SOME QUANTITATIVE AND QUALITATIVE STUDIES ON PLANT HORMONES

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BY

TAIICHI ASAMI

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SOME QUARGITATIVE AND QUALITATIVE STUDIES ON PLANT HOPEOVES

INTRODUCTION

Growth is a mysterious phenomenon of nature. A crystal grows, increasing its size quantitatively. The root and shoot of a germinated corn seed grow at the expense of the plant's own stored energy, while at it's maturation phase the milky substances are condensed with a loss of volume and thus energy is stored in the form of food in the seeds. When a mosquito bites our hand a swelling occurs, but no zoologist would designate this as growth, but many workers with plant hormones consider that the swelling of the plant body caused by biting insects or that due to any other causes, is the result of growth associated with hormones. Nany investigators are trying to demonstrate that the complex phenomenon of growth is theresult of the presence of some one or more hormones, even though there has been offered no definite nor satisfactory proof of their existence up to date. It is of course a difficult matter to determine whether or not all the phenomena related to growth can be attributed to the presence of hormones since growth is one manifestation of life itself.

Voluminous reports upon "plant hormones" have been published in recent years. There are two apparent reasons why so many workers are entering this field. Many complicated growth reactions such as tropisms, curvatures, abnormal growths, correlations, metaxenia, etc., may be explained by postulating the presence of hormone-like substances. Since hormones or chemical missengers have been demonstrated in the animal world, it would be desirable, if possible, to extend the idea to the plant world.

The term "hormone" in the vegetable world is used very ambiguously. The word was first suggested in connection with plants by Fitting (23) who found that a substance present in orchid pollen caused swelling of the gynostemium in the orchid flower. Starling (54) defined "hormone" as "any substance normally produced in the cells of some part of the body and carried to distant parts which it affects for the good of the body as a whole." Koogl (30) proposed the inclusive term "auxin" for growth substances that bring about cell enlargement. In comparatively recent years a number of terms not well defined have been referred to by various workers as growth hormones, phytohormones, auxins, plant hormones, etc. However, Boysen-Jensen (11) wrote that to determine the hormonal function is more i portant than to define hormone itself. In this paper the term hormone is used to include (a) laboratory synthesized chemicals which stimulate growth or cause bending of the coleoptile and, (b) chemically unknown substances extracted from plants which stimulate growth and produce curvatures.

The present paper is chiefly confined to the studies of the first type, for example, indole butyric acid and naphthalene acetic acid. A limited number of experiments have been performed both in the greenhouse and the field to determine the practical value of using these chemicals in the form of dusts on various seed and plant parts.

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HISTORICAL REVIEW

For the sake of clarity an effort has been made to arrange this review according to functional evidence and only those papers considered pertinent to the present problem will be discussed. Plants respond to certain stimuli such as chemical, thermal, mechanical, gravity, light, etc. Among these the reaction to light, known as phototropism, has stood out as a well known example since the time of Darwin. Darwin (21) carried on experiments with the coleoptile of <u>Phalaris canariensis</u> and concluded that the phototropic stimulus must be transmitted from the tip toward the base. Even today his demonstration of phototropism appears in various text books of general botany. Recently, Hoysen-Jensen (11) demonstrated that the transmission of a stimulus from the lighted side to the shaded one in the <u>Avena</u> coleoptile could be stopped by an incision.

In 1938, Went (63) reported that an agar diffusible substance from other plants caused bending of the <u>Avena</u> coleoptile when unilaterally applied. He also demonstrated the existence of a quantitative relationship between the concentration of the substance and the degree of bending. If the function of a hormone is to cause curvature in certain parts of a plant body, then Went must be given the credit of discovering "phytohormones." Rothert (47) reported that the removal of the coleoptile tip reduced growth in the stump. After 10 to 14 hours growth began again and the suggestion was made that this might be due to regeneration of the tip. Theorizing from this observation Boysen-Jensen (11) concluded that a substance is dispersed from the tip which promotes growth in the basal region. The writer believes, however, that it is probable that the temporary cessation of growth was due to the direct effect of

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injury by docapitation.

Weij (62) showed that if two agar blocks containing a growth-promoting substance were placed on eitherend of a cut coleoptile cylinder 2 mm. long, a decrease in growth substance took place in the upper block, but no increase was demonstrated in the lower block. "The most likely explanation of this and numerious similar observations is that it is consumed in growth," wrote Boysen-Jensen (11). The present writer believes it is just as probable that the hormone can be, so to speak, consumed through chemical reaction.

In 1934 Went offered the "Pea test method" for measuring the concentration of growth hormone in the solution form, and ennounced that it was just as reliable as the <u>Avena</u> test. Du Buy (15) reported that growth in the coleoptile is gradually retarded when the endosperm is removed. Some hormone workers (11) emphasized the importance of the supply of growth substance from the endosperm, and when this was absent, through being cut out, growth was retarded. The author suggests that the retarded growth may be due to a direct effect of injury rather than the removal of any hormone. Although <u>Avena</u> curvature occurs with the use of many synthetic chemicals and agar diffusible substances from living plant tissues, the response is, however, not always of the same nature nor always constant, and therefore "the use of <u>Avena</u> curvature values is not a reliable criterion either for the action or for the transport of growth substance in the tissue of other plants," wrote Skoog (50) recently(1938).

Very little is known of the chemical nature of hormones found in plants. At the present time the compounds which are obtained from plants and which stimulate Avena curvature are only three in number, Auxin "a",

Auxin "b", and heteroauxin. All of them were found first by Koogl and his co-workers. Auxin "a" (Auxentriolic acid) was isolated in 1933, from urine (31). It is characterized as follows: molecular formula $C_{18}H_{52}O_{5}$, molecular weight 238, crystals hexagonal, and stale in acid. The name Auxin "a" was given after Auxin "b" was found. Auxin "b" (Auxenolonic acid) was isolated from maize germ oil (32), with a molecular formula $C_{18}H_{30}O_{4}$, molecular weight 310, melting point 183, and is unstable to both acid and alkali, and easily decomposed by peroxides. Both Auxin "a" and "b" have recently been extracted from higher plants; for example malt, maize, peanut, sunflower, mustard, and linseed oils. Hetroauxin (3-indoleacetic acid) was prepared from urine (33) and also later found in yeast. Its molecular formula is $C_{10}H_{9}$ $O_{2}N$.

The presence of hormones in living tissues is at the present time chemically undetectable, and no quantitative or qualitative chemical tests are therefore available. For the determination of synthetic hormones, when diffused or injected into plants, Hitchcock and his co-worker (29) in 1958 reported that the <u>Avena</u> test or other seedling tip test were not reliable but that the Winkler and Petersen colormetric method (indole group test) was quite suitable. Identification of indole-butyric acid by the spectroscopic method is most applicable for such small quantities applied (59).

A mechanism for the formation of hormone-like substances has been offered by several workers. In 1932 Sakamura (48) reported that the formation of some growth substances was accelerated by temperature, and also by feeding amino acid in the c se where lower plants were used. In higher plants, however, very little is known of how the hormones are produced or

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the conditions which facilitate their development. In 1937 Avery (3) reported that hormone content was proportional to light intensity and also to concentration of CO_2 . It is clear that some substance which causes <u>Avena</u> curvature is increased, but whether the substance obtained by agar diffusion is a real hormone or merely some chemical by-product is not yet clear.

In 1934 Bonner (7) reported that mineral acids alone would cause curvature or would accelerate normal growth in Avena coleoptiles under certain conditions. Hitchcock (29) recently concluded that "There appears to be no specificity of action for plant hormones." In 1937, Bonner (8) carried out a very interesting experiment. Decapitated root tips of the pea, growing in nutrient solution, were cut and transferred into new cultures to eliminate the original thiamine (Vitamin B_1) that remained in the tissue. After repeating this transfer several times cell development was nearly stopped. Upon the application of Vitamin B_1 , growth again started. This experiment was designed to give some idea of whether or not any other hormone was necessary for the growth of roots. However, Thinann and his co-worker (53) concluded, regarding this work, that there was no doubt that Vitamin B1 was a growth hormone, "because it stimulates root growth." If one is led to believe from this that Vitamin B_1 is specific in its action on roots, it must be emphasized that up to date phytohormones have not been shown to be specific.

Cell division by hormones has been reported by several workers. In 1935, Snow (52) reported that the application of Auxin and E-indole acetic acid (heteroauxin) to the upper ends of decapitated Helianthus seedlings caused growth in thickness through cambial division. But other workers have postulated the existence of other special hormones for

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cell division (39). The role of ${}^{a}_{A}$ certain substance, bios, which "apparently does not belong in the same category with auxins", has been described by many investigators (43). The suggestion that hormones may act upon cell division has not yet been satisfactorily demonstrated (11).

Most investigators agree upon the inhibiting effect of hormones upon the growth of lateral buds. In 1938, Albaum (1) reported that, when a 1 per cent indole actic acid in lanolin paste was applied to the apical cut surface of certain prothallia it inhibited adventitious outgrowths. while removal of the landlin paste by excision caused the resumption of growth. This experiment is very interesting, because the inhibition by indole acetic acid in lanolin paste could be removed by the excision of the part of the plant upon which it was applied. This hormone seems to retard budding without much apparent diffusion into the tissue. Thus even if diffusion occurs at all, it must be very small and mainly limited to the point of application. (One hormone worker reported by Boysen-Jensen (11) mentions the presence of a hormone, produced by the terminal bud that promoted its own growth but inhibited lateral buds. If this is so, then one may ask how is it possible that a single hor one can serve dual purposes? Hitchcock (28) has shown that lateral bud inhibition can be brought about by the use of indole acetic and indole probionic acids, as well as with ethylene and propylene gases when applied to decapitated tobacco plants. Therefore bud inhibition is not due to a specific hormone because the inhibition may be caused by many other chemicals.

Maize seedlings growing in darkness usually show significant elongation of the first internode. Inge (36) reported that heating to 51°C one hour stopped the elongation, and from that he concluded growth inhi-

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tion was due to the destruction of the hormone. Such conclusions, often found in the reports of hormone workers (11), are inadequate and unjustified from the experimental evidence presented. Some apparently do not take into consideration the fact that conditions such as variations in temperature or treatment with x-rays have their direct effects upon protoplasm and must therefore be evaluated.

In 1935, Thimann (50) observed inhibition of root elongation and at the same time the initiation of new roots by the a plication of the analogous compounds such as indole acetic acid, 3-indene acetic and 1-counsryl acetic acid. In 1935, Zimmerman and his co-worker (67, 68) reported that several he mones, beta raphthalene acetic, 5-aceto naphthalene acetic, indole butyric, phenol acetic, fluorene acetic, anthracene acetic acid, and alpha naphthalene acetic acid and indole butyric acid were the most effective root producing substances. Zimmermann (66) showed that ethylene and propylene were effective in causing curvature of <u>Avena</u> coleoptile. Carbon monomide and ethylene also induced roots on stems in numerous species of plants when a plied in lanolin paste. This further supports the non-specificity of hormones for root initiation.

Several workers have reported that root growth was also increased by decapitation. Cholodry (16) concluded that Maize root tips produced a growth substance which inhibited its development and that if the root tip was removed, increase in length of the stump t ok place. The root and shoot grow in symbiotic relationship and one benefits from the other. The root is parasitic on the shoot for its corbohydrates and possibly Vitamin B₁ (59). On the other hand it might be hindered by the presence of the shoot. Roots are easily cultivated on special media and root growth

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without shoots could be stimulated under ideal conditions. Whether shoot growth could be stimulated under ideal conditions is problematical but theoretically possible. Shoot growth without roots and root growth without shoot when the inhibiting effect of the other is removed may be stimulated although this may only be temporary.

In 1934, Laibach (37) reported that callus formation appeared after the application of landlin paste containing an extract obtained from orchid pollinia or human urine. There are many reports concerning cellus or tumor formations and hormome enthusiasts have been trying to show that such all ormal growth phenomenon are connected with the presence of a hormone, especially in the case of tumor formation of leguminous plants. These abnormal growths, however, are not due to a definite hormone for such growth may be obtained with the use of many kinds of chemicals (11). Parthenocarpy induced by hormone application has been reported by several workers. In 1930, Gustafson (27) reported that definite chemical substances, which are not specific, caused the ovary of a flower to develop into the fruit without fortilization. He thought these substances were closely related to the auxins.

It was sug ested that if hormones affected metabolism in the plant body, they might affect the processes other than growth, such for exam le as respiration. In 1958, Pratt (45) reported that a concentration of heteroauxin varying from 0.0000% to 0.02% markedly accelerated the respiration of the <u>Triticum</u> embryo and strongly depressed growth. It seems that heteroauxin is capable of markedly accelerating some metabolic reactions of plants but decreases others, therefore, the cause could just as well be related to something other than growth promoting substances. Further, Boysen-Jensen and his co-worker (10) studied the effect of

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decapitation on the intensity of respiration in Avena coleoptiles and finally concluded that there was no effect of growth substances upon respiration. Thus higher concentration of hormones may cause charical injury while lower concentration of hormones exhibits no effect on respiration.

There are also numerous reports on the effect of hormones in normal plant growth. In 1956, Loehwing and his co-worker (39) published the only report, so Thimann and his coworker (59) say, that showed any marked increase in the elon ation of the stem due to heteroauxin application. They demonstrated that intact stock seedlings could grow in aqueous solution of a growth substance (0.07%) or in the soil, treated with certain growth substances. They also reported slight increase in top elongation of Avena seedlings grown in solution culture with heteroauxin. In 1937, Grace (24) observed increases in growth of wheat and several other young plants treated with hormone is the solution or in the powder form. Marmer (41) applied a wide range of concentrations of hormones. her results showed no stimulation in growth for wheat seedlings, but a decrease of primary roots and an increase in number of secondary roots. Thimann and his covorker (60) have recently report d that auxin treatment (indole acetic acid 0.01% solution) hastened the development of the photosynthetic area at a given time by 50-90%. The conditions under which this stimulating effect was obtained in the case of tomato plants. were not fully described, for in other series of the same experiment no effect upon growth was apparent. The variation might have been connected in some ay with water relations. Finally, Hitchcock and his coworkers concluded that Went's axiom, "Without growth substance, no growth," is

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difficult to prove fecause of the limitation of experimental methods. To quote further from these authors, the above axiom is essentially no different from that proposed by Avery, "No nitrogen, no growth," and could be said of other important substances such as P, K, Ca, Fe, etc.

The effects of hormones upon normal plant growth have been reported with conflicting results, some positive and others negative. Several workers have attempted to ex lain these variable results. Greenfield (26) proposed the hypothesis that "auxin satiated plants" have already sufficient auxin, and therefore an additional supply causes injury and consequently no growth. On the other hand "auxi. unsatiated plants" have not enough hormone, and consequently could take up an additional supply and increase growth. Wheat seedlings were taken as an example of "auxin satiated plants," as their growth was not stimulated over a wide range of concentrations of several different growth substances. Cholodny (17) presented the hypothesis that growth substances promoted the rate of development of growing cells but shortened the length of their individual life cycles. In the case of the cells in the root these matured quickly without increasing in length, and therefore resulted in retardation of growth. In the stem, on the other hand, the zone of cell stretching was greater, and growth continued for a relatively longer time. However, there is insufficient evidence to support this hypothesis at the present time (11).

