THE RELATION OF PLANT GROWTH HORMONES TO THE ACTION OF ETHYLENE UPON PLANTS

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FOREWORD

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PART I.

PREVIOUS STUDIES OF THE EFFECTS OF ETHYLENE ON PLANTS

Effects of Ethylene on Growth

Introduction: The attention of investigators was first brought to this subject by the damage done to street trees by illuminating gas. This was investigated by Girardin (1864) and Virchow (1870). Further studies along this line were made by Stone (1907, 1913) and Wilcox (1911). Among the effects described were wilting, yellowing, and falling of the leaves, and injury to the cambium.

Kny (1871) found variation among different species in their sensitivity to illuminating gas. Späth and Meyer (1873) found gas most damaging to plants which were actively growing. Wiesner (1878) did experiments on phototropism with seedlings of Vicia, Pisum, and Phaseolus. As a light source he used a gas light. He noticed that gas affected geotropism, and termed the effect "undulierende nutation". He considered this to be due to the disappearance of geotropism under the influence of ethylene. Molisch (1884) observed that ethylene affects geotropism in roots. He also investigated the toxicity to plants of tobacco smoke, and found that it is not due to nicotine. It was later shown (Knight and Crocker, 1913) to be due to ethylene.

Effects of Ethylene on Stem Growth and Geotropism: Neljubow (1901, 1911) studied the effect of illuminating gas and ethylene upon geotropism in Pea (Pisum) seedlings. He knew that ethylene was the active constituent of illuminating gas. According to him, pea plants brought into an impure (ethylene-containing) atmosphere began to grow horizontally. If placed horizontally in such air, they remained and grew in that position. If the tip of a horizontally growing stem was bent upward, it returned to the horizontal position. If a plant was slightly inclined from the vertical, it bent in the direction of the inclination. If a plant was pointed downward at any angle, it turned to a horizontal position. Stems growing horizontally in ethylene-containing air, when placed in pure air, grew again in a vertical direction. When plants were on a klinostat, treatment with ethylene caused no bending.

Neljubow considered these effects to be due to a change from negative geotropism to transverse geotropism. Richter (1903-1910) also studied the effects of ethylene on geotropism. He built further on the assumption of Wiesner, and in opposition to Neljubow, believed that ethylene caused the plants to be ageotropic, and that the change in direction of growth was caused by autonomic movements, which became very large when freed from the limiting influence of geotropism. Accordingly, he termed the horizontal growth of ethylene plants horizontal nutation. In contradiction to Neljubow, he said that under the influence of ethylene the sprout

of the seedling always bent away from the seed, regardless of its position with respect to gravity. Also he found the same type of growth when normal plants were placed on a klinostat, thereby removing the effects of gravity.

Richter also observed that illuminating gas in low concentrations caused a decreased growth in length and a thickening of the stem of bean seedlings.

A few years later, Crocker, Knight, and Rose (1913) studied the effects of ethylene on etiolated pea seedlings. They described what they termed the "triple response", consisting of reduction in rate of elongation, increased growth in diameter, and "diageotropism". Their seedlings were 2000 to 5000 times as sensitive to ethylene as the most delicate known chemical test. They found, in fact, that the epicotyl of a pea seedling would change from a vertical to a horizontal position when placed in an atmosphere containing four parts of ethylene to ten million parts of air. Furthermore, etiolated sweet pea seedlings would respond to an ethylene concentration of one part in ten million.

These authors also made tests of fifty other gases, including paint solvents, possible impurities in laboratory air, constituents of illuminating gas, and distillation products of coal tar. Carbon monoxide, acetylene, benzene, toluene, and some other substances gave the triple response, but not when in concentrations too low to be detected by other means.

According to Knight and Crocker (1913), sweet pea seedlings became more and more inclined from the vertical position as the concentration of the gas rose, until they reached a horizontal position.

Schwartz (1927) found horizontal nutation in Phaseolus seedlings, but failed to find it in Helianthus and certain other dicots. Elmer (1936) found that ethylene caused decreased longitudinal growth and an increase in diameter of potato sprouts, but mentioned no effect on the geotropic response of the sprouts. He also observed abnormal thickening of roots as a result of exposure to ethylene. From this and the foregoing paragraphs, it is evident that plants differ greatly in their response to small amounts of ethylene.

Ethylene also affects the geotropic response of roots (Molisch, 1884; Zimmerman and Hitchcock, 1933). The latter authors show a picture of a potted marigold plant (Tagetes erecta L.), which was treated with 0.5% acetylene (which, they explain, acts in the same way as ethylene). The treatment caused large numbers of roots to turn upward, coming out of the soil into the air.

Van der Laan (1934) took up the question from a growth hormone point of view. According to him, ethylene does not act directly but acts on the auxin in the plant. He believes that ethylene causes a decrease in production of auxin, and to this he attributes not only the decrease in longitudinal growth but the increase in diameter

found in stems of ethylene-treated plants. To account for the latter, he states that the lack of auxin causes the cell walls to lose their plasticity, and as a result longitudinal growth stops at an abnormally early age. The cells, apparently, continue to take up water, and as they cannot expand in a longitudinal direction, they expand sideways. As will be shown later, this theory is completely at variance with the facts.

Using the method described by Dolk (1930), van der Laan showed that ethylene not only inhibited redistribution or lateral transport of auxin in horizontally placed Vicia faba plants, but that it apparently caused a reversal of this lateral transport so that more auxin could be extracted from the top side of the stem than from the bottom. Since this effect was accompanied by a great decrease in the total amount of auxin, his conclusions as to lateral transport in ethylene-treated plants are based on measurements of rather small amounts of auxin.

He found that ethylene-treated plants were not limited to horizontal growth, but would grow in any direction, irrespective of gravity, and he considers, in agreement with Richter, that they are ageotropic - the absence of geotropism being due both to the absence of lateral transport and to the small amount of auxin present in such plants. He expresses no opinion as to what role may be played by reversed lateral transport, if such a reversal actually takes place. He believes also that the bending of the

ethylene-treated plants is autonomic, and that it is closely connected with the fact that these seedlings are bilaterally - not radially - symmetrical.

Epinastic Movements of Leaves: Ethylene influences not only the orientation of stems with respect to gravity, but the orientation of leaves with respect to gravity. Wächter (1905) was the first to report that ethylene induced epinastic movements of leaves (in Callisia repens). Molisch (1911), E. M. Harvey (1913), Doubt (1917), and Schwartz (1927) described this phenomenon further, and observed ethylene-induced epinastic movements of leaves of several different plants, including Ricinus communis (castor bean), Salvia, Datura, Helianthus, and Lycopersicon (tomato).

Schwartz also showed that ethylene-induced epinastic movements were growth movements. He also showed that they could be caused by carbon dioxide and acetylene. Some other substances, such as chloroform, benzol, and alcohol were also active, but only in relatively high concentrations. Placing the plant for three hours in warm water bath (35° C.) also caused epinastic movements. Removal of oxygen from the atmosphere surrounding the plant had no effect.

Crocker, Zimmerman, and Hitchcock (1932) treated 202 species of plants with ethylene. Of these, 89 showed leaf epinasty. Four of these, including Helianthus were sensitive to one part of ethylene in twenty million. Tomato, which was selected for further

experiments, was sensitive to one part in ten million. They also tested a number of gases for their ability to cause epinasty. Those found effective, followed by their relative minimum effective concentrations, are:- ethylene, 1; acetylene and propylene, 500; carbon monoxide, 5000; and butylene, 500,000. Since the corresponding saturated compounds had no effect, these authors attribute the activity of ethylene to the double bond. Ethylene chlorhydrin did not affect leaf epinasty, though, as will be mentioned later, plants sometimes react to it as they do to ethylene.

By means of motion pictures, it was shown that ethylene not only caused epinastic movements of the leaves, but inhibited the normal autonomic nutation of the stem, (also Zimmerman, 1935).

It was also shown definitely that these movements were not due to loss of turgor. When a plant was placed in an atmosphere containing ethylene the leaves pulled downward with a force equal to four to eight times the weight of the leaf.

Ethylene-induced epinasty was shown to be related to the orientation of petioles with respect to gravity. Ethylene had little if any effect on plants placed in an inverted position, and had only about 40% as great an effect on plants placed on a kløinostat. Excised leaves behaved in the same manner as leaves attached to the plant. Ethylene was therefore considered to modify the equilibrium position of the leaf with respect to gravity.

Using tomatoes as test plants, Zimmerman, Hitchcock, and Crocker (1931 a) showed that ethylene moved through plant tissue without difficulty. If one leaf of a tomato plant, without being removed from the plant, was sealed into a vessel containing a small amount of ethylene, the whole plant reacted. Furthermore, ethylene was given off from the leaves of such a plant in sufficient amounts to cause other plants to react.

Effect of Ethylene on Production of Roots: This question has been studied by Zimmerman and Hitchcock, (1933), who treated 27 species of plants, in 15 of which ethylene caused a definite rooting response. Ethylene was shown to be more effective than acetylene or propylene in causing rooting. It was also shown (Zimmerman, Crocker, and Hitchcock, 1933) that carbon monoxide was capable of producing a similar response.

The most evident effects of ethylene in low concentrations were:- initiation of roots on young stems, stimulation of pre-existing root primordia, and root formation on the under side of leaves. In some species the roots were evenly distributed along the stems. In others they formed at the nodes, and in some in the region of elongation. In Nicotiana tabacum, roots were formed on the part of the stem which was elongating when the plant was placed in ethylene, but no roots were formed on parts of the stem which went through their grand period of growth after the plant was in ethylene. Several species were found in which ethylene stimulated

root formation although the plants, before treatment, contained no root primordia.

Cuttings of Salix, when placed in water without ethylene treatment, formed roots only on the submerged part. When they were also treated with ethylene, numerous roots were produced both above and below the water. The cuttings used had numerous root primordia.

These authors found also that ethylene stimulated, in different cases, the formation of root hairs and of lateral roots on roots that were already formed.

The authors conclude that "Since the three chemicals (ethylene, propylene, and acetylene) did not induce shoots to form ---- these gases are specific for adventitious root formation." This statement, however, seems in disagreement with the statement of Zimmerman, Hitchcock, and Crocker (1931 b) that "Ethylene caused an abnormally large number of latent rose buds to produce shoots, 70 per cent of all buds producing shoots on the treated plants compared with 44 per cent for the controls."

Other Effects of Ethylene on Plants

Effect on Dormancy: Denny (1926) investigated the ability of ethylene to break the period of dormancy in potato tubers. Ethylene had only a slight effect, and a number of other substances, including ethylene chlorhydrin, ethylene dichloride, sulphur dioxide, ethyl bromide, and sodium thiocyanate, were found to be much more

effective. Dormancy was easily broken by several of these substances.

Vacha and R. B. Harvey (1927) confirmed and supplemented the work of Denny. They reported that ethylene-treated potato tubers sprouted somewhat more quickly and grew much faster. They also found ether and chloroform more effective than ethylene and propylene in breaking dormancy in gladiolus bulbs. Denny also worked with gladiolus (1930), and found, among other things, that exposure to ethylene had no effect at the beginning of the period of dormancy, but that it did at a later date.

Denny and Stanton (1928) did interesting experiments on the response of dormant woody tissue to chemical treatment. Potted plants such as Prunus, Syringa, Malus were treated in the fall with ethylene dichloride and ethylene chlorhydrin. Such treatment resulted in immediate breaking of dormancy. Malus plants were in full leaf in fifteen days after treatment; Syringa in full bloom after thirty days. Several other substances, of which propylene was the only unsaturated hydrocarbon, were tried and found effective. They were unable to break dormancy in Viburnum by any of these treatments.

They were also able to end dormancy in individual twigs and buds. Treated parts would grow and bloom, while the remainder of the plant would remain dormant.

The present author tried to stimulate germination of seeds of Avena sativa by ethylene treatment, but was unsuccessful. Ethylene dichloride and ethylene chlorhydrin also failed to stimulate germination (Degard, unpublished).

