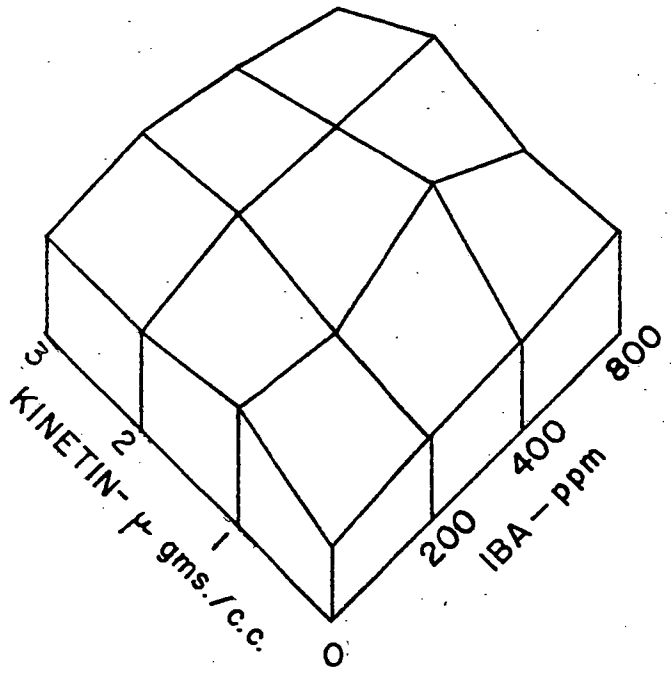
FIG.4



CHRYSANTHEMUM

WEIGHT OF ROOTS / 0.05gms.