Growth in plants, induced through the use of so-called plant hormones, has not indicated that any of them are specific, and therefore it naturally follows that the results are due to stimuli of chemical, electrical and mechanical, etc., nature. In 1937, Leonian (38) concluded that heteroauxin was a growth inhibiting rather than a growth pro oting

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substance. He interpreted many reports, including <u>Avena</u> coleoptile curvature, as plant responses to irritation which are equivalent to mechanical injury or to parasitic invasion. Purdy (46) reported for <u>Avena</u> that the initial curvature caused by wounding was positive and then was negative and for a second time became positive. —arotta (40) reported that sprouts of <u>Zea mays</u> grow readily in nutrient solution. If, however, the sprouts were slit (1 mu.) new roots would then appear. Also on addition of a Pb-salt to the nutrient solution, a retardation in growth occurred for the first few days, but after 11 days root growth was accelerated.

Recently, in 1938, Thimann and his coworker (60) reported a very interesting study. They vernalized Avena and Triticum seed by the cold treatment and compared their growth with hormone treated seedlings (C.OL/2 indole acetic acid in solution.) The result showed similarity of growth between the two treatments. Reviewing the literature and evaluating their results, they concluded that at first root inhibition was doubtless a general property of all auxin activity, but that later. growth was accelerated. Avena plants from hormone treated seeds, these authors found, would flower from three days to one week earlier than the controls. The writers believed that the growth of a plant especially in the early stage is dependent on the amount of water available, and therefore any increase in the amount of absorbing surface would naturally cause an increase in growth. In the case of Triticum, auxin treatment at an early developmental stage produced marked reduction in the size of the first leaf. In general it was found that the greater this reduction in size the greater was the subsequent acceleration of growth in the older leaves. The authors tentatively suggested that the vegetative effects of

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$y = \frac{1}{2} + \frac{1}{2} \frac{1}{2}$

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vernalization were due to the prolonged exposure of the seed to its internal auxin supply.

MATERIALS AND METHODS

Materials used in these experiments are listed in table 1. Hormone dusts were made by mixing fibely ground hormone constals with table powder. Mixing was facilitated by the use of a rotating machine run usually for a period of 20 hours at a time. Two concentrations of 3-indole-butyric acid (1 per cent and 20 per cent) were first made up and from these all the other concentrations were prepared (table 2). When very low concentrations were desired, a relatively high concentration was selected and the weaker ones made by dilution with talc powder. Lanolin pastes were prepared by mixing finely powdered crystals with lanolin. A ten percent paste was first hade and from this a three percent and a 0.1 percent were then prepared.

For practical purposes, it is important to know the optimum amount of dust adsorbed by seeds before using the seed dusting method. The amount adsorbed, as it is seen in table 3, is not constant, and therefore merely adding an excess amount of dust and shaking this off would not give the exact amount taken up by the seeds. Bernburg (5) reported a method for determining the optimum dust adsorbing power of 5 gram quantities of seeds. His method is not applicable for larger amounts of seeds, as for example a 100 gram sample. After several trials the following method was

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adopted. Various an unts of tale were added to various 100 gram samples of seeds. These were then mixed in a bottle, shaken vigorously by hand for five to ten minutes, and then transferred to a sieve and the encess powder removed with violent shaking for one minute. The results of this test are given in table 3, and the curves are shown in fig. 1. The princi le is this; the optimum point of adsorption is assumed to be that point where the amount added (Y) equals the amount adsorbed. There is therefore no excess (X), consequently the value of Y at X = 0 and this naturally is the optimum adsorption point. In the case of buckwheat the optimum point was calculated by solving the normal equation, which is given in table 4, and running through the calculations which all found in the subsequent paragraph. All the realts for optimum quantities of dust adsorbed by 100 gram sample are given in table 5.

From table 4 the following data are obtained for buckwheat

	n :	5		
Σ	у	= 11.57	Σ x ² =	31.48
Σ	x	8.78	Σ xy =	24.57.

The predicted equation is

$x = a \pm b y$,

where a and b are constants and x is excess dust remaining after mixing. The normal equations are

Substituting known values from the table, the normal equations are

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5 a + 11.57 b = 8.78

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11.57 a 31.48 b = 24.57.

Solving above equations alternately, the constants for the predicted equation are found, a = -0.278 and b = 0.878. The theoretical equation must be

x = - 0.268 + 0.878y.

When dust is completely adsorbed there will be no excess abount remaining after mixing it with the seeds, that is, at this point x = 0, and the equation becomes

From this, the optimum amount adsorbed (y) is solved,

y = 0.305 g./100g. seeds.

In the case of corn, large petri dishes (15 cm to 25 cm in diameter) covered with bell-jars, were used as moist chambers for germinating purposes. Filter paper was placed in the dishes on round glass plates adjusted to an angle of about 30°. This afforded more normal conditions for root growth. Wet cotton was placed around the glass plate to maintain moisture conditions. Ten or 15 seeds were placed on the filter paper near the upper edge. The bottom of the dish was covered with tap water, about one centimeter deep. Soon after germination had occurred only five to ten uniformly germinated seeds were lined up at definite distances on the filter paper. The whole set, arranged in an orderly manner, was placed on the table under diffused light. At a later date it was found convenient to use a much larger moist chamber, which is shown in Plate 1. The inside of this glass box or Wardian case (llx70x90 cm.) was lined with cloth to retain moisture, and the roof part was so hinged that adjustment could be made for temperature. The case was set upright in a galvanized iron pan in which about 5 centimeter of water was maintained.

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On one end of the case there is a swinging door. Seeds were mounted on filter paper resting on tilted glass plates placed in a shall pan (50 x 50 x 10 cm.) shown to the left of the Wardian case in Fig. 1. This shall pan was kept in the moist chamber during an experiment. All measurements of whatev r nature were mide in this chamber to prevent drying of young roots or root hairs. The case was placed in the botany greenhouse. Further details for other methods or modifications of the same will be given under each experiment.

IPPERIENAL

Grace (24) and others have mecentaly advocated the use of hormone dust on seeds and have reported increases in growth of roots and tops of certain species of plants. Hormone in the powder or dust form is said to be better than the liquid form especially on soil or sand cultures since the supply is available at greater dilution and over a longer period. A study of the literature fails to reveal the most effective hormoges or their best operating concentrations. It seems, however, that maphthalene acetic acid and indole-3-butyric acid are two of the best substances for root development (11). In 1938, Hitchcock and his coworker (29) reported that species of the same genus did not respond alike to a given treatment, and the same might be said of different varieties of the same species. However, they reported that in most cases the optimum concentration of indole butyric acid in solution was 0.05 percent or

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. ; less, and this was especially true for root production. According to Grace (24) wheat seeds dusted with 2:1,000,000 of indole-3-acetic acid (2 parts of hormone per million parts of seeds) increased root growth 65 percent at the end of a 30 day period and that soy bean seeds treated with 10:1,000,000 naphthelene acetic acid yielded greatest top growth.

In a preliminary experiment the growth of barley and corn roots was studied carefully in petri dish cultures every day for several days, but no significant differences could be detected at low concentrations such as 0.05-4 p.p.m. (prots per million parts seeds) when indole butyric acid was used. These studies showed the necessity of adhering to cortain definite procedures in such experimentation. The seeds are very sensitive to variations in external conditions, such as moisture and temperature, and seemingly more so than to hormone treatments.

When seeds are placed too far from or too close to the water level, germination is retarded. In the case of corn there is a day gained in germination when the seed is placed with the embryo next to the wet filter paper rather than in the reverse position. It was found necessary to adopt some definite procedure for observing root growth. Equal time periods for observations, and photographic exposures, etc. ware selected. The cultures were arranged according to concentrations, or according to age, etc. By such means other workers might easily be able to repeat the experiments. Root hairs of young seedlings are particularly sensitive to moisture changes. A 20-minuted exposure to dry air is sufficient to ruin seedlings for experimental purposes. Seedlings which have a delicate root system such as wheat or barley are not very suitable for experiments on root growth under the conditions obtaining. For such reasons most of the experiments were conducted with corn which seemed relatively more resistant to changes in moisture conditions.

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The relatively few seeds used in any one experiment may seem insufficient from which to draw conclusions, yet the nature of the experiment precludes the use of larger numbers of corn seeds in any one experiment. To increase the number of seeds, for example, would increase the time to make the necessary measurements and this in turn would produce greater variations in temperature, moisture and other conditions and would lengthen the time period for the study of each culture. Consequently the experiments were repeated a large number of time. In this case the experiments with corn seedlings on filter paper were repeated twenty times or more.

The use of any chemicals for disinfection was avoided for fear of secondary reactions arising. The effect of these might be difficult to distinguish from those due to hormones. Seeds were carefully selected and brushed with a clean dry cloth. If any molds appeared in the cultures the cultures were discarded. All filter papers were sterilized in dry heat and all glassware and dishes first cleaned with formaldehyde and then treated to dry sterilization.

EIFERINGES I. THE EFFECT OF HURIDHE ON STEDLE GS GROWING ON FILTER PAPER.

Before dusting, all seeds were fanned, cleaned, and colished in a cloth sac. Samples of 100 grams of seeds were mixed with dust in small 8 to 12 oz. bottles and kept thereaft r in the same containers. Dusting hormone powder on the filter papers was accomplished by spreading the stock powder on as evenly as possibly with a spatula.

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The data for the growth of Dent corn on filter paper treated with hormone are shown in table 0 and plate 7 illustrate the condition of growth. It is seen from the table that germination and slightly retarded at the higher concentration, for example 0.2 mg/paper, and also that root growth was inhibited significantly at a higher concentration than 0.02 mg/paper. Theoretically, hormones increase growth, consequently there should be a certain proportionality between growth increases and increasing concentrations, but no such evidence was apparent. It would appear then that one might just as wellattribute the slight variations to chance and not to hormone treatments. Hormone treated seeds do not show any marked tendency towards increased growth at low concentration, such as 0.05-2 p.p.m. (table 7). Relatively yo nger stages of growth are presented in these cases, because with older seedlings secondary factors would enter in, such as a limitation of stored food in the endosperm, for example.

In table 6 can be found the data of another corn experiment where in this case there is definite evidence of growth accelaration in both tops and roots where the concentrations of hormone were low. At high concentrations growth was significantly retarded. Fig. 2 shows the curves plotted from data in table 6. The graph shows that the inhibition in root growth is greater than that of to growth. This was repeatedly observed in the different experiments. In table 9 will be found the Sata for seeds treat d with hormone concentrations varying from 20-200 parts of hormone to a million parts of seeds. Top growth is not much affected but root growth is significantly inhibited at higher concentration.

At higher concentrations (2000 p.p.m.) as shown in table 10 both

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both tops and roots were similificantly inhibited while at 200 p.p.m. only the roots were inhibited. It was repeatedly noticed that when root growth was inhibited nonerous secondary roots developed from hear the seed. It was also observed that numerous root hairs developed on retorded primary roots. It seems that the root hair is more resistant to chemical injury of hormones. Meesters (23) reported, "The root hairs are less sensitive to the growth substance." The production of dense root hairs may be accounted for through the fact that hormones are not believed to affect cell division but will modify cell elongations. Others affirm that hormones inhibit both cell division and elongation, and if this is so, root hair growth might then be stimulated. Without furth r anatomical study other suggestions would have little value. Various stages of development in certain Speet corn seedlings can be seen in plates 3,4, and 5.

Table 11 shows the effect of light upon the Sweet corn seedlings growing on hormone treated filter paper. Marked differences can be seen in the length of the first internode. Darkness seems to favor elongation. Inhibitions of top and root growth through hormone activity is less in darkness than in diffused light. These reactions could just as well be due to effects of darkness or to several other possible favorable or unfavorable conditions. Many observations have led the author to conclude that hormone treatment has not significantly modified the growth of the first internode, whether in darkness or in diffused light. The length of the coleoptile was constant regardless of hormone treatment or light variation. It is suggested that retardation of germination while in darkness is due chiefly to alowering of the temperature.

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The effect of hormone on the rowth of buckwheat is shown in table 12. Buckwheat roots are also sensitive to variations in moisture, etc., and if exposed for even 20 minutes to room temperature growth will have ceased at the end of twenty-four hours. Later experiments with buckwheat were conducted in the Wardian case described above. Buckwheat seedlings show considerable variability in their growth habits. It was very difficult to obtain seedlings of uniform length and apparent equal vigor. as was possible to do with wheat or barley. The results of such experiments are plotted and shown in Fig. 3. At lower concentrations the curve indicated stimulation but this was found not to be the general rule when the experiment was repeated six times. At the higher concentrations there was always a retardation of top and root growth in the younger plants. At higher concentration (300-3000:1,000,000) both roots and tops were retarded; the former much more so. At a later date growth retardation of both tops and roots was greatly diminished, and complete recovery finally resulted.

Plates 8 to 11 show the various stages in the development of buckwheat seedlings. It followed inevitably that at higher concentration when primary roots were inhibited there was a heavy production of secondary roots which developed from the base of the stem. As compared with check plants, the total functional root mass was thesame or in some cases better. This can be seen on a study of plate 12. Experiment 1 is sumarized in table 13.

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ELPERIELT II. THE EFFECTS OF HORIONE ON SEELING OROWIN IN FLATS OR POTS IN THE CHEETH USE.

Plants were grown in pots or wooden flats and placed in concrete benches in the greenhouse. Watering was carefully done from below by running water into the bench until it reached a height of 5 to 10 cm. At the end of a few hours the water was drained off. Water was never applied on the top of the soil. As a result of preliminary studies it was found wise to dilute the hormone dust with 100 to 200 gram of dry soil and to distribute this as uniformly as possible over the surface of the soil. Seeds were then placed directly on this layer of soil and covered with approximately 2 cm. of sand. The soil used in these experiments was a Michigan sandy loam. More experiments were conducted with higher concentrations than with lower ones, since the latter had not been shown to have a stimulating effect.

In one of many experiments with buckwheat it was observed that growth of seedlings was greatly inhibited at 20 mg/pot concentration, but that when the flower stage was reached the rate of growth was markedly increased. At maturation the height of the plants was slightly more than in the case of the controls. Some of the possible explanations for this effect may be found by (a) assuming as some enthusiast would, that it is a direct result of hormone treatment; that (b) jarovization (10), is a likely cause; that (c) injury of a mechanical (46, 40) or chemical nature is operative; or that (d) it is a matter of chance. In view of the fact that stimulation effects are not usually indicated, this would point to the conclusion that natural chance might be the explanation. If jarovization is effective, then in the earlier stages of growth inhibition should be evident, and in the later

-23-



stages accelerations of growth would obtain. In this buckwheat emperiment it appears that jarovization might afford the explanation, but closer study shows that this is not probable, since the most significant characteristic feature of this type of vegetative modification is the fact that it hastens maturation. In the case of these buckwheat seedlings, maturation was retarded. A further consideration of jarovization leads one to wonder whether suchtreatments as implied would be conducive to normal growth. It was orginally claimed that yields of winter varieties were increased but in a number of recent tests this has not been realized.

Stimulation of growth through mechanical, chemical, and electrical injury, is admittedly possible. In some cases the mechanism appears to be one of enzymatic activity or oxidation while in other instances it seems impossible to learn the cause. Jarovization might also be classed under theheeding of injury since the nature of treatment is one similar to mechanical and chemical injury. It has often been observed in biological experimentation that unfavorable conditions of temperature and chemical proportions, etc., do induce accelerated growth either directly or indirectly, providing the injurious factors are not very strong. Living organs or tissues some times show an increased resistance a ainst a poison and later not only recover their original activity but in some cases become more active. But these examples are scarce, and it is rarely possible to find in nature all the optimum conditions at one time in one place to bring about the desired effects, or even to produce them artificially (11).

If the unfavorable stimuli are not severe, one will first notice inhibited growth which will soon be replaced by accelerated growth.