It is noteworthy that only a very few substances affect growth in the same way as does ethylene; and these substances are all (except carbon monoxide) chemically similar to ethylene. In contrast to this, dormancy is affected not only by ethylene and other unsaturated hydrocarbons, but by other substances which bear no similarity to ethylene.

Effect of Ethylene on Fruit: For a number of years ethylene has been used by fruit packers to promote the coloring of citrus fruits. According to Chase and Denny (1924), citrus fruits often remain greenish in color even after they have reached maturity. Ethylene often causes them to turn yellow, thereby increasing their market value.

Years ago it was customary to cure the fruit in "sweat rooms" heated with kerosene stoves. As it was believed that the fruit changed color under the influence of heat, some packing houses built steam heated "sweat rooms", and found immediately that fruit placed in them would not change color. As a result of this, Sievers and True (1912) showed that it was not the heat, but the fumes from kerosene stoves, that caused coloring of the fruit. It was not

until 1924 that Denny showed that ethylene was the active ingredient in these fumes, and ethylene in a concentration of one part per million of air would cause a color change in lemons in six to ten days. Treatment of citrus fruit with ethylene is now a standard procedure used by packing houses[1]. The usual ethylene concentration is about one part in five thousand.

Ethylene has also been reported to promote ripening in bananas (Wolfe, 1931), persimmons (Davis and Church, 1931) and other fruits. According to Mack (1927), ethylene accelerates the blanching of celery. To the author's knowledge, however, ethylene is used commercially only on citrus fruits.

Additional Effects of Ethylene: A few other effects of ethylene are mentioned in the literature. According to Schwartz (1927) ethylene has no effect on stomatal opening, but causes a slight increase in guttation. This is of interest, for the present author noted that one of the symptoms of injury due to ethylene is drying of leaves and stems.

Zimmerman and Hitchcock (1933) noted that ethylene sometimes causes abcission of leaves and flowers, and proliferation of lenticular tissue. This has also been noted by the author.

Wallace (1928) placed apple twigs in pure ethylene. This caused intumescences, which were characterized by breakdown of the cell walls and hypertrophy of the cells.

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1. "The Coloring of Citrus Fruits", published by California Fruit Growers Exchange.

Effect of Ethylene upon Metabolic Processes

Action on Metabolism and Chemical Composition of Plants: A number of authors have considered the effect of ethylene upon various metabolic processes in plants. E. M. Harvey (1915) investigated the action of ethylene on pea seedlings, and found a large number of changes. Ethylene treatment caused an increase of simple soluble substances at the expense of more complex soluble substances and insoluble substances. This was accompanied by an increase in osmotic concentration of the cell sap. The acidity of the tissue was not affected. Ethylene caused a slight increase in permeability. It retarded respiration, but did not alter the respiratory quotient.

Denny (1924) treated lemons with ethylene in concentrations from 0.1% to 0.001%, and found in every case an increase in respiration of the order of 200%. Ethylene in a concentration of 0.0001% was slightly less effective. Davis and Church (1931) reported a somewhat smaller increase in respiration in persimmons, and also found an increase in the ratio CO_2/O_2. Mack (1927) reported an increase in respiration in celery, but Regeimbal, Vacha, and R. B. Harvey (1927) disagreed with him. Huelin and Barker (1933) found that ethylene caused an increase in respiration of potatoes.

Mack and Livingston (1933) made an extensive investigation of the effects of ethylene on wheat seedlings. Ethylene caused no great acceleration of respiration, but its influence on respiration

was closely related to its influence on shoot elongation.

Chase and Church (1927) analyzed treated and untreated oranges and lemons for water content, pentosans, soluble solids in juice, reducing sugars, sucrose, and acid. None of these were affected by ethylene. Similar investigations have been made by other workers, (Regeimbal, ~~Vacha,~~ and R. B. Harvey, 1927; Davis and Church, 1931; and others), who also failed to find that ethylene caused significant changes in the chemical composition of fruits.

Effect of Ethylene on Enzyme Activity: R. B. Harvey (1928) claimed that the role of ethylene in living tissue was that of increasing enzyme activity. Englis and Zannis (1930) found that ethylene did not increase the activity of diastase and invertase in vitro. These views, of course, are not mutually exclusive.

Nord + Franke (1928a) suggested that small quantities of ethylene caused an increase in cell permeability, allowing an intensified interaction between enzyme and reactant. Nord and Franke (1928b) developed this theory also, and they believed further that ethylene also forms a protective film over enzyme particles. The capacity of ethylene to do this they attributed to its double bond, which they said was capable of taking the form $\begin{matrix} CH_2^+ \\ | \\ CH_2^- \end{matrix}$, thereby enabling ethylene to form an uncharged protective film over colloidal particles. This author knows of no other evidence showing that ethylene can do this.

Production of Ethylene by Plant Tissues: There is considerable evidence favoring the view that ethylene is produced in the normal life processes of plants and is given off into the surrounding air. Elmer (1932, 1936) showed that apples gave off a gaseous substance which caused abnormal growth in potato sprouts. It was shown by other workers that this substance also caused epinasty in tomato petioles, and that its effects on plants resembled in all respects those of ethylene. Gane (1934) identified this substance as ethylene. He also stated that the amount given off by an apple is very small, perhaps only one cc. during the life of an apple.

Kidd and West (1933) showed that ethylene was produced by an apple at the time of the so-called "climacteric" - a drop in respiration associated with ripening. The ethylene thus given off was capable of inducing the climacteric in other apples which had not yet reached that stage of ripening. This explained the previously observed fact that apples placed in a single closed container would all ripen at once; while, placed singly in separate containers, they ripened one at a time.

The production of ethylene may not be limited to ripening fruits, however. Denny and Miller (1935) and Denny (1935) obtained emanations causing epinasty in potato leaves from a large number of leaves, roots, tubers, immature fruits, and pistils, anthers, and petals of flowers. Denny (1936) compared the gas produced by vertically placed tomato stems with that produced by horizontally

placed stems. The latter was more effective in causing leaf epinasty in the potato. Zimmerman and Wilcoxon (1935) also found that addition of heteroauxin increased the capacity of tomato plants to give off the epinasty-producing emanation. The gas produced by these tissues was not identified as ethylene, but it was pointed out that there are very few other known possibilities.

The author observed a typical "triple response" of pea seedlings to emanations from apples. Response to rapidly growing Salix cuttings was weaker, but nevertheless very distinct.

Similarity in the Effects of Ethylene and Auxin

Introduction: Since the subject of plant growth hormones has been reviewed by several authors (Went, 1935 a; Boysen Jensen, 1936; Went and Thimann, 1937), it seems necessary here only to mention certain phases of the subject which are particularly related to work described in this paper.

The best known phytohormone is auxin, also known as growth substance and Wuchsstoff. It is produced in buds, leaves, and certain other parts of plants, including the tip of the Avena coleoptile, where it was discovered. It is necessary for cell elongation in the growing regions of stems and leaves; and without it cell elongation is not possible. Auxin is transported polarly downward in stems. Also it is destroyed, to a certain extent, by oxidative enzymes within the plant.

Auxin is found in all of the higher plants. It is to be distinguished from heteroauxin or 3-indole acetic acid, which is formed by certain fungi, and also artificially synthesized. Heteroauxin is not destroyed as rapidly as auxin by plant tissues (van Overbeek, 1936), but otherwise its action on the plant is the same as that of auxin. In subsequent parts of this paper it has been convenient at times to use "auxin" as a collective term meaning auxin and heteroauxin.

Effects of Auxin which Resemble Those of Ethylene: Auxin has several effects which resemble those of ethylene. Laibach (1935) observed swelling or callus formation in decapitated Vicia faba epicotyls, to which lanolin paste containing heteroauxin had been applied. Laibach and Fischnich (1935 a) found the diameter of the swelling to be dependent on concentration of heteroauxin in the paste applied. They also found that cell division took place in these swellings. Czaja (1935) found that lateral applications of heteroauxin paste caused increased growth in thickness and decreased growth in length. The cells in swellings thus produced were much shorter and larger in diameter than normal, thus tending to become isodiametric. From this and other experiments, it was concluded that cell enlargement takes place in a direction parallel to the direction of auxin transport; and that short, thick cells are a result of sideways transport of auxin into the stem from the point of application. In the light of present knowledge, this

theory is untenable. As will be mentioned later, these swellings resemble very closely those produced by ethylene.

According to Thimann and Koepfli (1935) auxin is identical to the root-forming substance of Went (1934 b). Laibach (1935) and Laibach and Fischnich (1935 b) produced roots on Coleus, Vicia faba, and Lycopersicon by applying heteroauxin paste to the surface of the stem. Zimmerman and Wilcoxon (1935) and Hitchcock and Zimmerman (1935) produced roots on the stems of a number of plants by applying heteroauxin paste and also by injecting a water solution of it into the stems. A number of workers (Michener, 1935; Cooper, 1935; Hitchcock and Zimmerman, 1936) have shown that, in certain cases, heteroauxin stimulates ~~growth~~ formation of roots in cuttings of woody species.

Auxin also causes epinastic movements of leaves. Fischnich (1935) observed that, when heteroauxin is applied to one leaf, epinastic curvatures occur in neighboring leaves. This has also been observed by the present author. The workers at the Boyce Thompson Institute (Hitchcock, 1935 a and b, and others) have found epinastic movements resulting from auxin applied as paste, injected into the stem as a solution, or taken up through the roots.

Crocker, Hitchcock, and Zimmerman (1935) have pointed out the similarity between the physiological effects of ethylene and those of auxin. They say that "the unsaturated, carbon-containing gases and heteroauxin are identical in their power to produce leaf

epinasty". They state further that these facts, together with the fact that ethylene is produced in plant tissues, suggests that ethylene may be one of the hormones controlling the growth of plants.

PART II.

METHODS

Experimental Material: The plants used most extensively in these experiments were etiolated seedlings of Pisum sativum, Zea mays, and Avena sativa. The first two were grown in moist sand, in a dark room, at a temperature of about 24° C. The Avena seedlings were grown under the same conditions, but in water instead of sand, by the method used in growing them for the Avena test.

Ethylene Treatment: Large plants were treated in a large incubator of about 180 litres capacity, in which the temperature was, again, 24° C. Light was excluded from the incubator. Ethylene was measured in a gas burette and added through a tube leading into the incubator. After addition of the ethylene the air in the incubator was stirred for a few minutes by means of a fan.

Smaller plants were treated in a glass desiccator of about ten litres capacity. For relatively high ethylene concentrations, (0.1%) the ethylene was added by means of a small gas burette, which could be placed inside the dessicator and opened after the desiccator was closed, by means of a wire leading through a stopper in the side of the dessicator.

For smaller ethylene concentrations it would have been somewhat difficult to measure out the proper amount of ethylene. It

was found much easier to make a saturated solution of ethylene in water. This could easily be pipeted in the required amount into a small bottle, which was placed in the desiccator. After closing of the desiccator, the bottle was tipped into a petri dish or onto the bottom of the desiccator, thereby allowing the ethylene to evaporate. Since one unit volume of water will dissolve about 0.11 unit volumes of gaseous ethylene, the advantage of the above method for measuring small amounts of ethylene can easily be seen.

Ethylene was used in concentrations varying between 0.001% and 0.2%. Its effects did not appear to vary greatly within these limits, except where the higher concentrations caused damage to the plants.

Application of Heteroauxin to Plants: In some experiments, heteroauxin was dissolved in lanoline and applied as a paste, according to the method of Laibach (1935). Otherwise blocks made of 1.5% agar were soaked in a heteroauxin solution and applied to the plant, as described by Went (1935 a). In all cases heteroauxin was used for such applications.