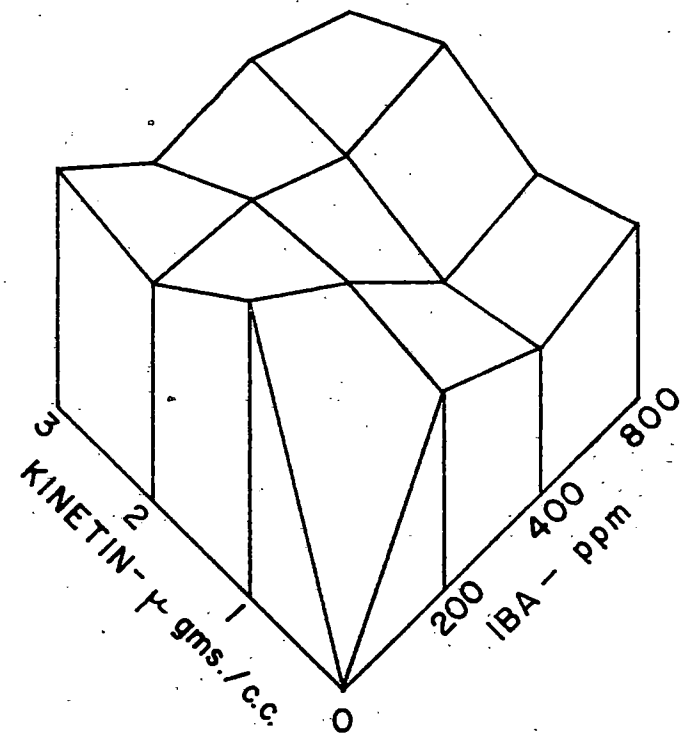
FIG.5



SULTANA

ROOT WEIGHT

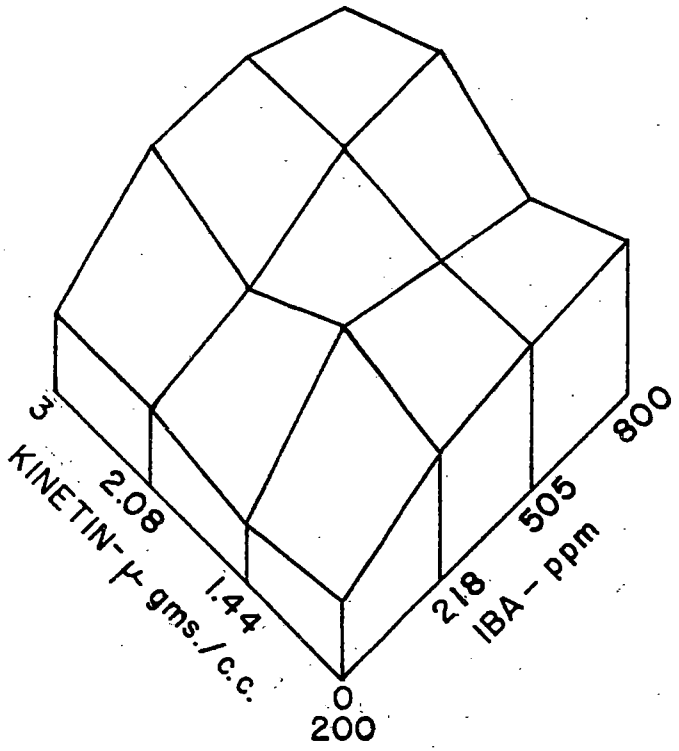
FIG.6



CHRYSANTHEMUM

WEIGHT OF ROOTS /0.05 gms.

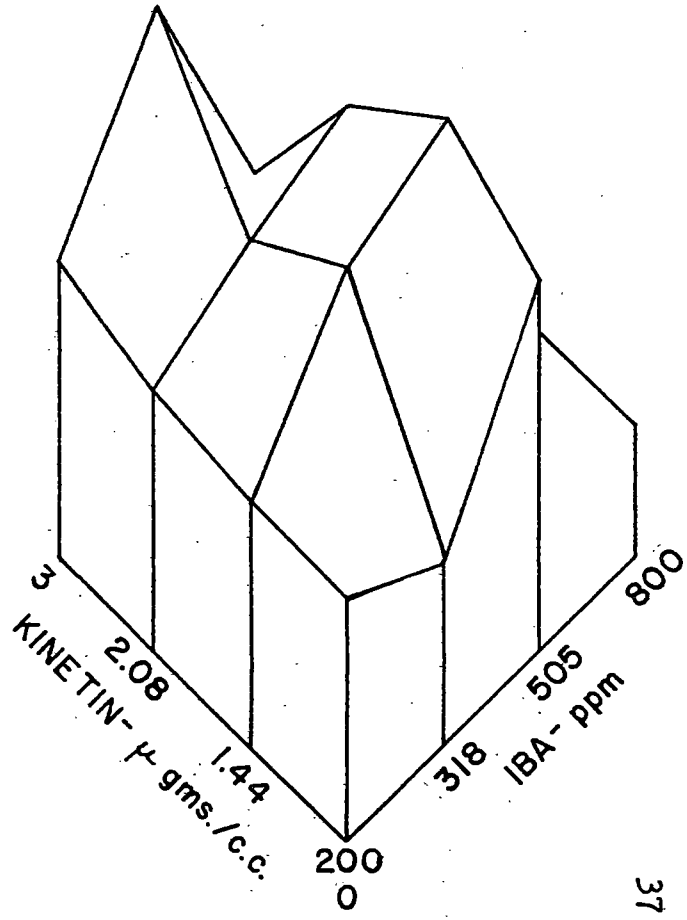
FIG.7



SULTANA

ROOT WEIGHT

FIG.8



CHRYSANTHEMUM.

With Chrysanthemums (Figure 2) the minimum number of roots occurred on the untreated cuttings and the maximum number on cuttings treated with either of two or three micrograms of kinetin and 400 ppm of IBA. The root count curve for cuttings treated with IBA alone rises significantly up to a range of 400 ppm. Similarly, the curve for kinetin treatment also tends to rise to one microgram level and then runs parallel to the base lines. The data do not represent any interaction effect due to the combinations of IBA and kinetin, but the most effective range of concentration according to Figure 2 seems to be 400 ppm for IBA and two micrograms for kinetin when treated as a mixture.

The second set of experiments was with the use of different concentrations set on a logarithmic scale. Almost all curves for root counts are parallel to each other and show no significant increase in the total root counts due to either substance alone. The root counts were significantly increased for a combination of 505 ppm of IBA and 2.08 micrograms of kinetin.

Similar treatments with Chrysanthemum cuttings show an increase in the total number of roots due to IBA alone up to a concentration of 505 ppm. Kinetin was found to have no influence upon the number of roots (Figure 4). The maximum number of roots was produced when IBA was used at 505 ppm and kinetin at 1.44 micrograms. The data do not show any

interaction between kinetin and IBA.

The next four illustrations (Figures 5 to 8) show the weight of roots produced for each set of cuttings treated alike. With Sultana cuttings there is significant increase in root weight when the cuttings were treated with kinetin alone at low concentrations. High concentrations of kinetin, as well as IBA, tended to decrease the weight of roots produced. The weight of roots was least at concentrations of 800 ppm of IBA and three micrograms of kinetin. The maximum weight of roots was produced by cuttings treated with one and two micrograms of kinetin and 400 ppm of IBA. This is shown in Figure 5. The data do not show any interaction between these chemicals.