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Now if this is continually maintained to maturity then obviously we have a true case of accelerated growth. However, conditions are rarely so combined that an increased growthrate can be continually maintained. Normal plants can sometimes be made into dwarfs by drastic changes in environmental conditions but this is not common under the general run of donditions for there are certain genetical limitations.

To conduct further experiments, it is important to determine whether or not hormone is essential for normal growth. If hormones are necessary then Went's (63) statement "Without auxin no growth," is correct. On the other hand if hormones are not essential for normal growth then Leonian (38) has the right interpretation.

Sweet corn seeds treated with hormone dust and grown in the greenhouse showed marked inhibition of growth at the early stages but later normal growth was attained. This is shown in table 15 and plate 14. The results with buckwheat are shown in tables 16 and 17, and summarized in table 18. The experiment shows that buckwheat at earlier stages of development was markedly inhibited by the higher concentration (100 mg per pot) while at a later date growth was only slightly checked. Low dry weight yields were obtained at high concentration. Plate 15 shows the appeareance of the plants at the beginning of the flowering stage. The conclusion drawn from this experiment is that buckwheat in the seedling stage is retarded in its growth approximately 40 per cent when computed with checks. The concentration of hormone dust in this case was 0.1 gram indole butyric acid per pot. After the flowering st ge and up to maturation there was a marked increase in growth. At maturation the height and vigor of the plant was like that of the check.

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Yet there was a decrease in dry weight, which decrease is believed to be due to the decrease in growth during the seedling stage which in turn was due to the hormone treatment.

Growth of the bean seedlings was markedly inhibited at 2000 p.p.m. of seed as shown in table 19. Germination was delayed two days behind that of the check in the field, while in the greenhouse it was delayed for seven days. It is suggested that the explanation of this lies in the mechanical removal of hormone in the field experiment. Grace (24) reported marked increase in growth of bean plants at 10 p.p.m. naphthalene acetic acid. In this experiment at the same concentration as in Grace's experi ent only three percent increase in length and dry weight was obtained and this is not considered significant. This is within the limits of natural chance. In the case of high r concentration (200 p.p.m.) inhibition was marked and significant.

Results for tomato seedlings treated with "Rootone" (naphthalene acetic acid) are found in table 20. The tomato plants were started from seeds planted in 8 cm. pots. When these were about 15 cm. high they were transplanted (October 4) into 25 cm. pots containing sandy loam. Root ne powder was added to the soil around the roots. Two weeks after, marked inhibition was observed in the Rootone treated seedlings. This inhibition was observed in the Rootone treated seedlings. This inhibition was observed in the Rootone treated seedlings. This inhibition consisted in a reduction of growth in height and number of branches. The photograph (plate 17) was taken one month after transplanting. About 6 weeks later, on December 20, the treated plant had not yet recovered.

In table 21 can be found the data for growth of wheat and millet in

-25-

wooden flats 50x35x10 cm. in the greenhouse. Rootone treated seeds were sown on the soil in the flats. When the plants were about 2 cm. tallthey were thinned out to 5 rows 35 cm. long and 10 cm. apart. The results at the end of a period of one month indicated that millet at higher concentrations was markedly retarded in its growth, while wheat showed an increase in dry weight in the lower concentration amounting to about seven per cent. The root system of the millet plants was found to be different from that of the control. The roots were increased in number but reduced in length in higher concentration (60). In the field experiments with wheat it was noted that tillering was better in treated plants. In this experiment increased tillering was noticed and it was determined that the number of tillers is also correlated with the dry weight. At the end of a period of one month 40 plants from each group were classed according to the number of tillers. The greatest number of tillers was three, but this high number occurred in fever plants. The larger number of plants had one or two tillers. The data are shown in table 22. The number of tillers are found in column headed (n) and the number of plants in each class under the column headed (N). The summation of these values (Σ nH) will give the total number of tillers in each group. The value of (5 nl) expressed in percentages, based on check is the relative value of total tillering for the group. This is given in the last column (table 22). It is seen that the number of tillers is correlated with dry weight. The dry weight figures are found in the last column in table 21.

This experiment shows that increased dry weight through hormone treatment (0.3g. Rootone per 100g. seeds) resulted because of the inc-

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rease in the number of tillers. In other words, hormone treatment increased the dry weight of wheat seedlings through increasing tillers. The writer finds no reference in the literature on this particular feature. It is suggested that this increase of dry weight can be explained in the following way. At first growth is retarded due to hormone treatment and then this in turn stimulated tiller formation. These results were obtained on one month old plants. If the plants had been selected at a much later date and the data collected, the above results might not have been obtained. It is quite probable that then the difference might not be so apparent. Cases are plentiful where the stimulating effects of hormone treatments wear of?, so to speak, and the plants are no better than the checks. In the case where inhibitions have been reported later test would indicate recovery and both treated and check plants would yield alike.

ELECTION III. THE OFFICERS OFFICERUNES IN THE FORM OF DOFF ON THE SUDD UPON THE SOLUTION GROWIN OF PLACES IN THE FIGHT.

From his results in the greenhouse, Grace (34) suggested the use of dusted seeds for field plantings. This led to our field experiments with dusted corn seeds. The seeds were planted by hand in rows 3 feet apart. The hills in each row were also 3 feet apart. Each row was about were 165 feet long. Bean, buckwheat, and wheat also planted in the field. Data were collected in such form as to be utilized for statistical treatment if necessary.

-27-

The data in table 25 show that Dent corn was slightly retarded in germi ation and significantly inhibited in its growth in height at 200 parts of indole butyric acid per million parts of seed, but not affected at lower concentrations. The results for Sweet corn (table 24) treated in the same manner show very plainly that at earlier stages, of growth, inhibition is signific at the higher concentration. Observations & weeks later showed that the effects of inhibition had disappeared.

Table 25 contains the data relative to the growth of buckwheat up to flowering time. At the higher concentration (3,000 p.p.m.), inhibition is apparent. After this period and during maturation no difference between check and treated plants was noticeable. At concentrations 0.15 to E p.p.m. and at the time the experiment was completed, there was found to be an increase in height amounting to an average of 5 percent and an increase in dry weight of about 4 percent. These differences in favor of hormone treatment are of no practical significance.

Fisher's "analysis of variance" was applied to the data for hormone treated corn as found in table 26. From the data in table 26 the following table of analysis of variance has been prepared.

Sources of Variance	Degrees of freedom	Sun of squares	Vari- ance	Standard deviation
Total	9 9	6219.0		-
Between means of trea	t. 4	1608.8	401.450	
Between lines.	19	1014.0	53.366	
Error	76	5 97.2	47 .332	6.89

Analysia of Variance for Testing Growth in Length of Corn.

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The data in table 32

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The standard error, S, of the difference between any pair of means is

Variability between treatments: the value of F in Fisher's table, corresponding to the degrees of freedom for 4 and 76 is, F = 3.56, while that for the sample is

F = 401.45/47.33 = 8.47.

The value, 8.47 demonstrates that variability between treatments is highly significant.

To determine the variability between line means or location means the following procedure is given. From Fisher's table, F = 1.67, and F sample = 63.368/47.33 = 1.12. This shows that variability between line means or location means is not significant, since the calculated value F is smaller than 1.67. The test for significance between two means is also given. For 76 degrees of freedom the values of the 5%and 1% levels are respectively t = 1.99 and 2.65. Hence a difference between treatment as large as 2.17 x 1.99 = 4.32 is significant, and a difference as large as 2.17 x 2.65 = 5.76 is highly significant. This shows that any mean difference geneter than 4.32 is significant, and any greater than 4.76 is highly significant. These data show that hormone treatments 20 or 16 are significant in causing growth inhibitions when compared to checks.

A study of table 27 shows that in general hormone treatment has little or no effect except in the case of soy bean at the concentration of 2000 parts per million parts of seeds, and wheat seedlings which indicated slightly more tillering inder hormone treatment. In table

-29-

27 a general summary of several preceding tables is found.

Parked secondary root development was indicated in the laboratory and greenhouse experiments and it was noticeable in the field experiment. This is not due to hormone treatment necessarily but is a natural development in the prowth of corn. The original main root system of the seedling soon dies and a large number of roots develop from the crown and these are responsible for the nutrition of the corn plant and its later development. This loss of seminal roots and the heavy production of crown roots was studied for the writer's own satisfaction by digging up at the end of the season about 50 Sweet corn and 20 Dent corn plants. Later, photographs were taken and same may be seen in plates 19 and 20.

EXPERIMENT IV. THE EFFECTS OF HORMONE ON PLANTS WHEN APPLIED IN THE FORM OF LANOLIN PASTE.

Naphthalene acetic acid, mixed with lanolin paste was injected below the epidermal cells on stems, petioles, and leaves of buckwheat plants at various stages of growth. These experiments were conducted in the greenhouse. In most all cases roots were produced at the point of injection. The effects were not so different except at the highest concentrations where cracking or bending of stem or petiole ocurred. After 10 days the wounds were healed, and after 2 weeks marked swelling ap-

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peared. Then several epidercal cells would form knobs, showing that the roots would soon push through. Consequently after 3 weeks, especially during the summer period the roots formed would be about 2 cm. long. In the winter period the elongation of these roots was very slow. However, root initiation followed application of hormones.

Significantbud inhibition was observed in hormone treated plants growing in the greenhouse, and plate 25 shows this. Tomato plants growing in 25 cm. pots in the greenhouse, with the temperature varying between 20-27°C., were treated with the lanolin paste (0.1° naphthalene acetic acid) on November 1. The average height of the plants at that time was 55 cm. (plate 22).

To study coleoptile bending, Zea mays was selected because this was found (Exp. 1) to be more resistant to moisture and temperature variations than Avena in its early seedling stage. In this experiment naphthalene acetic acid mixed with lanolin was applied on the coleoptile with a needle. At first the experiments were carried out in the dark room but the results were so irregular that the method was revised. At the higher concentration (10 percent) the bend was negative. The same was true at the lower concentration (3 percent, table 29). Later it was found that moisture and temperature conditions were very important for coleoptile curvature. The new method of procedure was as follows. Glass vials were filled with moist sand and one day old gerninated Zea seedlings were planted in these (plate 20). Twenty-five vials were placed in a dish containing 2 to 3 cm. of water. They were covered with an inverted flower pot. This afforded the proper moisture conditions. When coleoptiles were up to 2 to 3 cm. in height lanolin hormone paste was applied at various heights upon the coleoptile. The temperature (25-27°

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C) was higher then that in the dark room and more for vorable for coleoptile growth. In table 30 the results are shown. Applications at the node are more effective and for a longer period, than those on any other part of the coleoptile. Scally after 24 hours the coleoptile returned to its original position if the hormone paste, had not been too concentrated or the humidity too low.

The change in the dir ction of curvature and the degree of curvature was found to be controlled more or less by age of part, the amount of hormone paste, variations in moisture conditions, and changes in temporature. From a consideration of the above facts the author believes that the phenomenon is one of polarity rather than hormone activity. This will be discussed more in detail later.

The writer has noticed that under certain conditions the rate of growth of the coleoptile and seedling leaf enclosed is concurrent, but under other conditions just the opposite is true so that the coleoptile sometimes is empty. The latter condition obtains when hormone pasts has been applied. This paste affects the two structures differently. The enclos d leaf is sensitive to the paste and its growth rate is reduced while the coleoptile is more resistant (table 31). It is more resistant also to checked injury than the plumule (plate 23).

The effect of varying concentrations applied on Zea coleoptile was studied. The results are given in table 53. Higher concentrations often caused more curvature but the curvature due to age was more significent than the variations caused by concentration differences. The significant effects at higher concentration were more probably after

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effects. It higher concentration the curvatures remained longer and recovery was slover. It was found also that recovery was slover when the paste was applied on the internode. If hormone paste was placed symmetrically on intact coleoptiles it resulted in growth inhibition of the tip of the plusule and resulted in copty coleoptiles (plate 24).

Emperiments with decogitated coleoptiles are reported in table 33. Decepitation was carefully done by catting the coleoptile tip (0.3 to 0.4 cm.) with a razor blade. Hor one paste was placed on the cut surface unilaterally. The difference in the reactions to hormore paste between intact and decapitated coleoptiles was one of degree. Decapitated coleoptiles r soled quicker. At the end of a four hour pariod marked convature occurred in the decapitated coleoptiles while in intact coleoptiles it was difficult to see any curvature. Decapitation appears to make the coleoptile sensitive to hormone treatment. The optimum time for curvature depends upon conditions, and 6 to 13 hours in that of decominated coleoptiles.

Old coleoptiles do not react at any concentration of hormone while young ones will. Since symmetrical application of hormone did not change the growth of the coleoptile, it is an important matter to decide whether or not lending is due to growth. Reports are numerous that the bending of the coleoptile is due to the activity of a phytohormone. Few are the reports as to the direction of the curvature. This to the writer appears more important than the degree of curvature. Then and his coworker (CE) reported that usually in the case of phototropism Avera curvature was positive, that is towards the light

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source, but if the light intensity were high, then the curvature was negative. They concluded that this difference in reaction was due to the concentration of hormone. In the first case the hormone concentration was higher on the shaded side while in the second case it was higher on the illudinate' side.

Granick and his covorher (25) reported that it was difficult to predict the direction or degree of bending of various plant structures when a one percent 3-n-propionic acid in landlin paste was applied. A study of bending and cell elongation of various sturectures indicated that it was difficult to predict the direction or the degree of bending. The response to hormone treatment varied for the same tissues under different conditions of temperature, light, etc. In general, the younger and more repidly growing parts produced corvatures sooner.

The present experiment is in accelerent with this conception. In general it is believed that the negative curvature (inward) is due to direct injury and not to hormone tratment, since it has been repeatedly observed that the coleoptile when placed in lower moisture conditions excludits a negative curvature, and especially so when of higher concentration. A shrinking of the tissue on the concave side is apparent. The author concludes therefore that negative curvature is due to the injury of the tissue on the concave side. In the case of positive curvature, it is suggested that this is related to chemico-physical polarity. This will be considered later.

A summary of the experiments with the Zea coleoptiles is given below.



1. Humidity is one of the i port at factors in causing a chance in the direction of curvature. Low humidity often produces negative c rvatures, which the author believes is due to injury, probably chemical in nature.

2. The after-effects brising from the curvature are greater when the hormone is applied on the node than on any other part of the coleoptile.

3. Small variations in the amount of landlin paste used, had no . visible effects on curvature, but when the amount wis increased to ten or more times, then the direction of curvature was change:.

4. The age of the coleo tile is an important factor in the type of curvature made. Young parts react better than older parts.

5. When the concentration of the hormone was increased, recovery was that much more retarded.

6. The decapity ted coleoptile is slightly more sensitive than the intact coleoptile when the hormone is applied in the form of lenolin paste.

7. Symmetrically ap lied hormone on decapitated <u>Zea</u> coleoptiles had no effect on the elongation of the coleoptile but often inhibited the elongation of the leaf within.