Tests for Auxin: The test used most often in the experiments to be described here is the Avena test of Went (1928, 1935 a). This test is carried out as follows: Avena seedlings are grown in the dark, under standard conditions, (temperature = 23.8° C., relative humidity = about 85%), to a height of about three cm. They are then

decapitated. A second decapitation is made three hours later, after which a block of agar (2 x 2 x 1 mm.) is placed unilaterally on the resulting stump. Since the auxin is transported polarly downward, it goes down one side of the coleoptile. As a result, that side of the coleoptile grows while the other side does not, thereby causing a curvature of the coleoptile. The curvature produced is proportional to the amount of auxin in the block. The plants are photographed after 90 minutes (rarely 100 or 110) and the curvatures measured on the photographs. This test has been somewhat modified from the original method of Went (C. L. Schneider, unpublished).

In practice, large agar blocks (6 x 8 x 1 mm.) are soaked in auxin solution or used for collection of auxin by diffusion from plant tissue. These are cut into twelve equal parts, each of which is placed on one of a row of twelve plants. In the following experiments, the amount of auxin in the blocks is in most cases stated in terms of the number of degrees of curvature it produced in the Avena test.

Other tests for auxin have not been used extensively in these experiments, and they are described in connection with the experiments in which they were used. (See page 39 and page 72.)

Tests of Auxin Production, Transport, and Destruction: Auxin production by tips of Avena coleoptiles or other plants may be measured by removing the tips and placing them with their end surfaces on blocks of pure agar. After one or two hours the blocks

are tested by the Avena test. This method is described by Went (1928, 1935 a).

Transport of auxin may be tested by placing two blocks of agar, one with and one without auxin, in contact with the two ends of a section of Avena coleoptile or other plant organ. After some time, usually an hour, the blocks are tested for auxin. Since freshly cut sections always contain auxin, it is necessary to place them on wet filter paper for a time before the experiment, to allow this auxin to go out of them. (See Went, 1928, 1935 a; van der Weij, 1932).

If coleoptile sections containing no auxin are placed with their basal cut surface on agar blocks containing a known amount of auxin for one or two hours, and if the blocks are then tested, they will be found to contain less auxin than at the beginning. This is due to the previously mentioned destruction of auxin by plant tissue, (van Overbeek, 1935). As will be seen later, this destruction is comparatively small in Avena, but in Pisum it is so large that it causes great difficulty in experiments on transport and production. It is reduced to some extent if the cut surfaces of the sections or tips to be used are placed for a time in contact with wet filter paper, before they are placed in contact with agar blocks containing auxin. This presumably removes some of the oxidative enzymes from the cut surface.

Entry of Ethylene into the Plant: When it was found that plants do not react immediately to ethylene, it was thought that a considerable part of this time lag might be due to the time required for entry of ethylene into the plant. A calculation was therefore made of the time required for the ethylene to enter a pea stem. It was assumed that ethylene will diffuse as rapidly in water as in plant tissue, and that the stem may be represented by a solid cylinder of water, 1.4 mm. in diameter. It was found that, if such a cylinder were put in air containing 0.1% of ethylene, the time necessary for the ethylene in the water cylinder to reach such a concentration as to be in equilibrium with 0.001% ethylene in air would be only slightly over a second.[1]

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1. The calculation was made by means of Frick's law, which is:

$$d\,Q = - DA \frac{\partial u}{\partial x} dt \qquad (1)$$

"where dQ represents the amount of material diffusing in the time dt, during which all conditions may be considered to remain constant, across a plane of area A at right angles to the direction of diffusion, the concentration gradient being $\frac{\partial u}{\partial x}$." (Quoted from Jacobs, 1935). D is the diffusion coefficient, which is characteristic for a given substance and, to some extent, for a given concentration, but which depends mainly on the molecular weight of the substance.

Since the external concentration is constant and large compared to the internal concentration, $\frac{\partial u}{\partial x}$ may be considered to be $\frac{\Delta u}{\Delta x}$. Equation (1) may then be integrated, and becomes:-

$$Q = - DA \frac{\Delta u}{\Delta x} t \qquad (2)$$

or

$$t = - \frac{Q}{DA} \frac{\Delta x}{\Delta u} \qquad (3)$$

These quantities may now be evaluated.

D for N_2 in water at 22° C. is given by the International Critical Tables as 2.02×10^{-5}. Since N_2 and ethylene have the same molecular weight, they will have approximately the same diffusion coefficient. Hence D for ethylene at 24° is taken as 2.1×10^{-5}.

Since the "stem" under consideration is one cm. long, A is the area of a cylinder 1 cm. long and 1.4 mm. in diameter, or 0.44 $cm.^2$. The volume of the same is 0.015 $cm.^3$.

From the Henry's law constant for ethylene at 24° - 8.48×10^6 (International Critical Tables) - it may be calculated that the 0.1% ethylene will be in equilibrium with a concentration in water of 5×10^{-9} mols per cc. This may be taken as Δu, since it is large compared to the concentration inside the cylinder. It is negative, since the external concentration is larger than the internal.

The internal concentration will be 5×10^{-11}. From that and the volume, 0.015 $cm.^3$., may be calculated Q - the number of mols of ethylene passing from the outside to the inside of the cylinder. It is 0.75×10^{-12}.

The radius of the cylinder, or 0.07 cm., is taken as Δx.

When these quantities are substituted in equation (3), it is found that $t = 1.14$ seconds.

As to the validity of the above assumptions there is, of course, some doubt. The rate of diffusion will be much faster in air than in water, and the stem contains many air spaces. On the other hand, the cell membrane may decrease the rate of diffusion. This decrease may be considerable, but it seems unlikely that it would be extremely large, for ethylene is probably soluble in the lipoid layer of the cell membrane, and its molecules are small so that they should be able also to pass through the holes in the lipoid layer. This conclusion is supported also by the data of Collander and Bärlund (1933), who show that, in Chara cells, methyl alcohol and ethyl alcohol require, respectively 1.3 and 2.3 minutes to reach half of their

equilibrium concentration. These alcohols have molecules comparable in size to that of ethylene. It thus seems impossible that ethylene can require more than a few minutes, at most, to reach a physiologically effective concentration within the cells.

Effect of Ethylene on Auxin in Vitro: It is possible, though improbable, that ethylene can have some direct effect on auxin. To test this, agar blocks containing heteroauxin were placed for a time in an atmosphere of ethylene, and then tested by means of the standard Avena test. The results are shown in Table 1. In the first three experiments the treatment with ethylene lasted from 1 to 3 hours; in the last one, four hours.

These experiments therefore confirm the work of van der Laan, who found no effect of ethylene on heteroauxin in water solution (1933).

TABLE 1.

Effect of Ethylene on Auxin in Vitro

Experiment	Curvature Produced by: Ethylene Treated Blocks	Controls	Ethylene Concentration
1	8.3	8.5	0.01%
2	6.3	7.1	0.05%
3	9.8	7.9	0.1%
4	8.9	9.1	100 %

PART III.

EFFECTS OF ETHYLENE ON GEOTROPISM AND PHOTOTROPISM

Extensive experiments on geotropism and phototropism were not carried out. Nevertheless some interesting results were obtained.

Preliminary Experiments: It has already been shown that ethylene affects geotropic and phototropic responses of plants. In preliminary experiments, done with several kinds of plants, the most noticeable response was leaf epinasty. Ethylene produced marked (but not extreme) epinastic response in several dicots, including Pisum sativum, Carica papaya, and Malva parviflora. In Lycopersicon and Helianthus, the ethylene produced extreme epinastic movements, ~~(See Fig. 1.)~~ which caused the leaves not merely to take a vertical position, but in some cases to turn completely upside down. After the plants were removed from ethylene, the leaves returned to their normal position unless they were old or injured by the ethylene. Potted Avena plants, however, were not affected by ethylene, except that the growth of the stem was reduced ~~(See Figs. 2 and 3)~~.

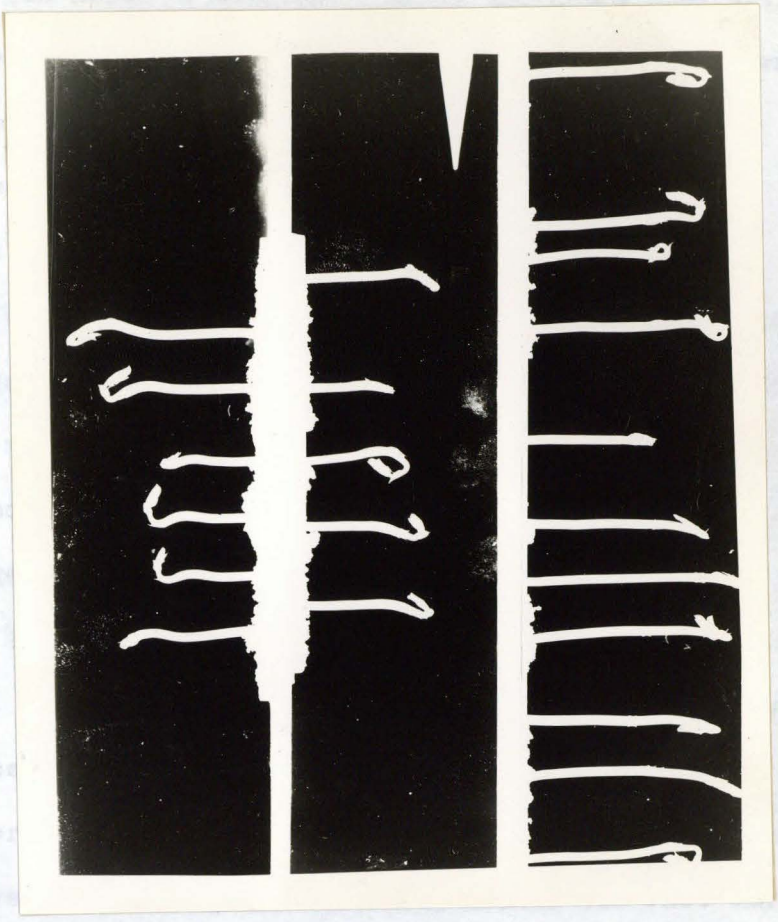
Geotropism: When seedlings of Avena and corn (Zea mays) were placed in a horizontal position in 0.1% ethylene, it was found that ethylene did not inhibit their geotropic reaction. The bending was somewhat less than in air, but the decrease appeared roughly proportional to the decrease in longitudinal growth caused

by ethylene. In older Avena plants the response to gravity was not affected by ethylene. ~~(See Fig. 3.)~~ On the other hand, ethylene was found to completely inhibit geotropism in pea (Pisum sativum) seedlings.

Some experiments were done to see how quickly pea seedlings regain their ability to respond geotropically after ethylene treatment. This was found to depend on the length of the treatment. Thus, plants treated for two hours responded normally two hours after being removed from ethylene. Plants treated for five hours were still somewhat slow in their response two hours after treatment. Plants treated for twelve hours were still somewhat slow in their response ten hours after the end of the ethylene treatment.

It is clear from this that the inhibitory effects of ethylene (at least some of them) last long after the ethylene has disappeared from the plant. It is consequently not directly dependant upon the presence of ethylene, but is connected with ethylene in some more remote way.

Tests were also made to see how rapidly ethylene acted to inhibit geotropism. Here the plants were placed in 0.1% ethylene in an upright position, in order to allow entry of ethylene into them. They were then placed in a horizontal position, in the ethylene. When placed horizontally after only five minutes in ethylene, they showed a perceptible geotropic reaction as soon as



A B C

Fig. 4. Negative geotropism caused by ethylene in pea seedlings.

A. Plants placed horizontally (in ethylene) after 5 min. in 0.1% ethylene.
B. Placed horizontally after 2 hrs. in ethylene.
C. Placed horizontally after 1 hr. in ethylene (plants slightly older).

In all cases, the photos were taken after the plants had been in a horizontal position in ethylene for several hours.

the controls (in about 50 minutes). After two hours they still were the same as the controls, but after that they turned down instead of up. Plants placed horizontally after an hour in ethylene showed little or no geotropic curvature.

The most interesting feature of this experiment is that ethylene not only inhibited upward curvature, but caused a slight downward curvature. This was most distinct in the plants mentioned above, which were treated only for five minutes before being placed in a horizontal position, (Fig. 4a). In plants treated for somewhat longer periods, the downward curvature was less distinct, (Fig. 4b and 4c). This is in agreement with the observation of van der Laan, who found that ethylene caused a reversal of the gravity-induced lateral transport of auxin in Vicia faba. It has not been observed in seedlings treated for long periods of time, i.e., for several days. This is probably due to the fact that long treatments with 0.1% ethylene cause an almost complete inhibition of longitudinal growth.