For Chrysanthemum (Figure 6) there was no significant increase of weight due to treatment with either IBA or kinetin. The minimum weight of roots was produced by control plants and the maximum root weight was measured for those cuttings receiving a treatment with 400 ppm of IBA and three micrograms of kinetin.

Using the second series of concentrations, the maximum weights of the roots were obtained on Sultana when it was treated with 318 ppm of IBA and 1.44 micrograms of kinetin. Apparently IBA within the range of 318-505 ppm together with kinetin at levels of 1.44-2.08 micrograms provides the satisfactory concentrations for producing heavy weight. The

minimum root weight was found for cuttings treated with 800 ppm of IBA and three micrograms of kinetin. The data do not show any interaction between these chemicals.

For Chrysanthemum, kinetin itself was found to have no influence, whereas IBA seems to be effective for increasing root weight up to 505 ppm. Maximum root weight produced was found to be in the same range as for the cuttings of Sultana. The cuttings produced the least root weight when treated with maximum concentrations of these chemicals.

II. DISCUSSION

With the Sultana cuttings it was found that IBA was effective at various concentrations ranging from 200 to 505 ppm. An increase in concentration did not give a proportionate increase in the number of roots produced. For cuttings of Chrysanthemum the most effective range of IBA was found to be the same as for Sultana. Plants treated with 800 ppm of IBA produced at least as many roots as when no IBA was applied.

Kinetin does not increase the number of roots produced by Sultana. There were apparently a few more roots produced by Chrysanthemum as the dosage rate of kinetin was increased. This, however, does not seem significant.

The weight of roots obtained for Sultana cuttings shows that kinetin does have an influence on increasing the

weight of roots. In the case of Sultana cuttings, the maximum weights of roots were for those cuttings that produced the maximum number of roots; but the weights of roots were not uniform so as to give an increased root weight in proportion to increased root numbers. This was true of Chrysanthemum, too.

There appears to have been an interaction between kinetin and IBA when mixtures of these chemicals were applied. Low concentrations of kinetin (1-2.08 micrograms) in combination with effective ranges of IBA (200-505 ppm) tend to produce the maximum number of roots within a given time. Furthermore, an increase in the weight of roots produced more uniform rooting and the emergence of roots from a greater area of stem tissue was observed. The two chemicals appear to function in such a way as to cimpliment each other. The use of high concentrations of kinetin (three micrograms) in combination with high concentration of IBA (800 ppm) had a tendency to reduce the total number of roots produced in the case of Sultana cuttings. This is suggestive of a possible inhibitive interaction of the chemicals, when applied above a range of 505 ppm of IBA and three micrograms of kinetin. Neither the root counts nor the root weights for Chrysanthemum cuttings showed much interaction response. The data suggest that a concentration of kinetin up to three micrograms in a mixture will not exert any inhibitive influence

on treatment with the cuttings of Chrysanthemum. The stem tissues of Chrysanthemum are much less open to absorption of materials in solution than those of Sultana. It is possible that the Chrysanthemum did not absorb as much of the kinetin solution (three micrograms) as did the Sultana and so showed the least injury.

CHAPTER V

SUMMARY

As a concept, the idea of plant hormones or growth regulating substances budded in science with the publication of Darwin's Power of Movement in Plants. Since then startling advances have been made. IAA, IBA, and NAA are some of the best known chemicals for inducing cell elongation. Kinetin is another chemical known for the ability to induce cell division.

The object of this study is to understand the effect of IBA and kinetin upon rooting of cuttings when treated individually and in mixtures. Further, it will enable one to understand the other possible responses of the treated cuttings.

An auxin is an organic substance which promotes growth along the longitudinal axis when applied in low concentrations to shoots of plants freed as far as practicable from their own inherent growth promoting substances. IBA is an auxin. Kinins are substances capable of stimulating cytokinesis when applied in very low concentrations. Kinetin is a specific kinin.

There are several theories explaining the mechanism of auxin actions. None of these theories has been proved yet. The mechanism of auxin action remains unsolved. The means by

which the auxin may bring cell enlargement is being hotly debated between those who feel that the action is primarily based upon the cytoplasmic uptake of water and those who feel that it is principally based upon the growth of cell wall.

Auxins can be treated to cuttings in lanolin paste by dipping in dilute or concentrated solutions of auxins, as dust application using talc or coal as a carrier, and by spraying on mother plants before the cuttings are taken.