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MPRIMERY. THE EFFERS OF MORULE OF FULL FULL

The effect of a 0.1 percent naphthal ne seekic acid in the form of a dust (Rootone) on the rooting of cuttings was studied. Stems of certain plants whre cut in lengths of 5 -> 10 cm. and all leaves wire stripped off except a few at the tip. The sut ends were i mersed in the "Rootone" and the excess worder shaken of ... The cuttings ware then planted in sand in flats to a depth of for or five ca. on October 4. and placed in the greenhouse. In some cases 0.2 ~. "Nootone" was scat cred along the rows (de cm. long) close to the cuttings and then covered with sand. Watering was always done from below and never from the top, in order to prevent any possible removed of the hormone. At the end of forty days ($\mathbb{I} \circ \mathbf{v} \cdot \mathbf{1} \in \mathbf{S}$), all the plants were washed out of the sand and classified into groups (plates 20, 27, and 28), according to the abundance of roots. All cuttings with many well developed roots were placed in group A. "Then a fewer number of roots but more than 5 were present and only moderate de elopment was indicated these were placed in group B. Those cattings with less than 5 roots, were put into group C, and in the final group, E, vare cost the puttings that were alive but had no roots. The results of the study of the groups are expressed in percentages and are found in table 34. Percentages are based on tot 1 number of plants alive. Some of the plats died but their death was due to causes other than the hormone treatment. The plants treated were Chrysanthomum, Mesembryanthemum, Mydranges, Azaloa, Kleinia, and Crassula.

Mesembryanthemum gave a better root development but none of the oth as were significantly benefitted by the hormone treatment. Whether rooting in this case was due to the hormone treatment. or sole fortuitous

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factor should be tosted further by repeating the experiment. It appears however that the treatments increased the number of roots in class B but did not accelerate root growth emerget in the case of Mesonbryanthemum (table US). Cuttings whose roots were extremely seant, such as in many hardwoods or in Azslea, were generally little improved with this particular hormone treatment (00). Rootone has not been widely used to date and its effectiveness has not been established.

ELECTION T VI: NO DAY APULICATION ON COLOHIOINE TREATED CORY SETDLINGS.

Colchicing treated <u>Dea</u> seedlings usually form bulbous tips on all the roots, and root elongation is markedly retarded. This suelling of the root is due to the formation of new polyploid cells. It was suggested that hormone application might stimulate growth of the polyploid tissues.

One day old Dent corn seedlings were soaked for 20 hours in Colchicine. These treated seedlings, which at this time showed matked smellings, were then washed in running tep wat r for 34 hours. They were now transferred into varying concentrations of maphthalene acetic acid in solution. They remained in this for 20 hours and then were worked in running tap water for 4 hours. Their further grouth was studied in petri dish cultures. On observation the next day no growth had occurred in 0.4 percent concentration, and no marked change in concentration of 0.004 percent. That is, the former was too strong and the latter too weak to cause growth or root development. At 0.04 percent concentration, hopever,

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new roots developed from the primary root near the seed. The proper concentration then stimulated secondary root development on the primary roots. The treatment, however, did not induce any further change in the polyploid tissue as was hoped, but perhaps this might have been due to terminating the experiment too soon. Treatments and observations are tabulated (table 36).

THE GROWTH OF CORN SEEDLING ORONA TROU NORMAL CORNERS.

The advantages in the use of a box with a glass side for observation of seedling growth are apparent. One day old seedlings, germinated in moist chambers (petri dishes), were placed on the glass side of the box (60 x 30 x 15 cm.) and covered with moist sand. Seeds had been previously treated with indele butyric acid in dust form. The left side of the glass face of each box was covered with black paper to procide darkness. The other side was exposed to the light of the greenhouse. Photographs were taken duily until the plumules had grown above the top of the soil surface. Three such photographs are presented (plates 30, 31, and 32).

The summary of this experiment follows:

1. Darkness favors the elongation of tops, and especially the internode; for root growth darkness is slightly letter.

2. Light retards top growth of young seedlings. This is mainly

due to the prevention of growth of the first internode. Elongation takes place to a greater extent in the dark than in the light.

5. Hormone treatment (indole butyric acid 2000 p.p.a.) inhibited primary root growth and stimulated the development of many lateral roots, and also root hairs.

4. Maximum root development of younger seedlings was attained in about 10 days. After this period crown roots start developing. These now function in the nutrition and development of the corn plant.

5. Retardation of growth in the highest concentration of hormone (2000 p.p.m.) was observed as early as twenty days after the experiment was started (plate 32).

EXPERIMITY VIII. EFFECT OF HORMONE ON CATALYTIC ACTION OF FERRIC SALT FOR THE DECOMPOSITION OF H₂O₂.

The effect of naphthalene acetic acid on the decomposition of hydrogen peroxide by ferric salts was studied. The importance of the catalytic effects of ferric salts in biochemistry have recently been noted (20). The possible effects of hormones on catalytic reactions in general have not been studied, as far as the writer is aware. The experiment described here was corried on according to the procedure of Daniel and his co-workers (20).

A 0.4 percent naphthalene acetic acid solution was prepared by dissolving 0.2g. of the crystals with 5 cc of 95 percent ethyl alcohol,

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and finally diluting this with distilled water. A 0.04 percent solution was prepared by diluting 5 cc of a 0.4 percent solution with distilled water. The excess alcohol in the surphyer solution was driven off by gentle boiling for 10 minutes while in the meaker solution boiling was continued for 10 minutes longer before dilutions with water were completed.

The solutions used in this experiment are indicated below.

- 1. 0.5 M FeC13 + 0.5 M HC1.
- 2. (1) + 0.04% raphthalene acetic acid.
- 3. (1) + 0.4% naphthalene acetic acid.
- 4. 0.047 naphthalen ac tic acid.

The first contains Fe as the catalyst. The second has the Fe catalyst and a weak solution of the hormone. The third is the same as the second but the hormone is ten times as concentrated. The last solution contains the hormone without the catalyst. In proparation for a series of titrations, commercial H_2O_2 was diluted approximately to a 0.3 percent concentration, and concentrated H_2SO_4 was diluted (1:4). Distilled water was made ready at hand. Five minutes before titrations were started 10 cc of H_2O_2 were mixed with 15 cc of the various solutions to be sented. From each of these was withdrawn 5 cc which was pipetted into Erlenneyer flusks each containing 15 cc of diluted (1:4) H_2SO_4 and 10 cc of distilled water. At the proper time periods indicated in table 36 titrations were made. These data appear in table 36. Curves are plotted for each solution as well as for the check "0" and are shown in fig. 4. Curve "0" shows the amount of $HinO_4$ used to titrate the H_2O_2 solution alone and the value is found to be constant throughout. Curve "1" shows the de-

composition of H_2O_2 at succeeding titration periods and here the effect of the ferric salt is clearly indicated. Curve "2" shows that the rate of decomposition is retarded and this must be due to the presence of naphthalene ace ic acid. Curve "3" shows that decomposition has almost stopped. In this case ten times as much naphthelene acetic acid was used as in the solution represented in curve "2". Curve "4" represents what happens when no ferric salt is pre ent. No decomposition is apparent. If there is no chemical reaction between $E \ln O_4$ and naphthalene acetic acid, then all the curves should meet on line "O" at zero time. While curves "1", "2", "3", and "4" do not meet at zero time, this may mean that either the alcohol used as a solvent, or the impurities in the naphthalene acetic acid, or the latter itself, may account for the slight discrepancies. Since the effect is greater the higher the concentration of naphthalene acetic acid used, it is believed that this is the cause of the result obtained rather than the little alcohol or the minute impurities. Further studies are planned.

The data plotted on logarithmic paper show that curves "1" and "2" are of unimolecular form, that is, the rate of change dC/dt is proportional to the concentration "C" of H_2O_2 at a given time, "t"

- dC/dt 🔊 C

 \mathbf{or}

$$- dC/dt = KC.$$

Differentiating between initial concentration $C_{\rm O}$ and concentration C at time t.

$$-\int_{c_0}^{c}$$
 dc/c = K dt

or
$$Lo_3(C_0/C) = Kt$$
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where K is the reaction constant which varies with the type of curve. The value of K was calculated from the above equation, and is shown in table 89. In these calculations initial concentration "C_o" was determined for each catalyst from the curves at zero time respectively.

DIBC'S I N

Germination of corn was not significantly addressed except when hormone concentrations were too high (2000 p.p.m.) and then it was reduced. These results do not confirm those of other workers. Corn embryos seem to be more resistant to hormone treatment than the seedlings. Soon after germination has started inhibition begins to appear. Thismann (60) reported that <u>Avena</u> seeds treated with indole acetic acid solution (0.001 percent) for 24 hours showed an increase in germination. The author has noticed that under ideal condition (temperature, moisture, and freedom from microorganisms, e.c.) good seed of corn and wheat will nearly always germinate 100 percent. This is possible when the embryo side of the corn kernel is kept moist until germination takes place. This is more important than keeping the endosperm always wet.

When concentrations were higher than 100 p.p.m. (indole-butyric acid) the growth of the primary roots of both corn and buckwheat was reduced. During a period of two to seven days this reduction amounted

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to fifty per cent but it was accompanied by an increased production of secondary roots and root hairs. At the end of a week the quantity of root growth was often remarkable. At this time regardless of the treatment, crown roots developed and the further growth of the plant depended upon these roots and not on the primary sectional roots. I_n the case of corn the primary root system has only a temporary function and, therefore, exerts very little influence on plant growth (fig. 19 and 20).

Hormone application to cuttings may be suitable for the succulent type of plant but not for the woody kind according to these studies. Maphthalene acetic acid has very little effect on rooting (table 35). Hormones increase rooting on young, succulent cuttings in the nondomant conditions, but produce very little or no effect on root elongation. The initiation could be attributed to chemical stimulation of cell division. To determine this morphological investigations are necessary.

If root systems are benefitted by hormone treatments, then naturally top growth should also benefit, and increased growth should follow. This is the opinion of many workers (11). The writer agrees with the statement that hormone treatment will initiate secondary root development but will inhibit primary root growth (59). As regards top gro th the author believes that in most if not all of these cases the emplanation is due to some other cause or group of causes. Fost of the hormones are more or less difficultly soluble and of low diffusibility. Tany hormone treatments have been performed in solutions and the effects on the root are more direct while the effect on the top is indirect. The

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concentrations r aching the tops are probably too weak to be effective.

It is well recommized that increased growth can be brought about by chemical, thermal and mechanical stimuli, and even by jarovisation. The latter is of limited value, however, and can have but little practical significance for the farmer. The writer has never heard of farmers making money by utilizing the method of jarovisation.

In the wheat experiment (Exp. 11) it will be remembered that tillering was increased and some might attribute this to hormone treatment, but such effects can be accomplished by chemical and mechanical means. In the northern part of Japan certain experiment station workers have advised farmers to tread upon seedlings of winter wheat in the field, in the hope that the pressure of the foot would induce tillering later. This proved to be a costly operation and never practical. There are many factors (genetic, nutritional, soil, climatic, etc.) that can explaid baneficial results on normal growth other than relying on hormones to bring about normal growth.

The most important studies of phytohormones has centered around the study of the effect of these growth substances on coleoptile curvatures and growth in length. If hormones do not increase growth of coleoptile (4, 34, 41), then there is very little to support the theory. In experiment IV (table 33), it was found that a 0.1 percent naphthalene acetic acid in landlin paste applied symmetrically on decapitated <u>Zea</u> coleoptiles resulted in an elongation of 50 percent after 12 hours, but that in the case of the check it was 90 percent. Instead of accelerating growth, it reduced it. If these results are correct, as the writer believes, then hormonesare of do btful value. In the case of

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plants, Smith (51) insisted "growth is the normal function of cells. They are always multiplying when they are not inhibited by one thing or another." His conception gives us a very good idea of what normal growth is.

Even auxin a, and b, and heteroauxin are absent from green tissues of higher plants (39). Hany experiments with higher plants show that a supply of hormone for normal growth is not necessary. There is no conclusive proof that phytohormones are specific, but specificity of animal hormones is established without doubt. It is well known that root inhibition, root hair development, top growth, bud inhibition, internodal growth, coleoptile bending, and parthenocarpy, etc., con be induced by various chemical, mechanical, thermal and electrical means.

It is necessary to make clear now what is meant by the term hormone. Reasoning from known facts in animal physiology one would expect phytohormones to be specific, but they are not. Consequently the term phytohormone is not justified. Roogl (35) has recently complained that "the term must be limited to biological catalysts of organic nature which are used by the organism itself to bring about the various physiological effects." If plants possess hormones these must be quite different from the animal type, since the functional variations between animals and plants are great. It might be asked, can lower unicellular animals possess any or all of the homrones found in the higher animals? This is doubtful, since hormones in higher animals are not merely by products. but secretions from special organs functioning in a definite way to control the highly organized animal body. Without hormones the maintenance of life is impossible. For higher animals the nervous system acts as a telegraph, the circulatory system serves as a traffic system, while the

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hormones function as messengers. Without these hormones the various systems are not properly organized and the living animal can not exist. Could life be expected in a man's legs or head picked up from a battle field and stuck into a nutrient solution? In certain conditions, certain parts of plants can live and grow in a nutrient solution, and so also can certain definite tissues from higher animals be cultured.

Growth promoting substances might be similar in their action to enzymes, salts, sugars, or some polar compounds which affect the free energy of surfaces. Many physiological processes in plants are carried on throuch surface boundaries which are well known sources of energy for the plants. The writer feels that the position taken by those advocating the existence of phytohormones is not well founded. From a functional and morphological view point, the higher plant is a much more simple organism than the animal and the correlations between the different parts and its hormones if any are also of a simpler type. The plant has no nervous system, or a circulatory system like the animal, and no chemical mescengers seem necessary to regulate and coordinate its various more or less simple reactions.

The author has made a list of those substances most often reported as exhibiting growth promoting characteristics, so as to make a study of their chemical structure. These are presented in table 40. They possess active groups, -COOH, -OH, etc., which have high affinity for water, and also nonactive groups, such as carbon chains or rings which have high affinity for fat solvents, but not for water. The suggestion is offered that when such polar compounds are applied unilaterally, they penetryte into the cell. Folar compounds by nature change the free energy

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of the surface and thus induce variations in permeability, changes in concentration, modifications in turgor pressure, etc., on one side of the colcoptile where applied, and thus bring about curvature. In addition to this function of polar compounds in modifying surface tension through molecular orientation, they also resculpte and originate electromotive forces (e.m.f.) in the plant body, since the total e.m.f. of a plant is the sum of the e.m.f.'s of the unit cells (18). Since a plant is male up of many cells, a shall change in e.m.f. in a unit cell will bring about a large change in the whole plant. The penetration of these polar compounds must be very slow and when they do penetrate, their effects are largely limited. The first effects are near or on the polar compound an e.m.f. is created, it is probable that some polar compounds and ions already present will migrate from one side of the coleoptile to that which was chanically treated. In this way curvature would result through modification of turgidity.

From the time of Vöchting (Went and Thimann Monograph) (1878-1908) to the present the existence of polarity in correlation phenomena has been definitely proven. This is not only so in the case of whole organs like one of the higher green plants, but for its parts, such as leaves, stems, root and fruit. Even each separate cell exhibits polarity. In more recent times electro-polarity has been demonstrated and is now generally conceded.

A number of possible differences in potentials existing in living plants together with their probable causes, are listed below:

1. Concentration potentials exist between solutions of different concentrations. Such are possible in the cell sap of different tissues.

2. Diffusion potentials are set up when miscible solutions diffuse

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into each other.

3. Liquid junction potentials. When liquid A is dissolved in a mixed solution of two indiscible substances D and C, then A is unequally distributed in D and C and consequently a potential difference is bound to exist.

4. Membrane potentials are provalent in plants. They may arise through (1) unequal distribution of the ions on either side of the membrane and (2) by the unequal penetration or diffusion of the ions through the membrane.

5. Injury potentials. It some cases the mechanism is unknown. In other cases it is suspected to be due to stimuli. At the moment of injury the potential is high and then fades away. The injured part is negative to the uninjured, due, indirectly, as some think, to increasing acidity.