Phototropism: Untreated pea seedlings, and seedlings treated for several hours with 0.1% ethylene, were exposed to light (about 4500 meter-candle-seconds). The treated plants were replaced in ethylene after treatment. They failed completely to respond phototropically, while the controls responded.

Avena seedlings treated with 0.2% ethylene were exposed to light, as shown in Table 2. Neither ethylene treatment before exposure to light nor during the response to light inhibited phototropism.

TABLE 2.

Effect of Ethylene on Phototropic Response in Avena

Time in Ethylene (hours)		Curvature (degrees)	
Before Light Exposure	During Reaction	110 m.c.s.	15 m.c.s.
0	0	10.4	5.6
0	1-3/4	9.0	5.6
5	0	12.6	5.6
5	1-3/4	11.1	4.3

Summary:

1. Ethylene inhibits normal phototropic and geotropic reactions in pea seedlings, but has very little effect on those reactions in Avena seedlings.

2. Under proper conditions, ethylene causes a slight negative geotropism in pea seedlings. This is in agreement with van der Laan, who found that ethylene caused a reversal of the gravity-induced lateral transport of auxin.

PART IV.

EFFECTS OF ETHYLENE ON LONGITUDINAL GROWTH OF PEA SEEDLINGS

According to van der Laan (1934), the inhibiting action of ethylene upon pea (Pisum) seedlings is due to the fact that there is much less auxin in ethylene-treated plants. The effect of ethylene on auxin in these seedlings has been investigated more extensively. Experiments have been done to show its effect on production, transport, and destruction of auxin, and on the sensitivity of the plants to auxin. Seedlings of the variety Alaska have been used where not otherwise mentioned. In a few experiments, seedlings of the variety Perfection were used.

Effect of Ethylene Concentration: In a concentration of five parts per million (0.0005%) ethylene caused slight bending of pea seedlings, and considerable reduction of growth in length. A concentration of 0.002% caused almost complete ageotropism, and great reduction in growth. The same effect was given by a concentration of 0.01%. A concentration of 0.1% sometimes gives a more marked response. Apparently, however, the response to ethylene does not vary greatly with the concentration, if the concentration is between 0.002% and 0.1%. It has also been noted that very young seedlings are more sensitive to ethylene than older ones. It is probable that age is a more important factor than ethylene concentration in the response of these seedlings to ethylene.

Production of Auxin: Tips, as well as sections from the middle and base of pea seedlings were used for these experiments. They were first cut and placed on wet filter paper for about ten minutes, in an effort to remove auxin-destroying enzymes from the cut surface. They were then put on blocks of pure agar (12 tips or sections per block) for one hour. The blocks were then tested for auxin content by the Avena test. In every case, sections and tips about one cm. long were used. The results of these experiments are shown in Table 3.

TABLE 3.

Effect of Ethylene on Production of Auxin in Pea Seedlings

Exp. No.	Ethy. Conc.	Time in Ethy. hrs.	Time Removed# hrs.	Auxin Extracted. (Avena test curvature in deg.) Ethylene	Controls	Part of Plant Used
1*	0.1%	18	$\frac{1}{2}$	3.8	12.5	tip
				2.6	4.7	middle
				3.4	1.3	base
2*	0.1%	14	1-1/4	2.0	16.5	tip
				0.6	7.5	middle
				-0.5	1.4	base
3	0.1%	$4\frac{1}{2}$	1-1/4	1.8	12.5	tip Plants grown
				1.7	9.3	base in light
4a	0.1%	$17\frac{1}{2}$	$1\frac{1}{2}$	0.1	3.5	tip
b	0.1%	12	7	1.0	3.5	tip
5	0.01%	16	1/4	1.8	5.8	tip (Alaska)
				0.5	5.0	tip (Perfection)

#Time between removal from ethylene and cutting of sections for experiment.

* In experiments 1 and 2, tests for auxin were made with de-seeded plants, by the method of Skoog (1936).

From this it may be seen that the ethylene causes a reduction of the auxin extracted from the tips to about 25% of the normal value.[1] The reduction in sections from the middle and base of the cuttings is somewhat less. This agrees with the results of van der Laan, and would easily lead one to believe that ethylene causes a decrease in the production of auxin. In the next section, however, it will be shown that such a conclusion cannot be safely drawn from these data.

Destruction of Auxin in Pea Seedlings: If ethylene affects only production of auxin, it should be possible to remove its effects by substituting an artificial source of auxin for the natural one. Accordingly, seedlings were decapitated and to the cut surface was applied lanoline paste containing 0.02% heteroauxin. Half an hour later the plants were placed in 0.1% ethylene, where they remained for $3\frac{1}{2}$ hours. An hour later a one-centimeter section was cut from below the top, and placed on agar blocks as described above. The blocks were tested and gave the following curvatures:

Ethylene-treated plants	0.4°
Controls	3.8°

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1. This calculation and subsequent ones like it are made on the assumption that the "zero" angle in the Avena test is -1.0 degrees. Since pure agar blocks, when placed on Avena test plants, give about this curvature, it seems better to make such calculations on this basis than to consider zero curvature as indicating no auxin.

The ethylene has caused a reduction to 28% of the normal value (again assuming the zero point to be -1.0 degrees). This is roughly the same as the decrease obtained in the experiments which were supposed to measure production. This shows definitely that ethylene affects either transport or the rate of destruction of auxin, and indicates also that it may have no effect on production of auxin.

Consequently destruction was investigated in a more direct manner; namely, by making up agar blocks with a known amount of auxin, placing sections of stems of pea seedlings on them for a time, and testing the blocks for auxin by the Avena test.

Preparatory to these experiments, the rate of destruction was tested in various parts of the pea seedling and in the two varieties of peas used. The results are shown in Table 4. Here, and in subsequent experiments on destruction, twelve sections were placed on each agar block, and allowed to remain there for an hour.

TABLE 4.

Destruction of Auxin in Pea Seedlings

Variety	Height	Position	Original Auxin conc. in block	Final Auxin conc. in block
Perfection	7 cm.	middle of stem	25	0.5
Perfection	7 cm.	top	25	3.2
Perfection	1 cm.	top	25	10.2
Alaska	10 cm.	middle of stem	25	13.4
Alaska	10 cm.	near top	25	14.9

From this and other data, it is evident that destruction is higher at the base than at the tip; that it is higher in old plants than in young; and that it is higher in "Perfection" peas than in "Alaska". The third statement is of interest, as Alaska seedlings are tall with thin stems, while Perfection seedlings are shorter, slower-growing, and have thicker stems. Is this not due to greater destruction of auxin in the latter?

In Table 5 are shown the results of destruction experiments in which the plants were treated for three to five hours with 0.01% ethylene. With the young plants, there is certainly no significant difference between the ethylene plants and the controls. With older plants with a higher rate of destruction, there is perhaps a small difference.

It was found, however, that if the treatment with ethylene was increased to fifteen to twenty hours, the rate of destruction was greatly increased. The results of such experiments may be seen in Table 6. It appears from this that the rate of destruction is increased by about seventy percent by treatment with ethylene for fifteen to twenty hours.

In the experiments on production, an ethylene treatment of $4\frac{1}{2}$ hours caused a drop in the amount of auxin extracted. In the experiments on destruction, an ethylene treatment of this length had very little effect. The reason for this is unknown, though it

TABLE 5

Effect of Ethylene on Destruction of Auxin in Peas (1)

Short Ethylene Treatment

Experiment	Auxin conc. in block (*Avena* test curvature)			Percent destruction due to ethylene		Time in ethylene hrs.	Size of plants
	Original	Final					
		Ethylene	Air	Ethylene	Air		
1	12.1	6.5	6.3	43	44	$4\frac{1}{4}$	1 - 2 cm.
2a	8.5	4.7	4.9	40	38	$3\frac{1}{2}$	"
b	8.5	3.2	3.6	56	52	$3\frac{1}{2}$	"
3	22.6	10.7	12.3	50	44	$3\frac{1}{2}$	"
4	19.0	11.3	13.2	39	29	5	"
5	19.0	11.3	9.3	39	49	5	" (var. Perfection)
Average				45	43		
6	12.1	3.3	5.0	67	54	$4\frac{1}{4}$	5 - 10 cm.
7	11.3	1.7	2.6	78	71	3	"
8	22.6	8.6	10.9	59	49	$3\frac{1}{2}$	"
Average				68	58		

TABLE 6

Effect of Ethylene on Destruction of Auxin in Peas (2)

Long Ethylene Treatment

Experiment	Auxin conc. in block			Percent destruction due to ethylene		Time in ethylene hrs.	Size of plants
	Original	Final					
		Ethylene	Air	Ethylene	Air		
1	19.0	8.9	13.2	50	29	17	1 - 2 cm.
2	25.4	7.6	13.0	68	47	16	"
3	19.0	3.5	9.3	78	49	17	" (var. Perf.)
4	25.4	1.0	4.8	93	78	16	" " "
5	17.4	2.8	6.4	79	60	15	" " "
6	15.2	2.6	11.8	78	21	20	7 cm.
Average				74	47		

may be a result of the higher ethylene concentration which was used in the experiments on production.

Effect of Ethylene on Transport: From the high rate of destruction of auxin by sections of pea stem, one could expect difficulty in testing transport of auxin in such sections. Also, assuming such experiments can be successfully performed, how is one to separate the effects of ethylene on destruction from possible effects on transport? In spite of these difficulties, transport experiments were attempted, using the method previously described. Five mm. sections of pea stem were placed vertically on a pure agar block, and on top of the sections was placed another block containing auxin. Where not otherwise mentioned, the sections were turned with their basal end down; i.e., on the pure agar block.

The difficulties were found as anticipated. It was impossible to get any auxin to come into the bottom block unless a concentration of 250 units was used in the top block. In the latter case, the bottom block received less auxin in the case of ethylene-treated sections than in the controls, but this is to be expected from the effect of ethylene on destruction.

It was then decided to use a low auxin concentration in the top block, and to consider only the amount of auxin removed from that block. Such an experiment gave the results shown in Table 7.

TABLE 7.

Effect of Ethylene on Transport of Auxin (1)

Auxin Concentration in top block (Avena test curvature)		Treatment of Plants
Original	Final	
11.2	4.8	0.01% ethylene
11.2	4.3	air

The ethylene plants were treated for three hours before being cut into sections, and were also placed in ethylene during the transport test, which lasted for one hour.

This method was further simplified by using only one block. Sections were placed inversely upon a block containing auxin. The auxin removed then represented transport plus destruction. Destruction could then be determined alone by placing sections in normal position upon a similar block. Such an experiment was performed in which the ethylene plants were treated for four hours before the test, as well as during the transport test which again lasted for an hour. The results are shown in Table 8. Ethylene therefore has no effect on transport of auxin in peas, when the ethylene treatment is short.

TABLE 8.

Effect of Ethylene on Transport of Auxin (2)

Auxin concentration of blocks (by *Avena* test curvature in degrees)			Difference between inverse and normal	
Original	Final			
	Sections inverse	Sections normal		
27	7.8	10.7	2.9	air
27	8.8	12.3	3.5	0.01% ethylene

Effect of Ethylene on Sensitivity of Pea Seedlings to Auxin:

For testing sensitivity to auxin, the pea test (Went 1934 b) can be used very satisfactorily. For this test, peas are grown to a height of about ten centimeters, decapitated, and the top three centimeter section of stem is removed. These sections are split longitudinally from the top almost to the base, and put in water. The resulting expansion of the pith of the stem causes the two halves to curve outward. If, however, the stems are placed in auxin solution, an inward curvature results after several hours, and the curvature is roughly proportional to the logarithm of the auxin concentration.