Some of the important factors governing the ability of cuttings to root are the age of the plant, age of cuttings, season in which the cuttings have been taken, the number of leaves retained on cuttings, and the environment provided for the cuttings to root.

On reviewing the several experiments conducted by numerous workers using different growth regulating substances on cuttings, as relatively dilute solutions (1-80 mgm/liter), as concentrated solutions (1-20 mgm/c.c.), and as powders (0.5-50 mgm/gram of talc), produce essentially the same rooting responses.

The types of cuttings were selected for this experimental study to include one with soft tissues and quick rooting habit (Sultana) and the other with semi-hard tissues (Chrysanthemum) and difficult to root. IBA and kinetin were the growth regulating substances used. Two series of mixtures

were prepared in 50 per cent alcoholic solutions within a range of 200 to 800 ppm of IBA and one to three micrograms of kinetin. The cuttings were planted on propagation bench into a medium of perlite after being treated for not more than five seconds in respective mixtures. The observations for Sultana were taken twelve days after planting and for Chrysanthemum, twenty-five days from planting.

The root counts taken for Sultana show that IBA had some influence in increasing the number of roots produced. It was not effective for increasing the weight of roots. Kinetin when used alone did not have any influence upon the number of roots, but it did have an influence on increasing the weight of Sultana roots. The maximum number of roots as well as the weight of roots was found with those cuttings treated with mixtures of these chemicals having the various concentrations ranging from 200 to 505 ppm of IBA and 1.44-2.08 micrograms of kinetin. High concentrations of these chemicals tend to exert inhibitive influences.

With Chrysanthemum cuttings the use of IBA alone was found to be highly effective for increasing the roots produced. Kinetin individually was ineffective. The most effective range of these substances when used in mixtures for maximum production of roots was the same as for Sultana. Neither IBA nor kinetin was effective for increasing the weight of roots when applied individually. The maximum weight

of roots was observed on cuttings treated with a mixture of 400 ppm of IBA and three micrograms of kinetin. Tissues of Chrysanthemum are much less open to absorption of materials in solution than those of Sultana.

There appears to have been an interaction between kinetin and IBA when mixtures of these chemicals are applied. Furthermore, an increase in the weight of roots, uniformity of roots, and emergence of roots from a larger area of the stem tiddue were observed. Thus the chemicals appear to function in such a way as to compliment each other.

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APPENDIX

TABLE I

ROOT COUNTS FOR SULTANA CUTTINGS

Kinetin in Microgram	Indolebutyric Acid as Parts per Million 0.0	200	400	800	Sum	
0.0	15	12	15	20	62	
	32	28	20	25	105	
	20	29	5	16	70	
	20	30	16	21	87	
Sum	87	99	56	82		324
1.0	6	20	17	21	64	
	10	21	51	22	104	
	17	10	12	18	57	
	21	13	17	29	80	
Sum	54	64	97	90		305
2.0	14	12	28	20	64	
	16	25	49	24	104	
	15	7	16	11	57	
	17	24	25	18	80	
Sum	62	68	118	73		321
3.0	10	17	20	6	53	
	21	25	23	10	79	
	11	21	18	15	65	
	16	24	27	16	83	
Sum	58	87	88	47		280
Grand Total	261	318	359	292		1230

TABLE OF VARIANCE

Source of Variations	Sum of Squares	Degrees of Freedom	Mean Squares
Total	4391	63	
Variations due to IBA	323	3	107.66
Variations due to Kinetin	76	3	25.3
Cuttings treated alike	1450	48	30.2
Variations due to interaction	1054	9	117.1

TABLE II

ROOT COUNTS FOR CHRYSANTHEMUM CUTTINGS

Kinetin in Microgram	Indolebutyric Acid as Parts per Million 0.0	200	400	800	Sum	
0.0	4	20	17	16	57	
	6	16	21	15	58	
	5	19	22	18	64	
	4	15	24	13	56	
Sum	19	70	84	62		235
1.0	26	24	20	16	87	
	22	19	16	15	75	
	19	22	21	18	76	
	17	18	22	13	70	
Sum	84	83	79	62		308
2.0	25	22	24	17	83	
	25	20	25	18	78	
	21	25	29	14	88	
	16	19	32	13	80	
Sum	85	86	110	49		330
3.0	21	18	27	12	15	
	20	17	31	10	18	
	19	24	29	14	11	
	14	17	26	13	17	
Sum	74	76	113	61		324
Grand Total	262	315	386	234		1197