6. Oxidation-reduction potential. In tissues metabolic processes are occurring at different rates and are not uniform. In consequence potentials rise. One can observe this under the microscope. Dyes such as methyleneblue can be injected into tissues and their further changes observed. Methylene blue changes to the colorless form when reduced. Some living tissues or organisms contain pignents that change to the leuco form on reduction. Quantitative tests can thus be made.

7. Electro kizetic potentials. These potentials arise from certain surface phenomena, such as selective adsorption and ionization of proteins, etc.

8. Potential variations within a cell are very well known. Cince the nucleus is more negative than the cytoplasm the cell as a whole is

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changel negatively, for example:

 $((nucleus)^{---} + (nucleolus)^{+} + (cytoplasm)^{+})^{--}$.

Protoplasm is said normally to be electro negative and through stimulation could increase this charge in the part encited and therefore increasing the potential and making the protoplasm or cer ain parts of the plant more active. Polarity might be affected in this way. The presence of a solt or segar (59) may also affect polarity and by analogy the bending of the coleoptile. For, as is well known, salts change the ionization rate and form, and segar stabilizes the colloidal micellae (50). Indirectly then, polarity is affected by electric potentials.

Thimann and his coworker (53) suggested that the activity of a hormone is due to its double bond. But double bonds appear to be of little importance in physiological processes. i-Counaryl acetic acid, has been reported (56) to affect root initation but not <u>Avens</u> curvature. Since this compound has two active groups, -COUM and -CH in the structure, its solubility must be too high to be polar. It diffuses too easily in all directions and hence can not bring about the elongation of one side of the coleoptile. The compound, by its effects, must be less polar.

Further explanation of the mechanism of polarity and of polar compounds is beyond the scope of this paper but the author would like to introduce here several brief reports which affirm the polar theory and the emistence of electric potentials. Drauner (13), in 1926, reported that in the case of horizontally placed plant parts the lower side became electro positive to the upper side. The shaded side of the

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spedling stem was electro-positive to the illuminated side. Photo energy, it may be said, has been transformed into potential energy.

Crauner and his covorker (14), in 1950, reported that <u>Avena</u> coleoptiles curved toward the positive pole in an electric field. Thesefore the convex side must be positive to the concave side.

In 1937, Clark (16) studied the polarity of plant parts in considerable dotail. The polar dyee can be classified, (a) into acid dyes, light green, acid green, methyl oran e, etc., and (b) into basic dyes, safranin, methyl violet, neutral red, gentian violet, etc. When <u>Vicia</u> faba roots were i jected with basic dyes (positively charged), the dyes accumulated on the concave side. These dyes are notably toxic and it would seem that the stimulus and high potentials would negative any results. Clark also reported that the intact <u>Avena</u> tip is electro negative to the base.

In 1935 Czaja (19) discussed the mechanism of membrane polarity. Cell walls of lower and higher plants sho ed affinity for basic dyes. In Spirogyra cells, the cation of basic dyes was first adsorbed by the cell wall and then as a dye salt passed into the cytoplasm. With diluted solutions of basic dyes, nearly the whole of the anion remained in the external solution. Adsorption of appropriate salts into the basic dye solution retarded the adsorption of the cation and inhibited its passage into the cell.

Thimann and his coworker (69) stited that the formation f the growth promoting substances which affect <u>Avena</u> curvature is limited to the tip, but when the tip had been removed, a new zone of sumin formation wis produced at the apex of the stup after two to three hours. This

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regenerated tip showed no histological differentiation, so that auxin formation was not necessarily associated with special cells. This fact it seems would indicate that the coleoptile itself exhibited electro polarity, and accumulated some chemicals which affected the bending of the coleoptile.

However, curvatures produced by external application of chemicals a are temporary, ssrule, at lower concentration, but at higher concentrations permanent injury results. Electro polarity will not entirely account for curvatures, yet it appears to be the best explanation for many tropisms.

SULLARY

1. The germination of certain seeds was not affected by the "phyto-hormones" used unless the concentration was very high and then it was markedly reduced. Two authorities have claimed that germination is speeded to such an entent as to be of practical value.

2. Primary root development as significantly reduced when higher concentrations were used but was in no way affected at lower concentrations. At concentrations high enough to be effective but not too high to be deleterious secondary root growth and root hair development was stimulated. In corn the seminal roots were highly accelerated in .

their growth by hormone treatment but the crown roots which replaced these later were not affected. Consequently the plant showed no later improvement.

Top growth of young plants was not affected by hormone treatment unless concentrated solutions were used and then inhibition occurred in the roots as well as in the tops. When the high concentrations did not kill the root, but both the root and tops were only inhibited there was later a recovery so that no significant difference could be detected in yield of fruit, dry weight, or a pearance when compared to checks. This was true of plants raised to maturity either in the greenhouse or in the field. This is not in agreement with the studies of Grace. Treatment of seeds with hormone in the form of dust before planting in the field will not give results of any practical value to the farmer.

3. When the hormone was applied to petioles, stems, and leaves (buckwheat) in the form of lanolin pacte, roots were produced. Such root initiation has been observed on other species. Significant bud inhibition was also obtained in the case of tomato. This confirms other workers' findings in the case of other species. Unilateral application of hormonized lanolin paste on <u>Zea</u> coleoptiles at different levels was more effective on curvatures when applied at the node than at any other place. Hormone lanolin pa te applied to the coleoptile has little or no effect on elongation but does inhibit that of the leaf within. After such treatment the coleoptile appears empty. No report of such a reaction by the plurule has been observed by the writer. Certain parts of plants seem to be more recistant to hormone treatment.

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The order below starts with the most resistant;

First internode coleoptile > root hair > plumule > secondary root > primary root.

4. Hormone treatment of wordy cuttings has little or no practical value. Hormone try thent of vegetative cuttings produces in most cases few roots which do not elongate to any entent.

5. Laphthalene acetic acid inhibited the decomposition of $\rm H_2O_2$ by a ferric salt.

6. Chemical me sengers (hormones) of the type occurring in animals have not yet been demonstrated in plants.

7. The most plausible explanation of coleoptile curvatures and related phenomena is that of (a) chemical etc. injury and (b) electro-pularity.

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I DEE ROMANES

Table

- 1. Description of chemicals and plants used.
- 2. Dust number, parts of hormone per million of seed, percentage of hormone, and quantities of intrt dust used to make up mintur s.
- 3. Optimum dust adsorbing capacity of 100 grams of seeds.
- 4. Calculations for the determination of optimum sust adsorbing capacity of buckwheat seeds.
- 5. Optimize dust adsorbing capacity of 100 gram seed samples.
- 3. Growth of Dent corn roots on hormone disted filter paper.
- 7. Growth of dusted Dent corn seeds on filter paper.
- 8. Growth of Dent corn on hormone dusted filter paper.
- 9. Growth of hormone dusted Dent corn on filter paper.
- 10. Growth of dusted Swest corn seeds on filter paper.
- 11. Growth of Sweet corn seeds on dusted filter paper.
- 12. Growth of dusted buckwheat wheat seeds on filter paper.
- 13. Summary of emperiment 1.
- 14. Some preliminary observations of plants growing in hormone treated spil in the greenhouse.
- 15. Gr wth of dusted Sweet corn seeds in the prenhouse.
- 10, 17. Growth of buckwheat in dusted soil in greenhouse.
- 18. Growth of buckwheat on dusted skil, sumary of tables 13 and 17.

19. Gro th of disted soy been seeds in greenhouse.

20. Growth of Rootone treated tomato plants in greenhouse.

- 21. Growth of Rootone dusted wheat and millet seeds in greenhouse.
- 2. Calculation for the significance of Pootone treatment on tiller

production of wheat.

- 23. Growth of Dent corn dusted seeds in the field.
- 24. Growth of Smeet corn dusted seeds in the field.
- 25. Growth of dusted buck heat seeds in the field.
- 23. Measurements of height of plan's grown from dusted Sweet corn seeds in the field.
- 27. Observation on different seedlings treated with homeone dust.
- 28. Summary of experiment 1/1.
- 20. Degree and direction of curvature of Eea coleoptiles at low handity.
- 30. Effects of neghthelene acetic acid in the form of landlin paste applied at various points on Zoa seedlings.
- 51. The effects of quantity of hormone and age of coleoptiles on direction of curvature.
- 32. The effects of various concentration of hormone in landlin paste on Zea coleoptile.
- .3. The effects of homine in lanclin piste on decapitated Zes coleoptiles.
- 34. Rooting effects on costings through the use of Rootone.
- 35. Rooting effects of cuttings due to Rootone, celculated in percentages based on total cuttings alive.
- 36. Effects of hormone on corn seedlings previously troated with colchicine.
- 37. Solution mixtures with and without cutalyst.
- 28. Titrations for decomosition of $H_{\odot}O_{2}$.
- 39. Resction velocity constant for decomposition of H_0O_{22}
- 40. Chemicals report i as hormone or as hormone-like in activity.

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IDEX TO GRAPHS

Fig.

- Weight of dust added and excess for the determination of optimum by 100 gram seeds.
- 2. Concentration of hormone and percentage growth of Dent corn on filter paper.
- 3. Concentration of homone and percentage growth of buckwheat on filter paper.
- 4. Decomposition of Hg0gtitrated with 0.1 M Kin04.

ILDEN TO PLATES

Plate

- 1. Large moist chumber and pan for germination.
- 2. Porous pot moist chamber.
- Seedlings from hormone dusted Sweet corn seeds growing on filter paper.
- 4. Seedlings from hormone dusted Sweet corn seeds growing on filter paper.
- 5. Seedlings from hormone dusted Sweet corn seeds growing on filter paper.
- Sweet corn seedling on hormone dusted filter paper under diffused light.
- 7. Sweet corn seedlings growing on hormone dusted filter paper in darkness.
- E. Seedlings from hormone dusted buckwheat seeds growing on filter paper (Aug. 15).
- 9. Seedlings from hormone dusted buckwheat seeds growing on filter paper (Aug. 19).
- Seedlings from hormone dusted buckwheat seeds growing on filter paper (Aug. 23).
- 11. Seedlings from hormone dusted buckwheat seeds growing on filter paper (Aug. 25).
- 12. Seedlings from hormone dusted buckwheat seeds growing on filter paper (Aug. 23).
- 13. Seedlings from hormone dusted buckwheat seeds growing on filter paper (Aug. 25).

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- 14. Seedlings from homone dusted Steet corn seeds growing in the greenhouse.
- 15. Euckwheat growing in hormone dusted soil.
- 16. Soy bean plants grown from hormone dusted seeds growing in the greenhouse.
- 17. Rostone treated tomato seedlings growing in pots in the greenhouse.
- 18. Root system of millet grown from Rootone treated seeds.
- 19. Root system of Sweet corn grown in the field.
- 20. Root system of Dent corn grown in the field.
- 21. Aerial roots produced on buckwheat stems and petioles by application of hormone in lanolin paste.
- 22. The effects of hormone in lanolin paste on tomato plants.
- 23. The effects of quantity of lanolin paste applied and the age of Zea coleoptiles.
- 24. The effects of various concentrations of hormone in landlin paste on Zea coleoptiles.
- 25. The effects of hormone in lanolin paste on decapitated Zea coleoptiles.
- 26. The effects of Rootone on roo in of cuttings of Chrysanthemum.
- 27. The effects of Rootone on rooting of Mesembryanthemum and Kleinia cuttings.
- 28. The effects of Rootone on the rooting of Hydrangea and Craesula cuttings.
- 29. Seedlings from colchicin treated Dent corn seeds retreated with hormone solution.
- 30. Dent corn seedlings growing in boxes with one side provided with glass.
 31. Dent corn seedlings growing in boxes with one side provided with glass.
 32. Dent corn seedlings growing in boxes with one side provided with glass.

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TABLE

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Table 1. Description of Chemicals and Plants Used.

Substances		From			
HORMONES AND CHEMICALS:					
3-Indole-butyric acid (5g.)		Dr.R.P.	Hibbard,	Bot.Dept.	
Naphthalene acetic acid (lg.)			11		
Talc powder (500g.)			11	11	
Colchicine (0.5g.)			11	11	
Colchicine 4% solution		Dr.E.H.	Newcomer	,Bot.Dept.	
Lanolin paste		Stock ro	oom, Bot.	Dept.	
Rootone(naphthalene acetic aci	d)	American	n Chem. Pa	aint Co.	
SEE DS:					
Zea mays (Dent corn) 19	37	Dr.R.P.	Hibbard,	Bot.Dept.	
Triticum vulgare (wheat)	11		11	tt	
Fagopyrum esculentum(puckwheat)"		11	• •	
Panicum miliaceum (millet)	H		11	11	
Phaseolus sp. (kidney bean)	**		11	11	
Pisum sativum (pea)	11		11	11	
Zea mays (sweet corn)	H	Farm cro	op dept.		
Soya max (soy bean)	11		11		
Lactuca sativa (lettuce)	Ħ	Ferry-Mo	orse Seed	Co.Detroit.	
CUTTINGS:					
Lycopersicon esculentum (tomat	0)	Dr.R.P.	Hibb ard ,	Bot.Dept.	
Chrysanthemum		Mr.W.A.	Frost, Ho	ort.De pt.	
Hydrangea opuloides		Prof. C.	.E. Wildor	1, "	
Azalea sp.			11	11	
Mesembryanthemum crystallinum		Prof.H.(C. Beeskov	", Bot.Dept.	
k leinia repens			++	11	
Crassula arborescens			17	*1	

Table 2. Dust Number Parts of Hormone per Million of Seed, Percentage of Hormone and Quantities of Inert Dust Used to Make up Mixtures.

Dust No.	Hormone	Wt. dust per 100g. seeds	Hormone 1n dust_
	p.p.m.	g	70
1 2 3	0.00 0.05 0.10	0.2	0.0 0.0025 0.0050
4 5 6 7 8	2 6 10 20 40	11	0.1 0.3 0.5 1.0 2
9 10 11 12 13 14 15 16	60 80 100 120 150 160 180 200	11	345678900 100
17 18 19 20	400 1000 1600 2000	0.1 0.16 0.2	100 100 100
BUCKWHE AT * 1 2 3	. 0.0 0.075 0.15	0.3	0.0000 0.0025 0.0050
4 5 6 7	3 30 300 3000	11	0.1000 1 10 100
SO⊻ BEAN** 1 2 3 4 5	0 2 10 20 2000	0.2 "	0.0 0.1 0.5 1.0 100.0

* Indole butyric acid.
** Alpha-naphthalene acetic acid.

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Seeds	n	Y (added)	X (excess)	Adsorbed
		g.	g.	g.
Dent Corn	1	0.507	0.245	0.262
	2	1.053	0 .7 85	0.268
	3	1.557	1.269	0.288
	4	2.065	1.775	0.290
	5	3.082	2 .7 36	0.296
Soy Bean	1	2.001	1.750	0.251
	2	2.554	2.301	0.253
	3	3.215	2.958	0.257
	4	3.754	3.500	0.260
	5	4.790	4.496	0.294
Buckwheat	1	1.054	0.571	0.483
	2	1,990	1.505	0.484
	3	2.010	1.560	0.483
	4	2.510	1.858	0.652
	5	4.010	3.270	0.720

Table 3. Optimum Dust Adsorbing Capacity of 100 g. of Seeds.

Table 4. Calculations for the Determination of Optimum Dust Adsorbing Capacity of 100 Gram Samples of Buckwheat Seeds.