For such an experiment, four groups of plants were used, as follows: (1) controls, (2) treated with ethylene during the experiment (i.e., while the sections were in auxin solution, (3) treated with 0.05% ethylene for seven hours before the experiment, (4) treated with ethylene both before and during the experiment. The curvatures were measured fourteen hours after the plants were placed in the solution. To eliminate possible error due to slow diffusion of ethylene into the auxin solutions in groups (2) and (4), solutions were used which contained the appropriate amount of ethylene. They were also placed in an atmosphere containing 0.05% ethylene. The results of this experiment are shown in Table 9.

TABLE 9.

Effect of Ethylene upon the Pea Test

	Solution Used		
	Water	Heteroauxin 0.6 mg./~~cc.~~ l.	Heteroauxin 3.0 mg./~~cc.~~ l
1. Control (air)	2°	107° ± 7*	304° ± 12
2. Ethylene during experiment	2°	84° ± 8	277° ± 14
3. Ethylene before experiment	0°	49° ± 7	145° ± 15
4. Ethylene before and during the experiment	0°	61° ± 4	155° ± 11
Average of 1 & 2	2°	96°	291°
Average of 3 & 4	0°	55°	150°
Reduction caused by ethylene treatment before experiment		43%	48%

* The probable error is used here and elsewhere in this paper as a measurement of precision.

The most obvious conclusion to be drawn from this experiment is that ethylene does not, by itself, stimulate growth. In other words, it does not act in the same way as auxin, and in the absence of auxin it has no effect upon cell elongation.

If group (1) and (2) (Table 9) are compared, it will be seen that ethylene treatment during the time in which curvature occurs causes only a slight reduction in curvature. Additional indication that such a reduction is significant is given by an earlier experiment in which plants placed in ethylene while the curvature

was taking place (but not before) gave slightly less curvature than the controls. If groups (3) and (4) are compared, it will be seen that ethylene treatment during the experiment has no effect if the plants have been treated with ethylene before the beginning of the pea test.

The important fact, however, is that in groups (3) and (4) the curvatures are much below those in groups (1) and(2). In other words, if the plants are ethylene-treated before the experiment, the curvature is reduced between forty and fifty percent, regardless of subsequent ethylene-treatment during the pea test.

It is now necessary to consider what causes the curvature in the pea test. Van Overbeek + Went (1936, ~~unpublished~~) have shown that growth occurs in the outside of the stem, while on the inside (cut surface) little or no growth occurs. The lack of growth on the inside is due either to inability of the auxin to enter through the cut surface or to destruction of auxin which does enter there. The curvature, therefore, is caused by the auxin which enters through the epidermis of the stem.

Since peas have a high rate of destruction of auxin, it seems certain that, in the pea test, part of the auxin is destroyed before reaching the point where it can cause cell elongation. Since ethylene has been shown to increase destruction, it is to be expected that it will cause destruction of a greater part of the auxin entering the stem in this test, and therefore decrease the curvature.

Since the curvature is due to auxin which enters from the outside, not through the cut surface, it follows that the ethylene-induced destruction of auxin takes place (partly, at least) within the plant tissue and not on cut surfaces. It is not necessary for this theory that ethylene should have the same effect (quantitatively speaking) on the tests for destruction as it has on the pea test. The latter depends on the auxin which is not destroyed, which is not, of course, proportional to the rate of destruction.

These experiments do not exclude the possibility that ethylene affects the pea test curvature through some mechanism other than destruction of auxin. As will be shown later, however, ethylene causes no decrease in sensitivity to auxin in Avena seedlings, in which it has little or no effect on auxin destruction.

Summary:

1. It is shown that ethylene increases destruction of auxin in pea seedlings.

2. It is impossible to make a determination of auxin production without having, superimposed on it, the effects of destruction. In view of this fact, evidence is lacking that ethylene has any effect on production; and it is probable that it has no large effect.

3. Ethylene appears to have no effect on transport of auxin, though the data on this are not extensive.

4. Ethylene-treated peas give about half as great a curvature in the pea test as do normal peas. This may be a true effect of ethylene

on sensitivity to auxin, but it seems probable, as will be explained later, that it is a result of increased destruction of auxin.

PART V.

ETHYLENE-INDUCED SWELLINGS ON STEMS: GROWTH FACTORS OTHER THAN AUXIN

Swellings of Pea Stems

Same Type of Swelling Produced by Auxin and Ethylene: As was mentioned in the introduction, auxin, when applied in high concentrations to the growing zone of the stem of a pea seedling, causes an enlargement or swelling of the stem. This enlargement may reach three or four times the normal diameter of the stem. Such swellings in the bean have been studied histologically by Kraus, Brown, and Hamner (1936). These authors found a great proliferation of cells, which largely remained parenchymatous, though there was some differentiation. Increase in cell size was noticeable, but not extreme.

It has long been known that ethylene will cause swellings similar to those described above. To determine whether swellings produced by ethylene and by auxin are actually the same, free hand sections of both kinds were examined. In such sections, no difference was detectable between the two kinds of swellings.

The histological features are somewhat different than those found by Kraus, Brown, and Hamner.

As described by Czaja (1935) and others, the cells fail to elongate properly, but round out and become nearly isodiametric. This change in shape of the cells is accompanied by increased cell

division, which takes place especially in certain places, which appear to develop into root primordia. In some cases, roots are later produced by these swellings.

Because of the similarity between the effects of auxin and ethylene in the formation of these swellings, it is natural to conclude that auxin and ethylene act in the same manner to form such swellings. In the light of evidence now to be presented, this conclusion seems true only in a limited sense.

Nature of the Swellings: It was shown that auxin in high concentration actually causes a decrease in the rate of stem elongation - presumably due to the fact that the cells round out instead of elongating in the normal manner. A lower concentration, however, will cause an increased rate of elongation. This was shown by applying auxin paste to one side of the stem (in the growing zone). As shown in Table 10, high concentrations give a positive curvature (toward the paste) while lower concentrations give a negative curvature.

From this it is evident that the swelling is closely associated with failure of the cells to elongate properly. That this is not the only cause of enlargement is shown by two additional facts. (1) In both ethylene- and auxin-induced swellings, the swelling extends slightly below the region of elongation of the stem, as shown by placing marks at one mm. intervals on the side of the stem.

TABLE 10.

Effect of Auxin on Longitudinal Growth of Pea Stems

Concentration of Paste		Curvature	Swelling
Relative conc.	mg. auxin per gram of paste		
1	.22	Slightly positive	Slight
1/4	.055	Slightly negative	Slight
1/16	.014	Strongly negative	Very slight
1/64	.0035	Slightly negative	Probably none
1/256	.00076	None	None
0	0	None	None

If plants are cut off near the base and the stumps treated with auxin, a swelling is formed. Since the cells here are certainly unable to change their size or shape, swelling must be due to proliferation of new cells. This evidently occurs in the inner parts of the stem, often resulting in splitting of the epidermis and cortex. Such splitting is uncommon in swellings formed near the top of the plant.

In a few cases, swellings of organs other than stems have been noted. Young petioles of peas will swell under the influence of high auxin concentration. When auxin paste was applied to pea buds, the buds did not grow normally but rounded out into globules ~~perhaps~~ about a millimeter in diameter.

As has been explained, when pea seedlings are treated with ethylene, swellings form. Generally, when they are removed from

ethylene, normal growth is resumed, so that the swelling is surmounted by a stem of normal diameter. Occasionally, however, the plant fails to resume normal growth and remains as when removed from the ethylene. The cause of this is not known, though it may be due to injury to the terminal cells.

Necessity of Auxin for Swellings: Auxin-induced swellings may be caused by applying auxin either to intact plants or to the tops of decapitated plants. Ethylene treatment will induce swellings on intact plants, but it will induce only very slight swellings on decapitated plants to which no auxin has been added. Decapitated plants given a large amount of auxin and also treated with ethylene formed swellings slightly larger than those treated only with auxin.

From this it is evident that ethylene caused swellings in every case except the one in which auxin was absent. It may therefore be concluded that auxin is necessary for formation of swellings.

Necessity of Roots for Auxin-Induced Swellings: Went (1937, ~~unpublished~~) has shown that the formation of swellings is dependant on the presence of the roots. If they are removed, auxin-induced swellings are very small or totally lacking.

It has been shown in two ways that these swellings are not due to root pressure. (1) If the roots are removed, root pressure certainly disappears almost immediately. Yet if the roots are removed and auxin applied immediately, swellings form which are

indistinguishable from those formed by plants with roots. If, however, the roots are removed but the plants are allowed to remain for two or three days without auxin, the "swelling substance" formed before the roots were removed disappears. Then the addition of auxin causes no swelling.

(2) A more direct proof that swellings have no connection with root pressure may be made by splitting off half of the stem above the seed, as shown in Fig. 5. Thus the plant may be placed over the edge of the tray of water, with the root and seed outside but a part of the stem in the water. Since the roots are not in water they certainly can give no root pressure.

The data from such an experiment are shown in Table 11. This experiment was done with corn (Zea mays) seedlings, which form swellings in the mesocotyl, as will be described later. The plants were placed in the position shown in Fig. 5., half of them "derooted", and treated with auxin or ethylene, within half an hour. The ethylene concentration was 0.2%; the auxin was applied as lanoline paste (0.2%) to the top of the coleoptile. There was no significant difference between the size of the swellings in the normal and derooted plants.

Necessity of Roots for Ethylene-Induced Swellings: The next step was to show that ethylene-induced swellings, like auxin-induced swellings, could not form without a substance produced in

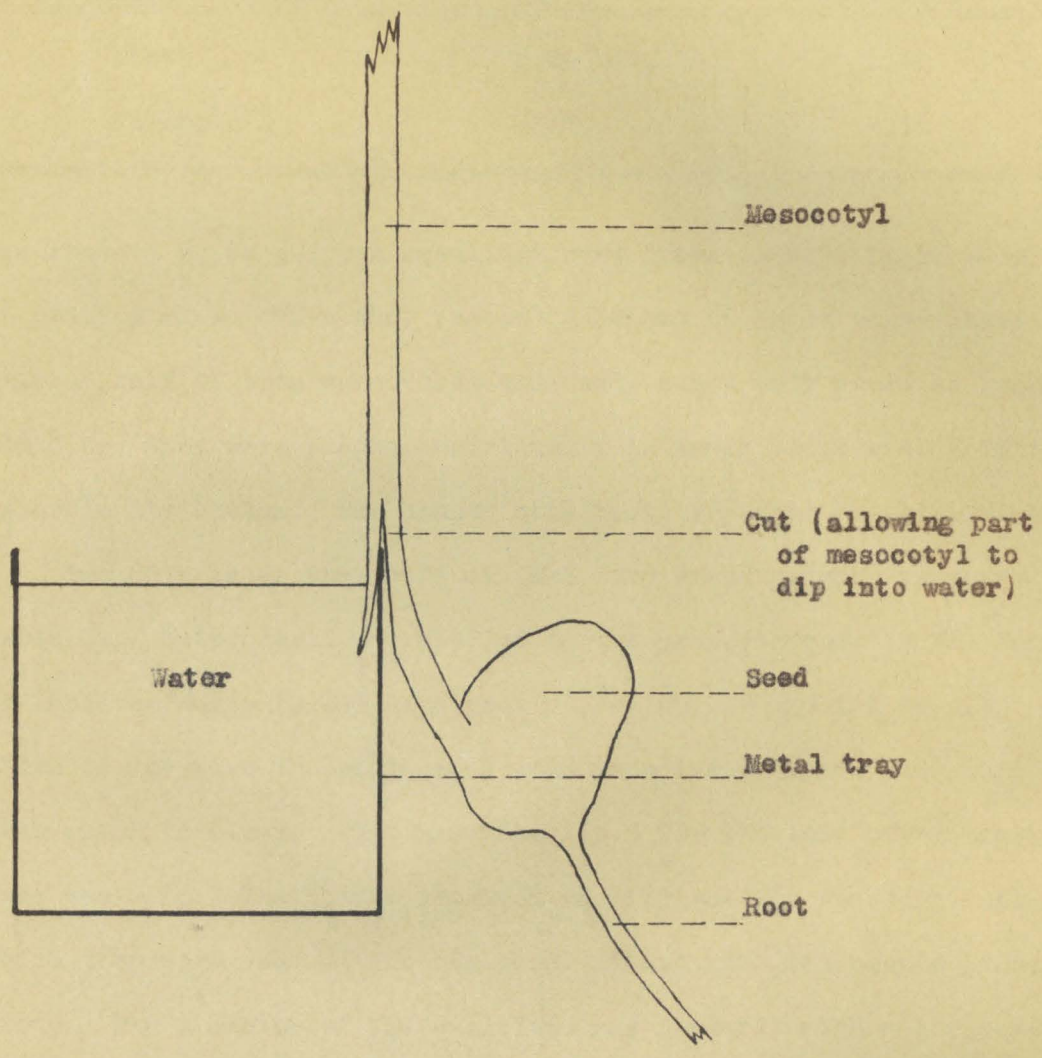

Fig. 5. Method of giving water to corn seedling while keeping the roots in moist air.