TABLE OF VARIANCE

Source of Variations	Sum of Squares	Degree of Freedom	Mean Squares
Total	2682.4	63	
Variation due to IBA	837.7	3	279.5
Variation due to Kinetin	360.2	3	120.0
Cuttings treated alike	1876.1	48	39.1
Variation due to interaction	677.2	9	75.2

TABLE III

ROOT COUNTS FOR SULTANA CUTTINGS

Kinetin in Microgram	Indolebutyric Acid as Parts per Million 200	318	505	800	Sum	
1.0	8	3	7	22	40	
	34	33	17	22	106	
	23	14	28	15	80	
	14	19	29	13	75	
Sum	79	69	81	72		301
1.44	14	12	13	11	50	
	29	18	18	19	84	
	14	18	20	11	63	
	11	26	12	25	74	
Sum	68	74	63	66		271
2.08	11	18	9	14	52	
	16	20	19	28	83	
	10	17	15	21	63	
	21	21	30	25	97	
Sum	58	76	73	88		295
3.00	11	6	15	21	53	
	11	10	16	23	60	
	11	17	8	18	54	
	28	31	25	13	97	
Sum	61	64	64	75		264
Grand Total	266	283	281	301		1131

TABLE OF VARIANCE

Source of Variations	Sum of Squares	Degree of Freedom	Mean Squares
Total	3176	63	
Variation due to IBA	38.4	3	20.2
Variation due to Kinetin	60.7	3	12.8
Cuttings treated alike	243.8	48	5.1
Variations due to interaction	144.7	9	16.8

TABLE IV

ROOT COUNTS FOR CHRYSANTHEMUM CUTTINGS

Kinetin in Microgram	Indolebutyric Acid as Parts per Million 200	318	505	800	Sum	
1.0	19	16	32	19	86	
	17	19	29	20	85	
	22	21	28	17	88	
	20	18	27	20	85	
Sum	78	74	116	76		344
1.44	24	32	32	15	103	
	22	26	30	16	94	
	21	28	31	13	93	
	25	29	32	17	103	
Sum	92	115	125	61		393
2.08	25	31	26	19	101	
	21	26	20	19	86	
	18	21	23	17	79	
	20	24	27	15	86	
Sum	84	102	96	70		352
3.00	24	33	29	16	102	
	19	28	24	19	90	
	21	26	23	14	84	
	22	30	28	12	92	
Sum	86	117	104	61		368
Grand Total	340	408	441	268		1457

TABLE OF VARIANCE

Source of Variations	Sum of Squares	Degree of Freedom	Mean Squares
Total	1688.5	63	
Variations due to IBA	1103.5	3	367.80
Variations due to Kinetin	87.5	3	29.10
Cuttings treated alike	1586.7	48	33.50
Variations due to interaction	395.7	9	43.94

TABLE V

ROOT WEIGHTS OF SULTANA CUTTINGS

Kinetin in Microgram	Indolebutyric Acid as Parts per Million 00	200	400	800	Sum
00	0.236	0.234	0.269	0.272	1.011
	0.178	0.209	0.212	0.266	.872
Sum	0.414	0.443	0.481	0.539	1.876
1.0	0.248	0.224	0.342	0.243	1.057
	0.375	0.310	0.374	0.268	1.327
Sum	0.623	0.534	0.716	0.571	2.384
2.0	0.226	0.246	0.236	0.268	.976
	0.298	0.391	0.352	0.298	1.336
Sum	0.524	0.637	0.588	0.566	2.315
3.0	0.239	0.238	0.218	0.042	.737
	0.319	0.294	0.182	0.210	1.005
Sum	0.558	0.532	0.400	0.252	1.742
Grand Total	2.119	2.146	2.192	1.867	8.317

TABLE OF VARIANCE

Source of Variations	Sum of Squares	Degrees of Freedom	Mean Squares
Total	0.14431	31	
Variations due to IBA	0.00778	3	0.00259
Variations due to Kinetin	0.03793	3	0.01130
Cuttings treated alike	0.08630	16	0.00540
Variations due to interaction	0.04059	9	0.00451