	Actual weights		Weights from	equation		
n	(added)	X (excess)	Y	X		
	g	g	g	g		
1	1.054	0.571	0.305	0.0		
2	1.990	1.505	1.050	Q. 654		
3	2.010	1.560	2.010	1.490		
4	2.510	1.858	4.010	3.250		
5	4.010	3.270				

Table 5. Optimum Dust Adsorbing Capacity of 100 gram Seed Samples.

	Wt. of Dust	per 100g. seeds
Seeds	Optimum	Applied
	g	g
Dent Corn	0.26	0.2
Soy Bean	0.24	0.2
Buckwheat	0.305	0.3
Wheat	0.3*	0.3

* Approximate value.

Table 6. Growth of Dent Corn Roots on Hormone Dusted

Filter Paper. Seeds previously soaked 12 hours and set on dusted filter paper (120 cm²) with 0.02g. indole butyric acid, April 23. Room temperature 22-27°C. Germination Apr. 25. Apr. 26 for No. 7. Nean of 10 plants.

Cor	Hormone ncentrations		Root length							
No.	mg/paper	Apr.2	27 (2)	Apr.2	Apr.28 (3)		(4)			
,		cm	70	сm	Po	cm	%			
1	0.00	7.3	100	13.5	100	16.0	100			
2	0.0005	7.2	99	11.3	31	13.5	84			
3	0.001	7.2	9 9	10.5	78	11.0	69			
4	0.02	6.0	82	8.0	60	9.5	59			
5	0.06	4.3	59	6.5	48	9.2	58			
6	0.1	3.0	41	6.2	46	8.8	55			
7	0.2	3.0	41	5.5	51	7.2	45			

(2) = Age in days, after germination.

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Table 7. Growth of Dusted Dent Corn Seeds Set on Filter Paper. Hormone used, indole butyric acid. Set in petri dish cultures May 7. Germination May 10. Room temperature 22-25°C. Mean of 10 plants.

H	lormone		Length of Top (T) & Root (R)							
Conc	entrations	Мау	13 (3)	May 1	5 (5)	(8)** May 18 N	(10) May 20			
No.	p.p.m.	Т	R	T	R	T	T			
		Сш	Сm	СЩ	сm	сш	Сщ			
1	0.0	6.7	14.1	12.9	19.1	18.5	20.1			
2	0.05	7.5	13.5	13.1	18.0	17.0	18.5			
3	0.1	5.5	12.9	12.2	16.5	19.0	22.2			
4	2	6.0	13.0	12.4	19.0	17.0	17.5			
6	10	7.3	14.0*	12.3	18.7	16.0	21.1			
7	20	6.3	11.0*	12.2	16.2	18.8	21.3			
7 • •		%	%	%	%	%	%			
1	0.0	100	100	100	100	100	100			
2	0.05	112	96	102	94	92	92			
3	0.1	82	91	94	86	103	110			
4	2	9 0	92	96	9 9	92	87			
6	10	107	100	95	99	86	104			
7	20	102	78	94	85	102	106			

Secondary roots appearing.
Root branching on primary root.
(3) = Age in days after germination.

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Table 8. Growth of Dent Corn on Hormone Dusted Filter

Paper. Seeds previously soaked 4 hours and set on paper May 7. Germination May 10. Hormone, indole butyric acid 0.02 g. on filter paper (120cm²). Room temperature 22-25°C. Mean of 10 plants.

Н	ormone	Length of Top (T) & Root (R)						
Conc	<u>entration</u>	May 1	3 (3)	<u>May 15</u>	(5)	(8) <u>Mag 18</u>	(10) <u>May 20</u>	
No.	mg/paper	T	R	T	R	T	<u>T</u>	
		cm	сщ	cm	cm	сщ	cm	
1	0.0	7.5	12.3	13.6	16.1	20.3	26.2	
2	0.0005	7.1	10.5	13.5	14.2	17.2		
3	0.001	7.0	9.5	14.1	14.5	21.1	26.5	
4	0.02	4.5	5.2	911	7.5	19.0	26.1	
6	0.1	5.2	3.2	9.2	3.9*	16.2	19.5	
7	0.2	5.1	2.5	8.3	3.7*	13.9	15.0	
	~~ ~~~~~~	%	ħ	76	n No	%	К	
1	0.0	100	100	100	100	100	100	
2	0.0005	95	86	99	83	85		
3	0.001	93	7 7	104	90	104	101	
4	0.02	69	42	67	45	94	100	
6	0.1	67	26	68	24	80	75	
7	0.2	68	20	61	23	68	57	

* Many secondary roots
(3) = Age in days after germination.

Table 9. Growth of Hormone Dusted Dent Corn on Filter

Paper. Seeds treated with indole butyric acid dust. Planted May 29. Germination May 30. Room temperature 24-27°C. Mean of 10 plants.

11-				Lengt	h of	Top (T)	& Roc	ot (R)	
HO	rmone							(11)	(15)
_Co	ncent.	June 1	(2)	June 2	? (3)	June	<u>5(4)</u> J	<u>un 10</u>	<u>Jun 14</u>
No.	p.p.m	. T	R	T	R	T	R	T	T
		cm	cm	cm	сщ	сщ	cm	cm	cm
1	0	1.0	4.2	3.2	7.3	5.2	10.3	26.9	29.5
7	20	0.8	4.3	3.1	7.4	5.1	10. 3	27.2	30.0
12	120	0.8	2.3	3.2	5.0	4.5	5.2		-
16	200	1.0	2.2	2.8	4.0	5.1	6.0	23.3	27.2
		%	%	%	%	<i>%</i>	%	%	%
F.	0	100	100	100	100	100	100	100	100
7	20	80	102	97	101	96	116	101	102
12	120	80	55	100	69	87	51		-
16	200	100	52	87	55	96	59	86	92

(2) = Age in days after germination.

Table 10. Growth of Dusted Sweet Corn Seeds on Filter

Paper. Seeds treated with indole butyric acid. Set June 30. Germination July 4, and July 5 for number 20. Room temperature 20-22°C. Mean of 10 plants.

	Length of Top (T) & Root (R)								
H	ormone					-			
Co	ncentration	July	6 (2)	July	7 (3)	July	8 (4)		
No.	p.p.m.	T	R	<u> </u>	R	T	R		
		CM	CM	CM	CM	CM	CM		
1	0	2.8	7.0	8.1	11.8	12.1	16.2		
4	2	2.5	7.1	8.3	12.3	10.5	14.6		
7	20	3.0	6.9	8.7	8.6	11.6	12.8		
16	200	2.6	2.6	8.6	3.2	11.8	6.6		
20	2000	1.7	1.5	4.5	1.8	5.9	1.8		
		# 	9%) //	%	%	%	%		
1	0	100	100	100	100	100	100		
4	2	89	101	102	104	87	90		
7	20	107	9 9	105	73	9 6	79		
16	200	93	37	106	2 7	98	41		
20	2000	61	21	55	15	49	13		

(2) = Age in days after germination.

Table 11. Growth of Sweet Corn Seeds on Dusted Filter

Faper. Germinated seedlings exposed to diffused light (A) and darkness (B). Indole butyric acid (0.1 g.) dust on filter paper (250 cm²). Set July 28. Covered with bell-jar (A), and inverted pot (B). Germination for (A) July 1, and July 2 for No. 16 and 20; for (B) July 2, and July 3 for Number 16 and 20. Mean of 10 plants.

E Con	lormone icentration	Measurement (July 17) of sixteen day old seedlings							
No.	mg/paper	To	0	Ro	oot	Inte: node	r Coleo- ptile		
(A)		сm	10	cm	<i>~</i>	cm	Сm		
1.	0.0	31.0	100	30.2	100	1.1	4.2		
4	0.1	27.8	90	27.0	89	1.0	4.5		
7	1.0	30.2	97	17.8	59	1.2	4.3		
16	10.0	16.0	84	17.1	57	1.2	4.2		
20	100.0	5.8	19	1.0	3 Av.	$\frac{1.3}{1.2}$	<u>3.8</u> 4.2		
(B)					. ~ ~ ~ ~ ~ ~				
1	0.0	33.0	100	20.4	100	6.8	4.5		
4	0.1	31.5	91	20 .0	98	6.2	4.4		
7	1.0	28.8	32	19 .1	93	6.0	4.4		
16	10.0	29.0	85	13.1	83	7.3	4.3		
20	100.0	15.9	4 4	0.5	3	7.1	4.5		
					A_{V} .	6.7	4.4		

Table 12. Growth of Dusted Buckwheat Seeds on Filter

Faper. Seeds treated with indole butyric acid dust. Set Aug. 15, in moist chambers in greenhouse. Temperature 23-27°C. Humidity 99-100 percent. Germination Aug. 17. Number 7 slightly poor in germination.

Ho	rmone	Top ler	ngth		Root le	ength	
No.	т. а.	Aug. 21	Aug.23	Aug.25	Aug.21	Aug.23	Aug.25
		сm	сщ	Cm	Cm	сm	сщ
1	0	6.7	8.7	11.0	15.6	16.7	20.7
3	0.15	7.1	9.8	11.6	16.2	17.6	21.3
5	30	6.5	8.8	10.3	14.6	1 5.1	15.8
6	300	6.2	8.8	11.5	7.0#	9.9***	15.9#
7	3000	3.4	8.3	11.0	3. 4**	8.6	10.3##
		5%	57 10	%	%	61 10	%
l	0	100	100	100	100	100	100
3	0.15	106	102	105	104	105	103
5	30	9 5	101	94	94	91	77
6	300	93	101	105	45	54	77
7	3000	51	96	100	22	52	50

* Primary roots about 0.7cm. Measured longest secondary roo t ** Primary root completed grwoth.

*** Some primary roots completed growth. # When primary root is short more secondary roots develope. ## Number of secondary roots 6 to 15.

Plants 1	able	Age	e Part		Hormone cent oí	c <u>k</u> onc growt	entra h base	tion a ed on	k per- check
				2	20	120	200	2000#	
Dent Corn	9 n n	2 4 2 4	T " R	 	80 96 102 116	80 87 55 51	100 96 52 59		
Sweet Corn	10 " "	2 4 2 4	Т 11 R 11	89 87 101 90	10 7 96 99 79		93 98 3 7 41	61 49 21 13	
				0.15	; 30	300	3000*		
Buckwheat	12 " "	4 8 4 8	T " R	106 105 104 103	95 94 94 77	93 105 45 77	51 100 22 50		
			0.00	005	0.001	0.02	0.1	0.2**	
Dent Corn	8 11 11 11	35835	T " " R	95 99 85 86 83	93 104 104 77 90	69 67 94 42 45	67 68 80 26 24	68 61 68 20 23	
				0.1	1.0	10	100*	*	
Sweet Corn Ligh Dark	t 11 "	16 "	T 11	90 91	9 7 82	84 85	19 44		
Ligh	t H	11	R	89 98	59 93	57 88	3		

Table 13. Summary of Experiment 1, Effect of Hormone Treatments on the Growth in Length of Seedlings.

Hormone, indole butyric acid. Age in days after germination. T = top length. R = root length. * p.p.m. ** Hormone in 0.2 mg. on 120 cm² paper Table 14. Some Preliminary Observation of Plants Growing

in Hormone Treated Soil in the Greenhouse. Seeds planted after soaking 4 hours, June 18; sand was added on top to the depth of 2.5 cm., after roots were out; pot covered with glass. Greenhouse temperature 27-32°C. Plants per pot, 5, but 10 for millet. Treatment duplicated.

	Hormone		Date emerg	of gence	Data of	Veccure
Plants	No.	Conc.	Root	Top	Flowering	Sep. 17.
		P.P. m .	June	June	July*	Top Length
Swee t Corn	1 4 7 16 20**	0 2 20 200 2000	21 " " "	23 " " "	22 " " "	55 cm. 54 55 53 38
	m	g/pot			~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Air dry wt.
Buck- wheat	1 4 7 16 20***	0 0.2 20 200	20 " " "	22 " 23 29	16 17 20 22	45.5g. 38.2 41.0 21.2
		g/pot			,	
Soy Bean	1 4 7 16# 20##	0 0.2 20 200	 	22 " 28 3 0	27 25 25 26 	
	m	g/pot				
Millet	1 4 7 16*# 20	0 0.2 20 200	20 " " "	22 " " "		
	-			•		

* Date tassel appeared on corn.

** Dwarfed at maturation.

*** Rapid growth after flower formation.

Growth inhibited.

Dead July 10. *# Younger stage only inhibited.

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Table 15. Growth of Dusted Sweet Corn Seeds in the

Greenhouse. Seeds with indole butyric acid dust. Planted Oct. 1, in 25 cm. pots. Germination began Oct. 7, but later in number 20 which was very poor. Temperature 20-27°C. Mean of 10 plants.

Нс	ormone	Silk	<u>Height</u>			
No.	p . p . m .		Oct. 21		Nov. 23	
			cm	80	Cm	%
1	0	Nov. 23	35.2	100	61.5	100
4	2	n	38.8	110	61.7	100
16	200	11	32.5	93	60.8	99
20	2000	24	30.7	87	59.6	98

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Tables 16, 17. Growth of Buckwheat in Dusted Soil in Greenhouse. Surface of the soil in pots (25cm in dia.) was covered uniformely with the 200 g. air dry soil mixed with dust containing certain concentrations of hormone; seeds were on the surface of this soil, and then covered with dry sand, to a depth of 2cm, Oct. 1. Temperature 27°C. Humidity 66-84%. Tops, ovendried at 85°C on Nov. 19. Mean of 10 plants per pot.

ł	Hormone Height and date of measurement.						nt.
Ro.	mg/pot	Oct.	21	Nov. 1	.9	<u>Oven</u> dr	y wt.
POT	A	Ciù	<i>6</i> /0	Сш	10	g	<i>j</i> 0
1	0.0	13.5	100	59.2	100	3.39	100
2	0.1	13.5	101	55 • 3	94	3.34	8 6
3	1.0	18.2	99	59.0	100	3.54	91
4	10.0	17.0	92	54.1	91	3. 06	79
5	100.0	11.7	63	50.5	35	2.11	54
R	0.3	13.4	99	56.8	96	3.64	94
R'	3.0	12.1	66	52.1	83	3.22	83
POI	с В						
1	0.0	19.2	100	51.5	100	3.46	100
2	0.1	18.7	93	51.7	100	3.52	102
3	1.0	15.4	96	52.1	101	3.43	100
4	10.0	17.2	90	45 .3	39	3.02	83
5	100.0	11.1	58	44.7	37	1.63	49
R	0.3	13.3	98	51.5	100	2.58	7 5
R'	3.0	12.6	66	49.3	97	2 . 54	74

Dust No. 1-5 = indole butyric acid R = Rootone 0.3 g. per pot R' = " 3.0 g. per pot. Germination began Oct. 6, except for No. 5 and R which germinated on Oct. 7.

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Table 13. Growth of Buckwheat on Dusted Soil.

Summary from tables 16 and 17. Average percentages in height based on check as 100 percent.

	Hormone	Heig	sht ·	Dry wt.
No.	mg/pot	Oct.21	Nov. 19	Nov. 19
			70	<i>ij</i> o
1	0.0	100	100	10 0
2	0.1	99	97	94
3	1.0	93	100	95
4	10.0	91	90	84
5	100.0	61	65	51
R	0.3	99	9 3	35
R'	3.0	66	93	79

Table 19. Growth of Dustod Soy Bean Seeds in Greenhouse.

Naphthalene acetic acid mixed with dust. Seeds planted in 25cm pot, Oct. 1. Germination Oct. 8 and Cct. 16, for number 5 (No. 5). Temperature of greenhouse 20-27°C. Harvested Nov. 10. Roots carefully washed; roots and tops dried together in oven. Mean of 10 plants.