TABLE 11.

Effect of Root Pressure on Swelling Formation

Treatment of Plants	Average Diameter of Swelling in mm.	
	Auxin	Ethylene
Roots	3.52	3.80
No roots	3.47	4.15

the roots. To do so, pea seedlings were grown on wet filter paper in petri dishes. When they reached a height of about three centimeters, half of them were "derooted" and, since they would no longer stand up, they were placed individually in small vials with a little water in the bottom. The plants with roots remained in petri dishes.

Two days later they were divided into eight groups, as shown in Table 12. Heteroauxin was applied to the proper groups in the form of lanoline paste (0.02% heteroauxin), which was applied on all sides of the five mm. section of stem immediately below the sharp bend which is always found near the tip. The ethylene concentration used was 0.1%. The plants remained in ethylene for two days, after which they were removed and measured. There were ten plants in each group. The diameter of the swellings was measured with a low power microscope equipped with ocular micrometer. Also a three centimeter section, including the swelling, was cut from each plant, and each group of ten sections was weighed. This data is tabulated in Table 12, and represented in graphic form in Figs. 6 and 7. (See also Figs. 8-12.)

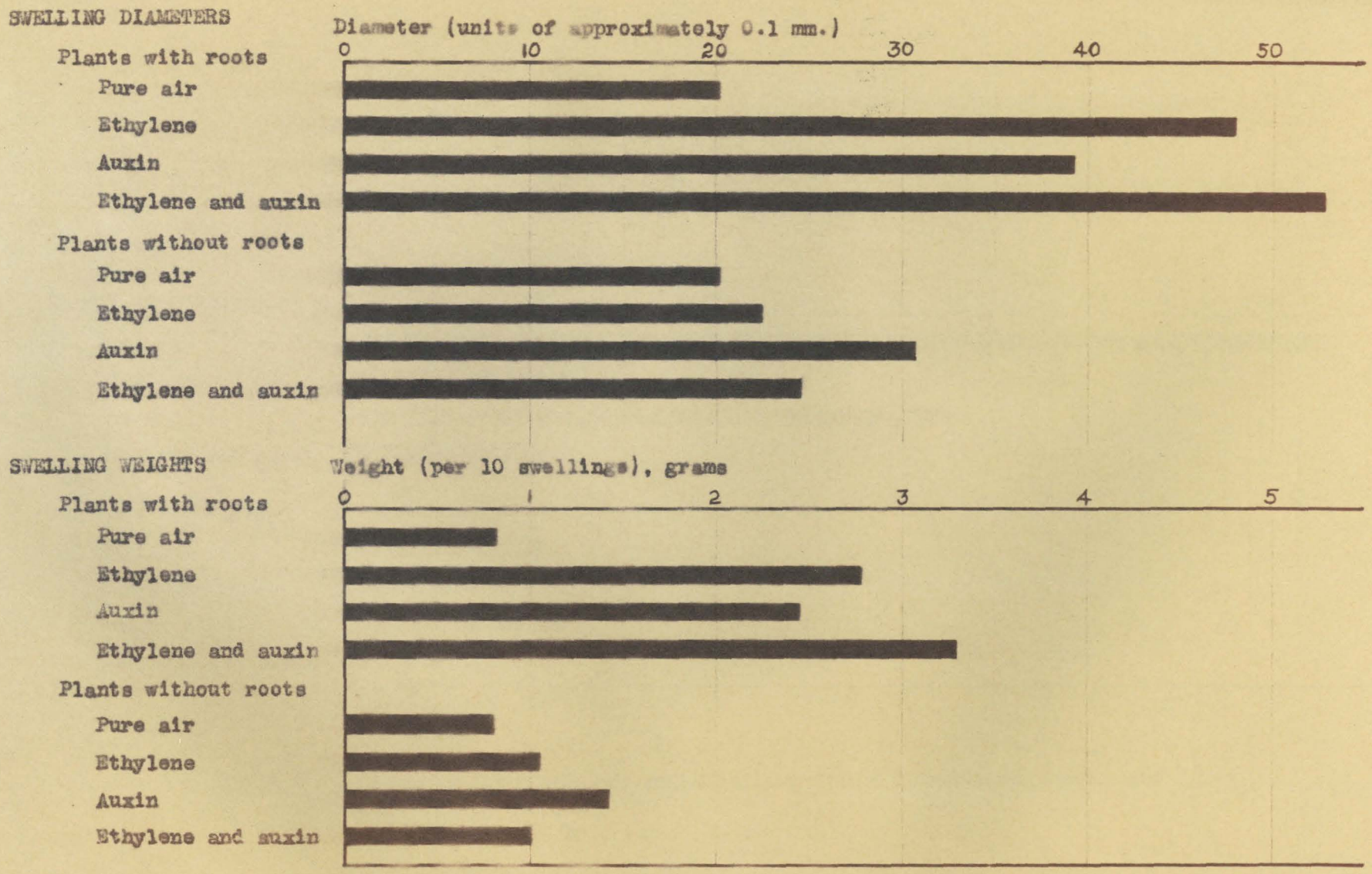

Fig. 6. A. Effects of ethylene and auxin on diameter of the stem, in normal and derooted pea seedlings.
B. Weight of a three three cm. section of stem (including the swelling in the treated plants).

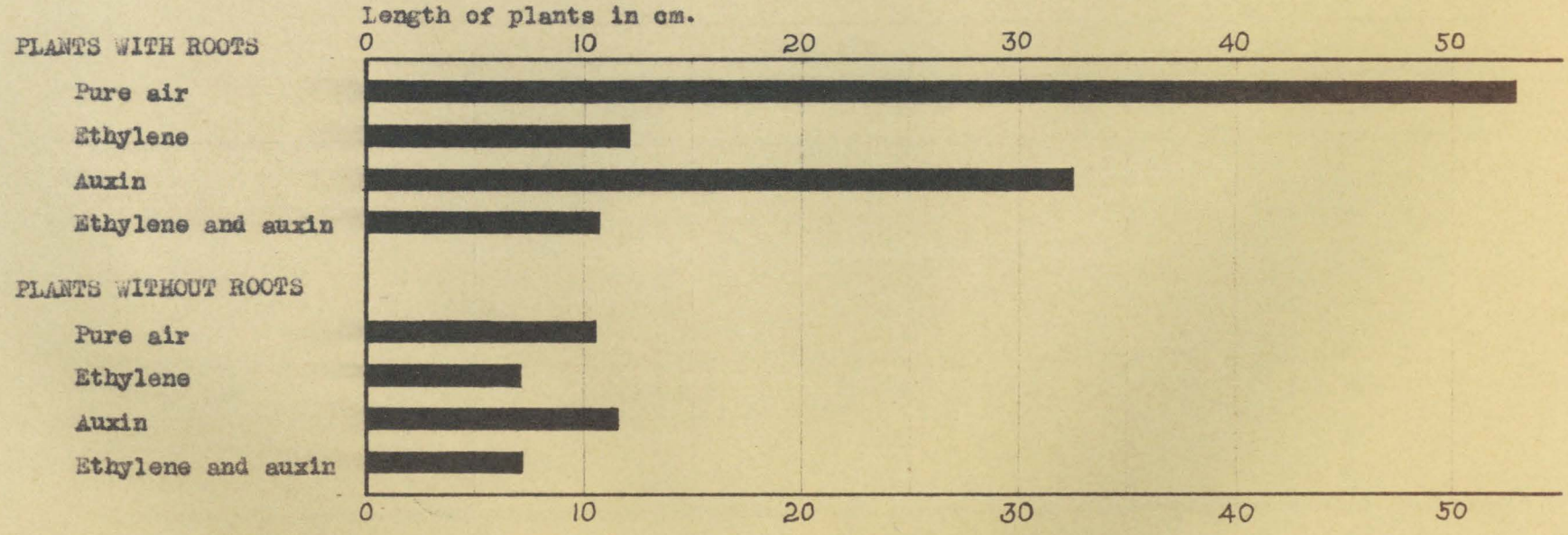

Fig. 7. Effects of ethylene and auxin on longitudinal growth in normal and derooted pea seedlings.

TABLE 12

Pea Stem Swellings Caused by Ethylene and Auxin

		Treatment of plants			
		No auxin		Auxin	
		Pure air	Ethylene	Pure air	Ethylene
Swelling diameter*	With roots	20.1 ± 0.3	48.0 ± 1.1	39.2 ± 1.4	52.9 ± 0.9
	No roots	20.1 ± 0.3	22.4 ± 0.7	30.8 ± 1.0	24.6 ± 1.0
Swelling weight (gm./10 plants)	With roots	.82	2.78	2.46	3.30
	No roots	.81	1.06	1.42	1.00
Length of stem (cm.)	With roots	52.9 ± 0.3	12.0 ± 0.4	32.4 ± 2.7	10.7 ± 0.3
	No roots	10.6 ± 0.6	7.0 ± 0.2	11.5 ± 0.8	7.1 ± 0.1
Number of lateral buds growing/10 pl.	With roots	0	2	0	2
	No roots	13	0	6	0

* Diameter in units of about 0.1 mm.

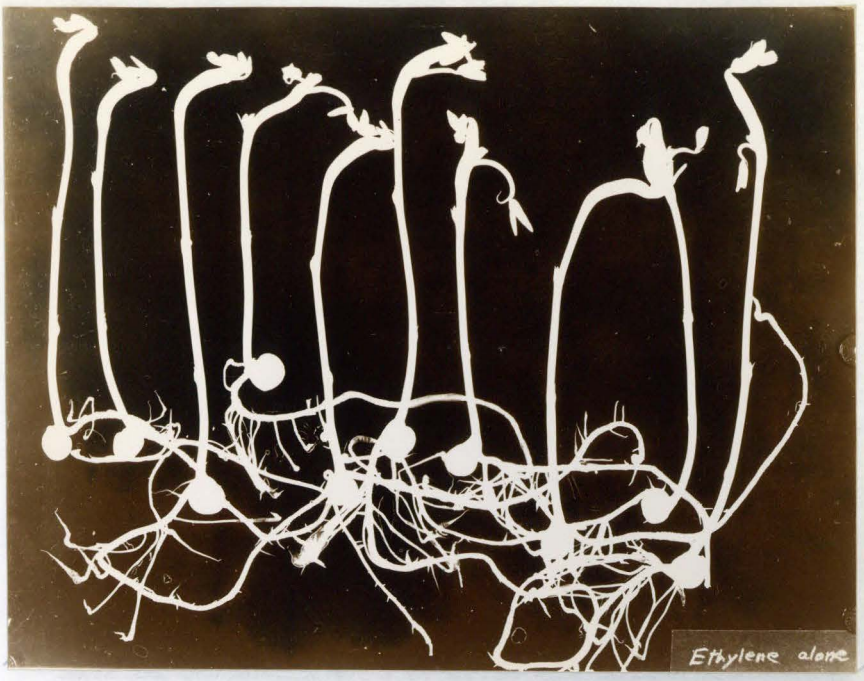

Fig. 8. Swellings due to action of ethylene on pea seedlings with roots. Note effect of ethylene on geotropism of roots in lower left hand corner.