TABLE VI

ROOT WEIGHTS OF CHRYSANTHEMUM CUTTINGS

Kinetin in Microgram	Indolebutyric Acid as Parts per Million 0.0	200	400	800	Sum	
0.0	0.008	0.292	0.151	0.247	0.698	
	0.009	0.254	0.164	0.214	0.641	
	0.009	0.292	0.242	0.248	0.791	
	0.007	0.218	0.257	0.203	0.679	
Sum	0.033	1.056	0.808	0.912		2.809
1.0	0.606	0.399	0.190	0.184	1.379	
	0.538	0.198	0.162	0.172	1.070	
	0.207	0.361	0.160	0.162	.890	
	0.189	0.221	0.166	0.163	.739	
Sum	1.540	1.179	0.678	0.681		4.078
2.0	0.448	0.357	0.278	0.045	1.187	
	0.398	0.309	0.294	0.041	1.115	
	0.210	0.284	0.276	0.056	1.405	
	0.131	0.165	0.557	0.051	.193	
Sum	1.128	1.042	0.826	0.904		3.900
3.0	0.396	0.160	0.312	0.124	0.992	
	0.412	0.152	0.401	0.150	1.115	
	0.285	0.316	0.321	0.128	1.050	
	0.171	0.161	0.303	0.156	.791	
Sum	1.264	0.789	1.337	0.558		3.948
Grand Total	3.965	4.066	3.649	3.055		14.735

TABLE OF VARIANCE

Source of Variations	Sum of Squares	Degrees of Freedom	Mean Squares
Total	1.0757	63	
Variation due to IBA	0.0319	3	0.0106
Variation due to Kinetin	0.0621	3	0.0207
Cuttings treated alike	0.4750	48	0.0990
Variation due to interaction	0.3810	9	0.0423

TABLE VII

ROOT WEIGHTS OF SULTANA CUTTINGS

Kinetin in Microgram		Indolebutyric Acid as Parts per Million 200	318	505	800	Sum	
	0.0	0.216	0.342	0.296	0.342	1.196	
		0.225	0.342	0.442	0.475	1.534	
Sum		0.441	0.684	0.788	0.817		2.730
	1.44	0.202	0.301	0.276	0.251	1.030	
		0.125	0.571	0.413	0.302	1.411	
Sum		0.327	0.872	0.689	0.553		2.441
	2.08	0.178	0.253	0.344	0.401	1.176	
		0.241	0.286	0.462	0.371	1.360	
Sum		0.419	0.539	0.806	0.772		2.536
	3.00	0.246	0.283	0.441	0.423	1.393	
		0.178	0.496	0.315	0.114	1.103	
Sum		0.424	0.779	0.756	0.537		2.496
Grand Total		1.611	2.874	3.039	2.679		10.203

TABLE OF VARIANCE

Source of Variations	Sum of Squares	Degrees of Freedom	Mean Squares
Total	0.3940	31	
Variation due to IBA	0.1531	3	0.0510
Variation due to Kinetin	0.0103	3	0.0031
Cuttings treated alike	0.2224	16	0.0139
Variation due to interaction	0.0590	9	0.0038

TABLE VIII

ROOT WEIGHTS OF CHRYSANTHEMUM CUTTINGS

Kinetin in Microgram	Indolebutyric Acid as Parts per Million 200	318	505	800	Sum	
1.0	0.295	0.242	0.518	0.181	1.296	
	0.283	0.251	0.427	0.192	1.153	
	0.346	0.234	0.441	0.172	1.193	
	0.351	0.246	0.462	0.188	1.267	
Sum	1.275	0.973	1.928	0.733		4.909
1.44	0.311	0.534	0.586	0.176	1.607	
	0.294	0.507	0.574	0.166	1.541	
	0.310	0.491	0.584	0.161	1.546	
	0.360	0.512	0.586	0.171	1.629	
Sum	1.275	2.044	2.330	0.674		6.323
2.08	0.541	0.469	0.494	0.212	1.715	
	0.284	0.412	0.367	0.232	1.295	
	0.291	0.382	0.458	0.201	1.332	
	0.286	0.391	0.501	0.198	1.376	
Sum	1.402	1.653	1.820	0.843		5.718
3.00	0.488	0.662	0.274	0.131	1.553	
	0.321	0.462	0.281	0.141	1.205	
	0.386	0.566	0.197	0.136	1.285	
	0.371	0.681	0.204	0.128	1.384	
Sum	1.666	2.371	0.956	0.536		5.429
Grand Total	5.518	7.041	7.034	2.786		22.379

TABLE OF VARIANCE

Source of Variations	Sum of Squares	Degrees of Freedom	Mean Squares
Total	1.4140	63	
Variations due to IBA	0.7490	3	0.2490
Variations due to Kinetin	0.0590	3	0.0190
Cuttings treated alike	1.0545	48	0.0220
Variations due to interaction	0.2465	9	0.0274