	Hormone	lieie	lieight and date				Oven dry wt.		
No.	p.p. %.	Oct.	21	Nov.	10	Nov.	10		
		Ciù	10	Сщ	;0	g	6,0		
1	0	19.5	100	56	100	3.33	100		
3	10	20.7	106	58	103	3.48	103		
4	20	16.1	83	56	100	2.84	84		
5	2000	4.6	23	*		1.06	31		

* Dead Nov. 1.

Table 20. Growth of Rootone Treated Tomato Plants in

Greenhouse. Rootene powder (0.2 gram) per plant placed around roots. Flowers appeared (50 percent) Nov. 3, in check plants, and Dec. 15 in rootene treated plants. Temperature 20-27°C. Height measurement to tip of leaves. Mean of 6 plants.

Date		Check		Rootone (0.2g/plant)		
		Cm	%	Clî	70	
October	4	15.6	100	16.7	107	
**	21	48.3	100	36.8	7 6	
Nov.	29	13 5. 3	100	93.2	70	

Table 21. Growth of Rootone Dusted Wheat and Millet Seeds in Greenhouse. Temperature 20-27°C. Mean of 40 plants.

Ro No.	otone g/100	Late Emera	of Tence	Oct.	Heig 21	ght Nov. 8		Top of dry we	plant ight
\[he	at:	Begin	50%	Ciñ	60	CIL	5.0	g	07 10
1	0.0	Oct.6	Oct.7	33.3	100	42.6	100	6.11	100
2	0.3	11	18	35.1	105	45 .3	106	6.28	102
3	3.0	*	8	33.5	100	44.1	104	6.08	99
[[1]	let:								
1	0.0	0ct.7	Oct.9	11.1	100	26.9	100	4.62	100
2	0.3	11	**	10.8	9 3	30.1	1 12	4.70	102
3	3.0	*	11	10.9	9 9	27.9	100	1.96	43

* Germination, poor or nearly one day behind.

g/100 = grams of Rootone per 100 grams of seeds.

Hormone conc. g/100	n Number of tillers*	N Number of plants	nN	nN	5%
g. O	1	20	20		
88	2	12	24		
11	3	8	<u>-24</u> 68	68	100
0.3	1	13	13		
11	2	17	34		
"	3	10	<u> </u>	77	103
3.0	l	14	14		
11	2	20	40		
"	3	6	$\frac{18}{72}$	72	9 9

Table 22. Calculation for the Significance of Rootone Treatment of Tiller Production of Wheat.

* Total number of tillers per plants

Number of plants for each group 40.

nN = Total number of tillers in 40 plants.

g/100 = Grams of Rootone per hundred grams of seeds.

Table 23. Growth of Dent Corn Dusted Seeds in the Field. Hormone, indole butyric acid. Planted June 3. Emergence June 9. Measurement to the top of flowering tassel on Aug. 8. Mean of 20 plants.

	Hormone	hogaelg#	Flower	Height.	
No.	p.p.m.	- 355C 15;r	in full		
				СШ	/0
1	0	Aug. 3	Aug. 8	212	0
4	2	••	t1	215	+ 1
7	20	*1	n	215	+ 1
10	30	11	n	218	+ 3
12	120 `	11	11	206	- 3
14	160	**		207	- 2
16	200	- 41	11	1 95 *	-8

* Statistically significant.

Tassel appeared in 80 % of plants.

% Bercentage difference from check.

Table 24. Growth of Sweet Corn Dusted Seeds in the Field.

Hormone, indole butyric acid. Planted July 5. Emergence from ground July 10 except for number 20 which was one day behind. Tassel appeared in 80% of plants Aug. 8. Flower in full Aug. 18. Height measurement to the top of flowering tassel, Aug. 20. Mean of 20 plants.

	Hormo	ne		Height			
No.	p.p.m.	July]	18	Aug.	3	Aug. 2	0#
1	0	21.7	0	75.7	0	107.2	9
4	2	21.2	-2	79.8*	+5	106.3	-1
7	20	21.5	-1	74.1	- 2	105.6	- 2
16	200	21.5	-1	68 .0*	-10	106.5	-1
20	200 0	17 . 6	-12	71.4*	-7	109.3	+1

* Statistically significant.

* " highly significant.

Variation within the row was statistically significant.

Table 25. Growth of Dusted Buckwheat Seeds in the Field.

Hormone, indole butyric acid. Planted June 30. Emergence from ground Aug. 6, except number 7 which was one day delayed. Flower start Aug. 23. Mean of 20 plants.

	Hormone	. <u></u>	Height						
No.	p.p.m.	Aug.	8	Oct. 3		Dry wt.			
		Ômí	6%	cm	%	g	%		
1	0.0	5.5	0	35.2	0	192.6	0		
2	0.075	5.4	-2	82.8	-3	186 .1	-3		
3	0.15	5.8	+5	90.0	+5	200.2	+4		
4	3.0	5.3	-4	90.2	+5	202 .]	◆ 4		
5	30.0	5.3	-4	83.1	-3	185.5	-4		
6	3 00 .0	5.4	- 2	81.5	- 5	195.6	-4		
7	3000.0	5.1*	-7	86.1	+1	194.4	+1		

* -longation retarded significantly.

.

	Hormo	ne conc	. and	measureme	ents
Lines	l	4	7	16	20
	Cli	сш	Cm	cm	cm
1	69	86	72	63	б 9
3	70	38	73	68	68
5	33	91	74	72	63
7	87	90	80	72	63
9	36	90	80	73	73
11	82	89	78	65	70
13	81	32	77	64	73
15	71	6 7	77	71	82
17	72	8 3	74	64	84
19	7 5	73	73	70	78
21	77	74	72	70	81
23	77	76	71	64	68
25	80	75	76	66	65
27	ôO	65	8 2	72	81
29	75	84	78	67	61
31	74	8 3	65	58	78
33	64	69	6 7	69	69
35	67	70	67	68	68
37	7 5	71	3 9	76	62
39	64	87	58	68	72
Ave.	75.7	79.9*	74.1	68.0**	714*

Table 26. Measurements of Height of Plants Grown from Dusted Sweet Corn Seeds in the Field (Aug. 3, Table 24).

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Table 27. Observation on Different Seedlings Treated with Hormone Dust. Seeds planted in the field, Aug. 20.

Plants	Hormone concent.	Germination Start 80%		Remark s
	p.p.m.			
Soy bean	0	Aug.27	Aug.28	
	2	34	89	
	10	**	**	
	20	11	11	
	2000	64	30	Germination poor.
	R	11	28	
heat 1llet	Check	24	26	
	R	11	11	Tillering appeare
	Check	**	**	Sep. 15
	R	11		
ea.	Check	27	29	
	R	11	11	
dney bean Check		26	28	
·	R	11	11	
ttuce	Check	27	30	
	R	FT	11	

Soy bean seeds were dusted with napthalene acetic acid mixed with talc. R = Seeds dusted with Rootone and excess shaken off.
Table 28. Summary of Exp. 11.

Plant	Dent corn	Sw	eet cor	'n	Bu	Buckwheat			
Table	23		24		<u></u>	25			
Age (days)	30	8	30	40	2	40	40		
p.pum.	ijν	70	6/0	¢1 10		c/0	ħ		
2 to.3	+1	-2	+5	-1	- 4	+5	+ 4		
20-30	+1	-1	≡ 2	-2	- 4	+3	-4		
200-300	-8*	-1	-10**	-1	-2	-5	-4		
2000-3000		-12**	-7*	+1	-7*	+1	+1		

Percentage differences based on check

* Significant.

****** Highly significant.

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Plants	Hormone concent.	Degree of cur- vature	Number of tip empty coleoptiles
	%		
6	0	0	Normal
**	0.1	+68	Normal
n	3	-3 3	4 (0.5cm)
11	10	- 35	5 (0.4cm)

Table 29. Degrees and Direction of Curvatures of Zea Coleoptile at Low Humidity.

Place, dark room. Cultures covered with pasteboard box. Temperature of room 21-25°C. Humidity low (22-42%). Coleoptiles were used when length was 2 to 3 cm, and hormone application in the form of lanolin paste at the middle. Measurements taken 10 hours after application. Date of observation Sept. 24 (12:10 A.M). Degree of curvature determined by means of shadowgraphs on bromide paper. Positive curvatures are indicated by (+) and negative by (-). Hormone used naphthylene acetic acid.



Table 30. Effects of Naphthalene Acetic Acid in the Form of Lanolin Paste Applied at Various Points of Zea Seedlings.

		Hours after application							
No. Location		8 hrs		12hr	8	20hrs	34 hrs		
heck							Plumule	appeared	
.5cm	from tip	++	G	++*	+	₽mp.	+-		
id.	coleop.	++	P	++#	+	Emp	+-		
ode		++	P	++	+-	+	, ++		
id.	internode	++		++	-	+	+-+		
	Loca heck .5cm id. ode id.	Location heck .5cm from tip id. coleop. ode id. internode	Location 8 heck .5cm from tip ++ id. coleop. ++ ode ++ id. internode ++	Location 8 hrs heck .5cm from tip ++ G id. coleop. ++ P ode ++ P id. internode ++	Location 8 hrs 12hr heck .5cm from tip ++ G ++* id. coleop. ++ P ++* ode ++ P ++ id. internode ++ ++	Location 8 hrs 12hrs heck .5cm from tip ++ G ++* + id. coleop. ++ P ++* + ode ++ P ++ ++ id. internode ++ ++ .	Location 8 hrs 12hrs 20hrs heck .5cm from tip ++ G ++* + Pmp. id. coleop. ++ P ++* + Emp ode ++ P ++ + + id. internode ++ ++	Location 8 hrs 12hrs 20hrs 34 hrs heck Plumule .5cm from tip ++ G ++* + Pmp. +- id. coleop. ++ P ++* + Emp +- ode ++ P ++ + + + ++ ++ id. internode ++ ++ +-	

Experiment conducted in laboratory in porous pot damp chambers. Room temperature 23-27°C. Humidity of seleding environment high. Hormone applied Oct. 12 (10 A.M). Length of coleoptile 2 to 3 cm. Number of seedlings used 40. Positive curvature indicated as (+), negative curvature as (-), and recovery indicated (+-). P. means sharp curvature at point of application. Empty coleoptiles indicated by Emp. Two negative curvatures recorded here also(*).

Group.		A		В		C		D
Av. Length Coleop. at start (cm)	3.5		3.0		2.7		2.0	
Quantity of paste	L	S	L	S	Ľ	S	L	S
Curve after 12 hrs	0	0	-	, +	-	+	-	+
					-	+	-	+ +

Table 31. The Effects of Quantity of Hormone and Age of Coleoptiles on Direction of Curvature.

Hormone used, 3 percent naphthalene acetic acid in lanolin paste. Room temperature 23-27°C. Number of plants 32. Hormone paste applied at middle of coleoptile. Positive curvature (+), and negative (-). See plate 23.

Table 32. The Effects of Various Concentration of Hormone in Lanolin Paste on Zea Coleoptile.

No.	1	2	3	4	5	6
Percent Hormone	0	0.1	3.0	10	BothSide 0.1	Inter: node 10
Cur ve after 12 hrs	9 0	++	++	++	0	++
" " 24 "	0	+-	+-	+	0	++

Number of plants and conditions of the experiment similar to that recorded in table 30. Place of hormone application 0.5 cm below tip. Plus signs indicate medium or strong curvature. Plus and minus signs together indicate recovery. See plate 27.

Hormone		<u>Older</u>	coleop.	Younge	Younger coleop.						
No.	Conc.	Begin	24 hrs	Begin.	12	hrs	24 hrs	ţ			
		СШ	D . D	Cm			сщ				
1	0.0	3.3	3.7	1.3	2.5	0	3.5	0			
2	0.1	2.8	3.2	1.4	3.3	++	3.3	+-			
3	3.0	2.9	3.4	1.6	2.8	++	3.5	+-			
4	10.0	3.3	3.9	1.7	2.7	++	3.6	+-+			
5*	10.0	2.8	3.4	1.7	2.7	++	3.7	++			
6**	0.1	2.7	3.4	1.5	2.6	0	3.4	0			

Table 33. The Effects of Hormone in Lanolin Paste on Decapitated Zea Coleoptiles.

Plants and conditions of the experiment similar to that shown in table 30.

* Lanolin paste applied at internode.

** Lanolin paste applied symmetrically on decapitated coleoptiles. Plus indicates positive, and minus negative curvatures. No effects indicated by O, and recovery by (+-).

Table 34. Rooting Effects on Cuttings Through the Use of Rootone. Temperature of the greenhouse 25-32°C. Placed in propagating bench Oct. 4. Examined Nov. 15.

		Nu	mber (of plan	its	Total	plants
<u>Plants</u>	Rootone	A	З	C	D	Alive	Used
Chrysanthem	um Check	26	6	7	0	39	40-50
**	Tal-S	20	0	8	4	32	**
11	R	21	1 8	6	0	45	11
17	R-S	16	12	8	2	38	11
Mesembry ant.	he- Check	12	12	8	4	36	30-40
H	mum R	15*	15	0	0	3 0	64
Hydrangea	Check	21	8	6	. 0	35	40
11	R	20	12	6	0	38	**
Kleinia Ste	m Check	12	6	6	8	32	30-40
t1 11	R	8	10	5	7	30	**
" Lea	f Check	6	7	7	9	29	19
17 18	R	5	9	6	8	28	
Crassula St	em ^C heck	10	12	3	0	23	25
11 11	R	9	14	3	0	26	11
" Le	af Check	12	8	0	0	20	11
11 11	R	11	10	0	0	21	81
Azalea	Check	0	0	0	0	20	40
11	R	0	0	0	0	10	H

* Root development significant.

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Class A = many roots well developed.
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B = Medium developement of roots, more than 5, rather short. C = Few roots, 1-5. D = No roots. R = Rootone.

R-S = 0.2g. Rootone for each row (35cm) in flat.

 $T\mathbf{\hat{q}}\mathbf{1}-\mathbf{S} = 0.2g$. talc powder without hormone for 35cm rows.

Table 35. Rooting Effects of Cuttings Due to Rootone Calculated in Percentages Based on Total Cuttings Alive. Data from Table 34.

		Percent	tage of	plant	rooting	Plants
Plants	Rootone	A	В	C	D	alive
		%	%	%	of p	Þ
Chrysanthemur	n Check	66	15	18	0	100
11	Tal-S	63	0	25	13	100
n	R	47	40	13	0	100
H	R-S	42	31	21	5	100
Mesembryanthe	e- Check	3 3	33	2 2	11	100
mi 11	am R	40	50	0	0	100
Hydrangea	Check	60	23	17	0	100
**	R	53	3 2	16	0	100
Kleinia Stem	Check	37	19	19	25	100
F8 F9	R	27	3 3	17	23	100
" Leaf	heck	21	24	24	31	100
11 11	R	18	32	22	29	100
Crassula Ster	n Check	44	5 2	13	0	100
88 87	R	35	54	12	0	100
- "Lea:	f ^C heck	6 0	40	0	0	100
11 f1	R	53	48	0	0	100
Azalea	Check	0	0	0	0	100
H	R	0	0	0	0	100

R = Rootone

R-S = Rootone on soil.

Tal-s = Talc without hormone.

Table 36. [⊥]ffects of ^Hormone on Corn Seedlings Previously Treated with Colchicine. ^Laboratory temperatures 20-25°C. Plants in petri dish moist chambers.