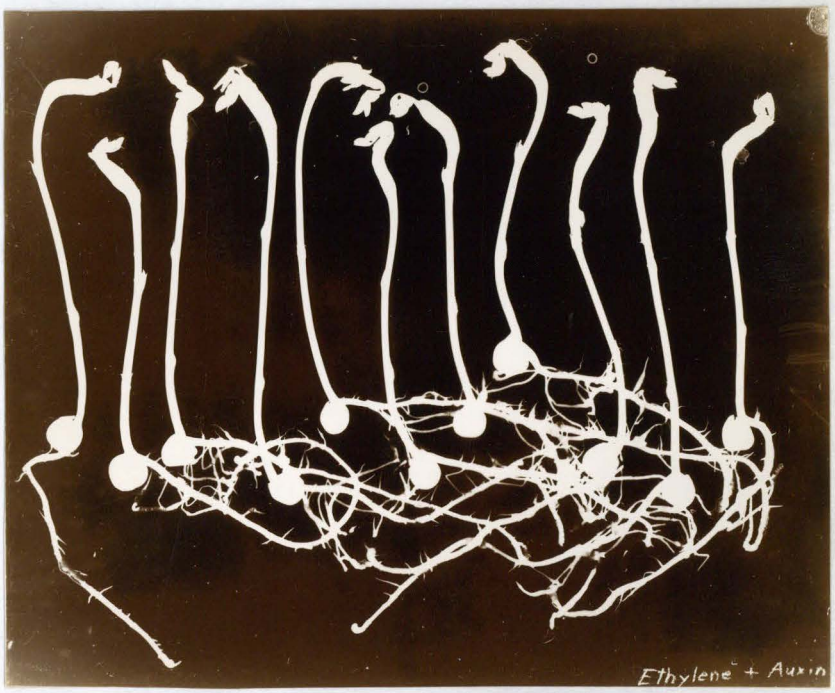

Fig. 9. Pea swellings caused by action of ethylene and auxin together.

Fig. 10. Derooted pea plants, not treated with ethylene or auxin. Note growth of lateral buds. The small roots have grown in the four days since the beginning of the experiment.

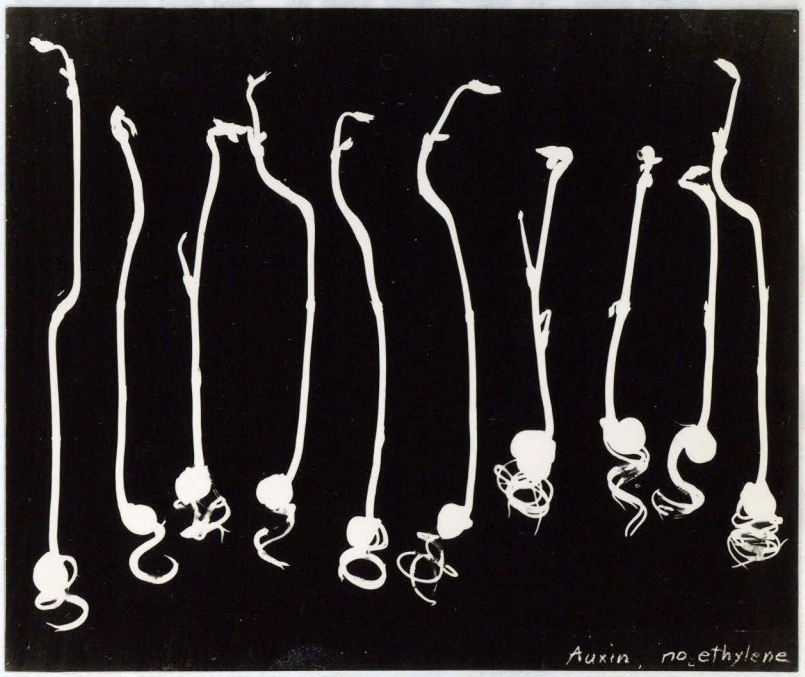

Fig. 11. Derooted pea plants treated with auxin in high concentration. Only slight swellings are formed.

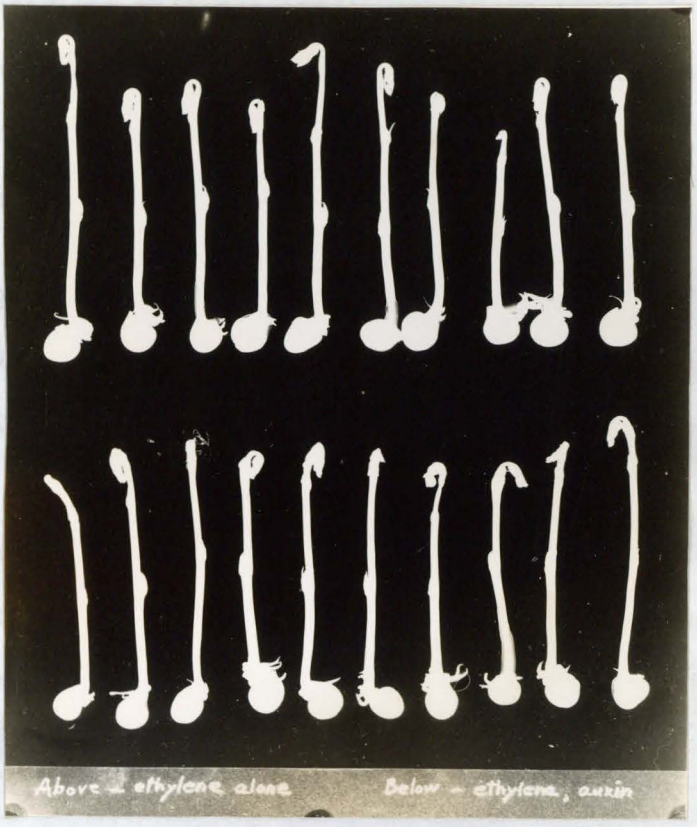

Fig. 12. Above. Derooted pea seedlings treated with ethylene.

Below. Derooted pea seedlings treated with ethylene and auxin.

Examination of Fig. 6 will leave no doubt that removing the roots greatly reduces the size of the swellings. It may therefore be concluded that ethylene will not induce swellings except in the presence of both auxin[1] and a "swelling substance" produced by the roots. This substance almost completely disappears from the plant within two days after the roots are removed.

Effect of Roots on Growth in Length: Comparison of the length of normal and derooted plants (the ones not treated with ethylene or auxin) will show that longitudinal growth is greatly decreased by removing the roots. (Fig. 7.)

From this it may be concluded that there is a substance coming from the roots which is necessary, with auxin, for the growth of stems. The existence of such a substance has already been shown by Went (1937, ~~unpublished~~). Data will be offered later which indicates that this is not the "swelling substance", but a second substance from the roots.

It may be seen in Fig. 7. that not only the derooted plants, but the plants with roots which are treated with ethylene or auxin, show a decreased growth in length. It might be thought that this "second root substance" is involved in this growth reduction also. It will later be shown, however, that such is not true in corn.

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1. Auxin is included, since it has been shown that ethylene alone will not cause swelling formation in decapitated plants.

There is no reason for thinking it is true here, for it has already been shown that swelling is accompanied by reduction in growth in length. The important fact is that removal of the roots causes great reduction in longitudinal growth ~~in~~ and the absence of swellings.

It is not surprising that ethylene reduces elongation more than does auxin. In the first place, it destroys auxin. Thus, to the effect of the swelling on elongation is added the effect of reducing the amount of auxin. In the second place, ethylene acts on the whole tip of the plant (as well as other parts), while the auxin is applied several mm. below the tip. It is quite possible, therefore, that in the ethylene plants the whole tip swells, while in the auxin-treated plants there is a short region remaining which elongates above the swelling. Whether this is true or not, it is in agreement with the appearance of the plants; for the ethylene plants were terminated by the swelling while in the auxin-treated plants the swelling was in the same position relative to the seed but was surmounted by a long section of normal stem. (This phenomenon may be seen, but less clearly, in the derooted plants, Figs. 10 and 11.)

One further point in connection with these data must be mentioned. In the derooted plants treatment with auxin has no effect on growth in length. This is undoubtedly because second root substance, not auxin, is the limiting factor in growth.

Swellings in Corn Seedlings

Description of Swellings: Seedlings of corn (Zea mays) have an intercalary meristem near the top of the mesocotyl (first internode). If auxin is applied in high concentration to the coleoptile, swelling of this meristem occurs. These experiments have also shown that ethylene causes similar swellings, which are indistinguishable from the auxin swellings. (See diagram of corn seedling, Fig. 13.)

Experimental Method: These experiments were carried out in nearly the same way as those with pea swellings. The plants which were not derooted were grown in sand in zinc trays. The derooted ones were again placed in small vials. The experiment was begun (i.e., auxin and ethylene treatment begun)when the plants were about four days old (3 or 4 cm. above the sand). Plants were derooted when they were very small (two days old), in order to give two days before the addition of auxin or ethylene. It was found advisable to use plants as small as possible, for they lost their ability to form swellings as they grew older, - probably of the disappearance of the intercalary meristem in the mesocotyl. Auxin was again applied (as lanoline paste containing 0.2% auxin) to the tip of the coleoptile. Ethylene was used in a concentration of 0.1%. Measurements were made at the end of a two day period.

The data for these experiments are presented in Table 13, as well as in the graphs in Figs. 14 and 15. These data show several

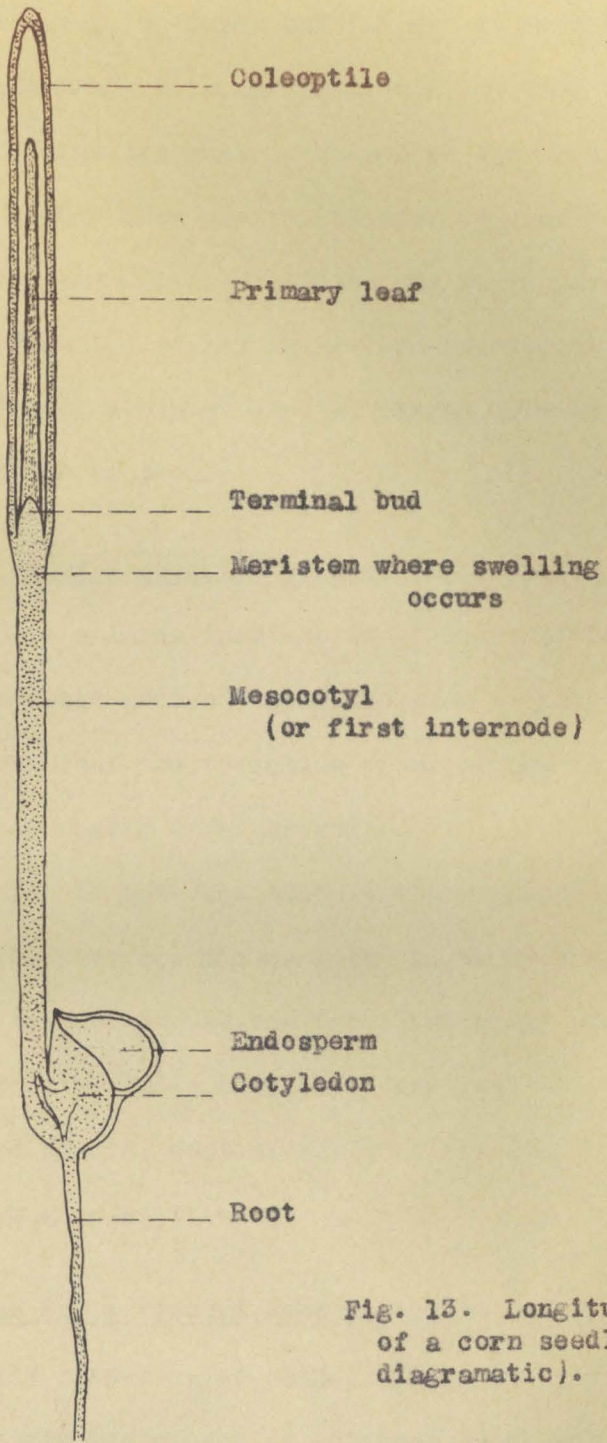

Fig. 13. Longitudinal section of a corn seedling (semi-diagramatic).

points of interest, as described below. It must be kept in mind that the experiments with normal plants and with de-rooted plants were not done simultaneously. Therefore they are not directly comparable, though they are placed side by side so that it will be easier to see what effects ethylene and auxin have on the two groups. In Fig. 15, it was found more practicable to plot the variation of each group of treated plants from the controls, as explained in the caption.