Treatment & Observation	Check	Hormone treated.
Seeds germinated	Nov. 1	Nov. 1
Colchicine O.1% sol.	Nov. 3	Nov. 3
Washing (tap water)	4 hrs	4 hrs
Hormone (0.04% naphthalene)		Nov.4 (20hrs)
Washing		4 hrs
Petri dish culture	Nov. 3	Nov. 5
Lateral roots appeared	Nov. 8	Nov. 7
Lateral rooted plants	2	12
n n 11 %	10	60

-

No.		0	l	2	3	4
			(0.5M) (0.5M)	(0.04%)	(0.4%)	(0.04%)
		Blank	FeC13 + HC1	1 + Naph.	1 • Naph.	Naph.
•	·····	cc	cc	cc	CC	cc
FeC13	нсі	0	10	10	10	0
Naph.	0.04%	5 O	0	5	0	5
11	0.4%	0	0	0	5	0
нон		_15	5	0	0	10
Total	cc.	15	15	15	15	15

Table 37. Solution Mixtures With and Without Catalyst.

Table 38. Titration for Decomposition H_2O_2 .

Mean	٥f	four	titrations	with	0.1	N	KMn04:	room	temp.	25°C.
	U L	TOUT	OT OT YOT OND		••-				· · · ·	-

No		0	1	2	3	4
Minutes		cc	CC	cc	cc	cc
5	נ	5.10	14.50	15.20	16.50	15.85
15]	5.15	12.50	14.00	1 6.65	15.90
30]	15.10	10.20	12.20	16.7 ୦	1 5. 88
45		15.10	7.90	10.95	16.70	15.82
60		16.20	6.35	10.00	16.69	15.80
90	:	15.20	4.20	8.10	16.65	15.86
120		15.17	2.90	6.60	16.50	15.85
180		15.10	1.25	4.25	16.50	15.84
240		15.17	0.60	2.85	16.43	15.86
	Av.	15.14				15.85

Table 38. ^Titration for Decomposition H₂O₂. Mean of four titrations with O.1 N KMnO4. Room temperature 25^oC.

No.	0	1	2	3	4
Minutes	cc	CC	cc	cc	cc
5	15.10	14.50	15.20	16.70	15.85
15	15.15	12.50	14.00	16.65	15. 90
30	15.10	10.20	12.20	16.70	15.88
45	15.10	7.90	10.95	16.70	15.82
60	15.20	6.35	10.00	16.69	15.30
90	15.20	4.20	8.10	16.65	15.36
120	15.17	2.90	6.60	16.50	15.85
130	15.10	1.25	4.25	16.50	15.34
240	15.17	0.60	2.85	16.43	15.86
Av.	15.14				15.85

No.		1	2
Co		15.14	15.85×10^{-4}
Minutes		K	K
5		8 7. 7	78.5
15		84	85.5
30		108	86.4
45		131	82.0
60		172	77.3
90 ·		132	73.0
120		133	74.0
180		135	73.4
240		139	70.0
	Av.	107x10-4	78x10-4

Table 39. Reaction Velocity Constant for Decomposition H_2O_2 .

Chem icals	Stimulate on	Reported by
Auxin a	Avena test	Koogl (1935)
Auxin b	"	11
ⁿ etero a uxin	11	n
1-Methy1-3-acetic acid		11 .
δ -3-Indole provionic ac	id "	11
Pyruvic acid	11	11
3-Indole pyruvic acid	Avena test	Haagen Shit
Phenyl acetic acid	"	& Went (1955) "
Phenyl propionic acid	II ~	N
Isatinic acid		n
3-Indole acetic acid	Pea test & some Avena test	Zimmerman & Wilcoxon (1935)
b (b)-Naphthalene acetic	2 11	11
3-3-Indole butyric acid	a "	11
Indole propionic acid	II	11
Phenyl acetic acid	11	11
Fluorene acetic acid	n	·11
Anthracene acetic acid	11	н
δ -Naphthyl acetonitrile	е и	II
Ethylene gas	Root, Stem.	Croker, Zimmerman&
Glutathion	Root	
Sulphanilaride	Root	Grace, N.H. (1937)
♂-Phenyl butyric acid	11	II.
Ascorbic acid (vit.C)	rea seedling	Hausen, S.V. (1935)
Ascorbic acid (vit.C)	Fea seedling Root initiatebu not Avenacoleop	Hausen, S.V. (1935) t Thimann,K.V.(1935) file)

fable 40.	Chemicals Reported as Hormone or as Hormone-like in Activity.	

GRAPHS

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1 - 4



						Fig.	2						
		PEI	RCENT	CC AGE GE	NCENT RC HT H	RATIC DENT	ON HOI CORN	RMONE ON FI	AN D LITER	PAPEI	R		
						· · · · · ·							
100													
· · · · · · · · · · · · · · · · · · ·													
									· · · · · · · · · · · · · · · · · · ·				
80													
													₿
													mr
- 60													13
				(able_	3							
				T3 =	Top,	3 day	s 010						
				R 3 = R5 =	Root,	3 1 5 1)						
							· · · · · · · · · · · · · · · · · · ·						
											X		
20				me	s/pape	er (1:	20 cm	5)					R5 R3
	0		0.0	0005		0.00	01		0	.02		0	.2





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PLATES 1-32 .

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LARGE MOIST CHAMBER AND PAN FOR GERMINATIOM



Right: Moist chamber (100x70x90cm). Left: Pan (50x50x10cm) containing filter paper on glass plates for germination of seeds.

POFOUS POT NOIST CHAMBER



SEEDLINGS FROM HORMONE DUSTED SWEET CORN SEEDS GROWING ON FILTER PAPER (July 5-Table 10)



Indole acetic acid dust.

SEEDLINGS FROM HORMONE DUSTED SWEET CORN SEEDS GROWING ON FILTER PAPER



The same seedlings as in plate 3, but older. Rrimary root growth inhibited but root hairs developing.

SEEDLINGS FROM HORMONE DUSTED SWEET CORN SEEDS GROWING ON FILTER PAPER (July 8-Table 10)



The same seedlings as in plate 3, but older. Secondary roots are appearing.

SWEET CORN SEEDLINGS GROWING ON HORMONE DUSTED FILTER PAPER UNDER DIFFUSED LIGHT.

(July 17-Table 11)



Indole butyric acid dust spread on filter paper (r = 9cm)

> 1 = Check4 = 0.1 mg/paper7 = 1.010 = 10.020 = 100.0

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SWEET CORN SEEDLINGS GROWING ON HORMONE DUSTED FILTER PAPER IN DARKNESS.

(July 17-Table 11)



Indole butyric acid dust spread on filter paper (r = 9cm)

1 = Check4 = 0.1 mg/paper7 = 1.010 = 10.020 = 100.0

SEEDLINGS FROM HORMONE DUSTED BUCKWHEAT SEEDS GROWING ON FILTER PAPER (Aug. 15-Table 12)



Indole butyric acid dust spread on filter paper (r = 9cm)

> 1 = Check 3 = 0.15 p.p.m. 5 = 30.00 6 = 300.007 = 3000.00

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SEEDLINGS FROM HORMONE DUSTED BUCKWHEAT SEEDS GROWING ON FILTER PAPER

(Aug. 19-Table 12)



Same seedlings as in plate 8 but older. Indole butyric acid dust spread on filter paper (r = 9cm).

> 1 = Check. 3 = 0.15 p.p.m. 5 = 30.00 6 = 300.007 = 3000.00

SEE DLINGS FROM HORMONE DUSTED BUCKWHEAT SEE DS GROWING ON FILTER PAPER

(Aug. 21-Table 12)



Same seedlings as those in plate 9 but older. Indole butyric acid dust spread on filter paper (r = 9cm).

> 1 = Check 3 = 0.15 p.p.m. 5 = 30.00 6 = 300.007 = 3000.00

SEEDLINGS FROM HORMONE DUSTED BUCKWHEAT SEEDS GROWING ON FILTER PAPER (Aug. 23-Table 12)



Same seedlings as those in plate 9 but older. Indole butyric acid dust spread on filter paper (r = 9cm).

> 1 = Check. 3 = 0.15 p.p.m. 5 = 30.00 6 = 300.007 = 3000.00

SEEDLINGS FROM HORMONE DUSTED BUCKWHEAT SEEDS GROWING ON FILTER PAPER

(Aug. 23-Table 12)



Seedlings 6 and 7 from plate 11. Showing primary roots.

Left: 300 p.p.m. Right: 3000 p.p.m.

SEEDLINGS FROM HORMONE DUSTED BUCKWHEAT SEEDS GROWING ON FILTER PAPER

(Aug. 25-Table 12)



Seedlings 1 and 7 from plate 11.

Left: Check Right: 3000 p.p.m.

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SEEDLINGS FROM HORMONE DUSTED SWEET CORN SEEDS GROWING IN THE GREENHOUSE

(Nov. 5-Table 15)



Indole butyric acid.

Left: Check Middle: 2 p.p.m. Right: 20000 p.p.m.

BUCKWHEAT GROWING IN HORMONE DUSTED SOIL.

(Nov. 6-Table 16)



Left: Check Middle: Indole butyric acid 100 mg/pot. Right: Rootone (0.1% naphthalene acetic acid) 3g/pot.

SOY BEAN PLANTS GROWN FROM HORMONE DUSTED SEEDS GROWING IN THE GREENHOUSE

(Nov. 5-Table 19)



Left: Check. Middle: 20 p.p.m. Right: 2000 p.p.m. All seedlings in right hand pot dead Nov. 11.

ROOTONE TREATED TOMATO SEEDLINGS GROWING IN POTS IN THE GREENHOUSE (Nov. 5-Table 20)



Left: Check. ^Right: 0.2g. Rootone spread on soil around roots.

ROOT SYSTEM OF MILLET GROWN FROM ROOTONE TREATED SEEDS (Nov. 8-Table 22)



Left: Check. Right: 3g. Rootone/100g. seeds.

ROOT SYSTEM OF SWEET CORN GROWN IN THE FIELD (Oct. 6-Table 24)



Showing the point of attachment of the seed. Crown roots have developed, and the primary root has completed its development.

A = Check. B = Indole butyric acid, 2000 p.p.m.

ROOT SYSTEM OF DENT CORN GROWN IN THE FIELD (Oct. 6-Table 23)



Showing seed attachment from which primary roots developed.

- A = Check.
- B = Roots of plant grown from seed dusted with indole butyric acid (200 p.p.m.)

AERIAL ROOTS PRODUCED ON BUCKWHEAT STEMS AND PETIOLES BY APPLICATION OF HORMONE

IN LANOLIN PASTE

(Nov. 20-Exp. 1V-A)



Hormone in lanolin paste injected, Nov. 11. Plants growing in 25cm. pots in the greenhouse. Temperature 20-27°C.

- A = Check, lanolin paste only.
- B = 0.1% Naphthalene acetic acid in lanolin paste.
- C = 10% Naphthalene acetic acid in lanolin paste.

EFFECTS OF HORMONE IN LANOLIN PASTE ON TOMATO: PLANTS (Nov. 18-Exp. 1V-B)



Right: Inhibition of terminal bud through treatment with 0.1% naphthalene acetic acid in lanolin paste. Lateral shoot growth increased. Left: Tip untreated, but axial treatment with the same hormone in landin paste.

Lateral shoot growth was retarded.

EFFECTS OF QUANTITY OF LANOLIN PAST APPLIED AND THE AGE OF ZEA COLEOPTILES

(Table 23)

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Three percent naphthalene acetic acid in lanolin paste applied at points indicated by arrows. Older coleoptiles at right. Right hand coleoptile of each group received the heavier application. Photograph taken 12 hours after application of the paste.

THE EFFECTS OF VARIOUS CONCENTRATION OF HORMONE IN RLANOLIN PASTE ON ZEA COLEOPTILES. (Table 32)



Photograph taken 20 hours after application of naphthalene acetic acid paste.

> 1 = Check. 2 = 0.1% 3 = 3% 4 = 10% 5 = 0.1% (applied on both side) 6 = 10% (applied on internode)

THE EFFECTS OF HORMONE IN LANOLIN PAST ON DECAPITATED ZEA COLEOPTILES

(Table 33)



Photograph taken 24 hours after application of naphthalene acetic acid paste.

No. 1 2 3 4 5 Conc. paste, 0 0.1 3 10 10 %

Group on left are older coleoptiles. Group on right are younger coleoptiles. Showing responde with age and location of lanolin applied; although the picture taken at later stage and most of coleoptiles recoverd from bendings, yet bendings are seen on younger group (right); No. 5 were treated on inter-nod.

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THE EFFECTS OF ROOTONE ON ROOTING OF CUTTINGS OF CHRYSANTHEMUM

(Table 34)



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Left: Check.

Middle: Cut stem treated with Rootone.

Right: Rootone applied to soil, about

the cut end of stem.

Classification:

A = Many roots well developed.

B = Medium developed roots, 5 to 10, short.

C = Few roots, 1 to 5.
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THE EFFECIS OF ROOTONE ON ROOTING OF MESEMBRYANTHEMUM AND KLEINIA CUTTINGS.

(Table 34)



A	=	Many roots were de	eveloped.				
B	=	Medium developed 1	roots but short,				
		5 to 10 in number.					
C	=	Few roots poorly (leveloped and 1 to				
•		5 in number.					
)					
•	_	Megembryanthemum	check.				
h	-	mesembry anonemus,	Rootone treated.				
D	-	7	ster Bostone treated				
C	=	Aleinia repens,	stem Rootone treated.				
d	=		leaf Rootone treated.				

THE EFFECTS OF ROOTONE ON THE ROOTING OF HYDRANGEA AND CRASSULA CUTTINGS.

(Table 34)



Top row: Hydrangea cuttings. Middle row: Crassula leaf. Bottom row: Crassula stem.

A = Many roots well developed.
B = Medium developed roots, but short and 5 to 10 in number.
C = Few roots, poorly developed and less than 5 in number.

SEEDLINGS FROM COLCHIGIN TEREATED DENT CORN SEEDS RETREATED WITH NORMONE SOLUTION (Table 36)



All four seedlings treated with 0.1% colchicine at the beginning. Later the two on the left given an additional treatment of naphthalene acetic acid (0.04%). The two on the right not treated with naphthalene acetic acid.

DENT CORN SEEDLINGS GROWING IN BOXES WITH ONE SIDE PROVIDED WITH GLASS

(Nov. 7-Exp. V11)



Top: Check.
Bottom: Seeds dusted with indole butyric acid (2000 p.p.m.)
Left side of each glass face was covered with black paper to exclude light.
Right side remained uncovered.
Planted Nov. 3. Greenhouse temperature 20-27°C.

Plate 31

DENT CORN SEEDLINGS GROWING IN BOXES WITH ONE SIDE PROVIDED WITH GLASS.

(Nov. 10-Exp. V11)



Same cultures as in plate 30 but seedlings are 3 days older. Top: Check. Bottom: Seeds were dusted with indole acetic acid in talc. Left side of each glass face was covered to exclude light. Right side left uncovered.

DENT CORN SEEDLINGS GROWING IN BOXES WITH ONE SIDE PROVIDED WITH GLASS.

(Oct. 30-Exp. V11)



Left box: Sand only. Right box: Sandy loam. Left face of each culture covered; right face left uncovered. For each group plants (10 plants) Left 5 plants: Check. Right 5 plants: Indole butyric acid (2000 p.p.m.) Planted Oct. 10. Greenhouse temperature 22-26°C. ROOM USE ONLY

Jul 5'40	ROOM	USE	ONLY		
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1.19					
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