Formation of Mesocotyl Swellings: In normal plants, ethylene and auxin cause a large increase in diameter of the mesocotyl. When the roots are removed, this increase is only slight. Thus it is again shown that the formation of swellings is dependent upon some substance coming from the roots.

It is also of interest that swellings caused by auxin, or by auxin and ethylene, are not as large as those caused by ethylene alone. Can this be due to the fact that auxin greatly increases coleoptile growth, while ethylene decreases it? Perhaps, in the auxin-treated plants, most of the swelling substance goes into the rapidly growing coleoptile.

Evidence for a "Second Root Substance": Let us now consider the coleoptile diameter, as shown in Fig. 14. In the plants with roots, the auxin has caused an increase in coleoptile diameter. This increase is not large, but it is nevertheless significant, and

TABLE 13.

Swellings on Normal and Derooted Corn Seedlings

	Treatment	Mesocotyl Diameter#	Coleoptile Diameter#	Mesocotyl Length mm.	Coleoptile Length mm.	First leaf length mm.
Exper. I Plants with roots.	no auxin, air	28.7±0.7	29.0±0.8	32.2	26.4	53.1*
	no auxin, eth.	38.2±0.4	29.3±0.4	16.8	17.1	30.3*
	auxin, air	33.3±9.8	33.8±0.4	31.9	41.5	33.3*
	auxin, eth.	34.3±1.0	34.7±1.0	31.1	38.2	26.5*
Exper. II Plants with roots.	no auxin, air	26.6±0.5	32.0±0.6	19.5	26.7	76.5
	no auxin, eth.	40.0±0.5	30.9±0.3	11.2	18.2	28.6
	auxin, air	32.7±0.4	35.9±0.6	22.9	36.7	32.3
	auxin, eth.	32.5±0.4	34.7±0.6	14.7	35.6	27.5
Exper. III Plants without roots.	no auxin, air	30.2±0.2	26.8±0.3	44.9*	35.1*	42.8*
	no auxin, eth.	32.9±0.9	26.6±0.4	42.5*	32.1*	30.7*
	auxin, air	32.6±0.8	28.5±0.8	45.4*	40.3*	38.7*
	auxin, eth.	33.1±1.0	27.5±0.5	43.1*	38.2*	26.1*

Diameters in 0.1 mm. units.

* Total length, not growth during experiment.

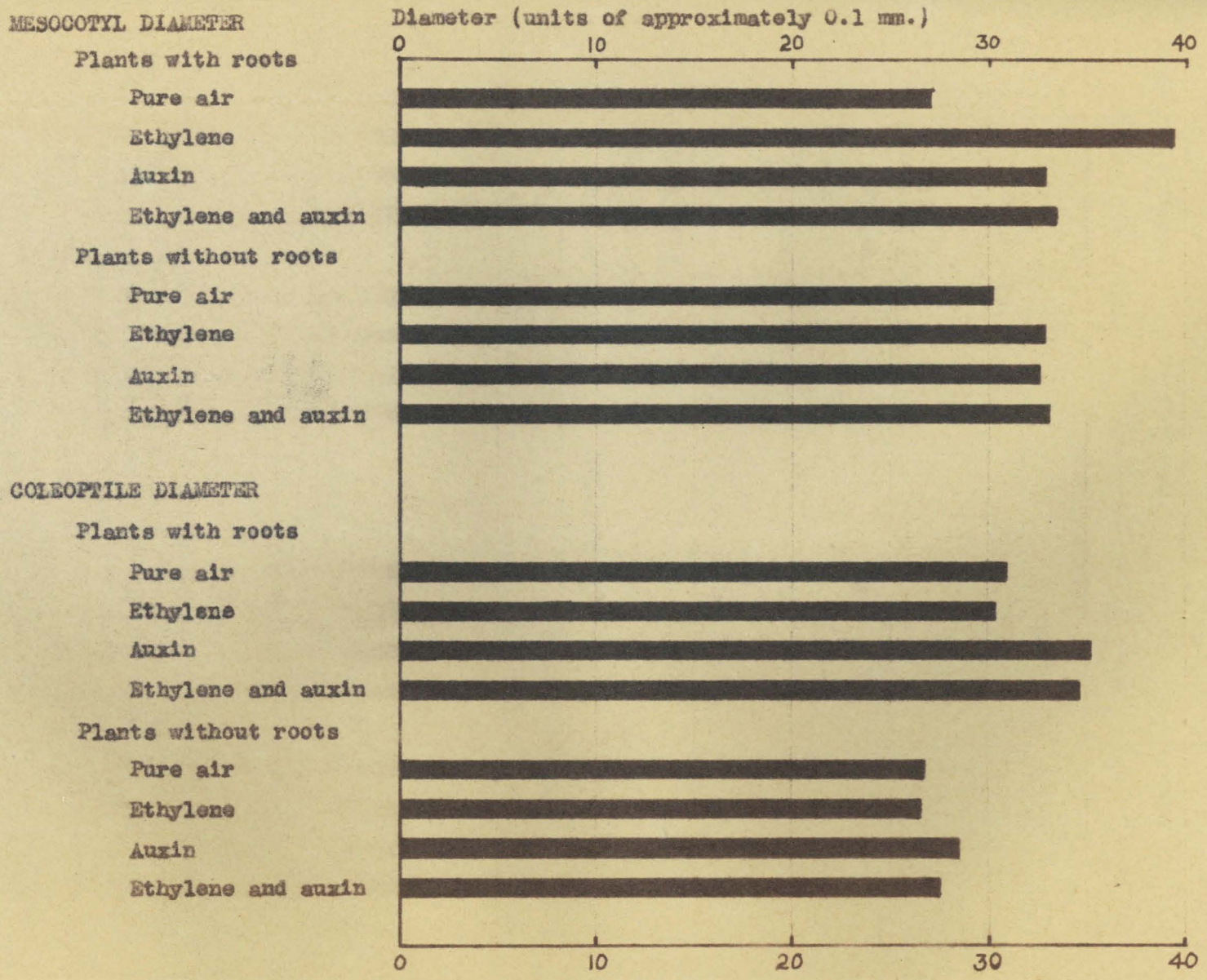

Fig. 14. Effect of ethylene and auxin on mesocotyl and coleoptile diameter in corn seedlings.

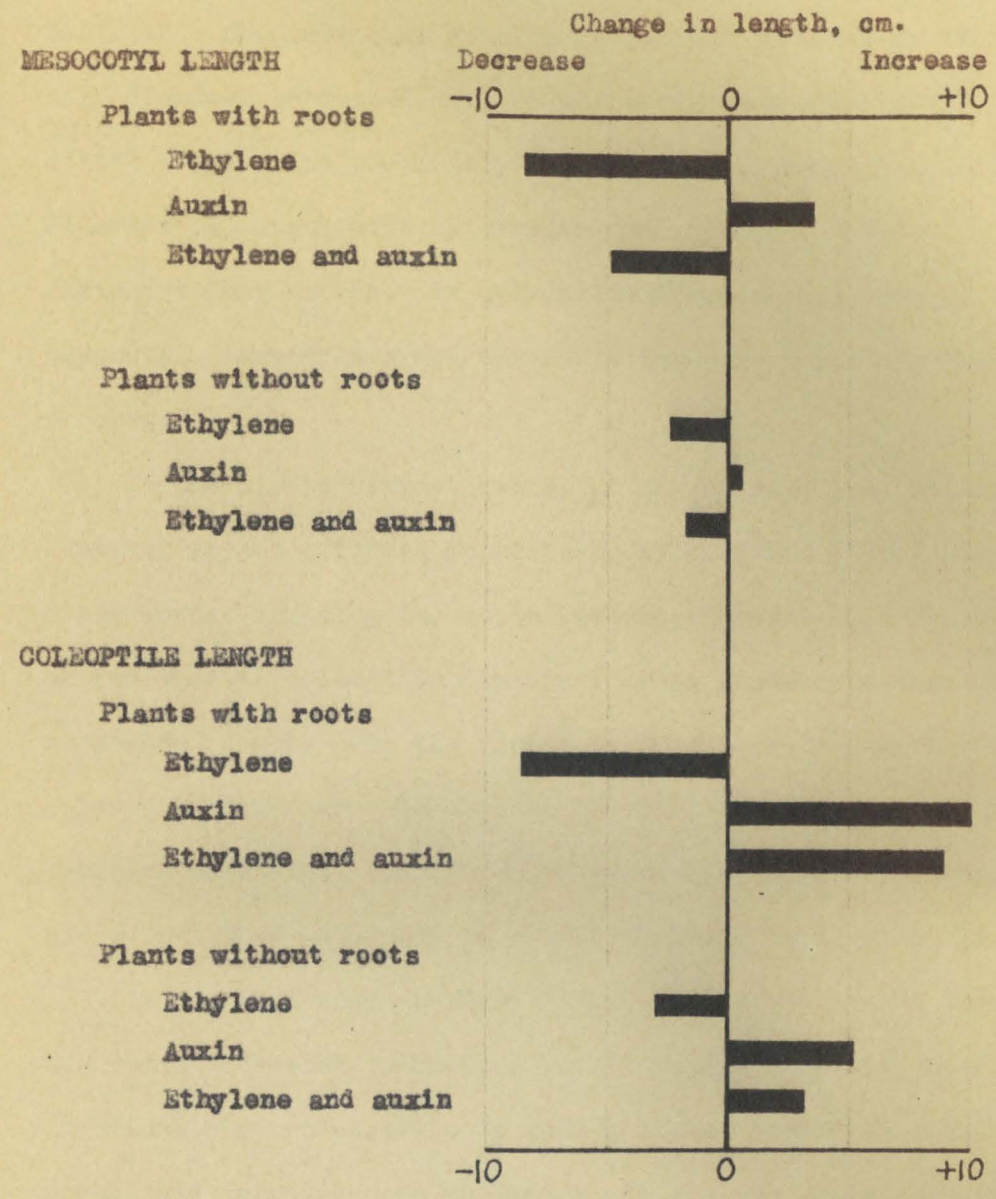

Fig. 15. Effect of ethylene and auxin on mesocotyl and coleoptile growth in length in corn seedlings. Each bar represents the increase or decrease in growth (over that of the controls) which is caused by the designated treatment.

it was found in each of two independent experiments (as shown in Table 13. The important point here is that this effect on coleoptile diameter is caused only by auxin, ethylene alone having no effect - while the previously described increase in mesocotyl diameter is caused both by ethylene and by auxin. This, then, indicates that increase in coleoptile diameter and increase in mesocotyl diameter are not caused in the same way, but are different processes.

In the plants without roots, it may be seen that coleoptile diameter is not affected appreciably by addition of auxin. In other words, removing the roots removes whatever is necessary for an increase in coleoptile diameter. This probably means that a substance, coming from the roots, is necessary for increase in coleoptile diameter. This must, however, be different from the swelling substance; since the latter is made active by ethylene alone, and this substance causing coleoptile enlargement is not.

It has been shown by Went (1937, ~~unpublished~~) that removal of the roots decreases coleoptile growth in Avena seedlings. It is unfortunately not possible to make a direct comparison here of normal and derooted corn seedlings, as the two experiments were not done simultaneously. It may be seen in Fig. 15, however, that in the normal plants, coleoptile growth in length is greatly increased by auxin, even in the presence of ethylene. This is as would be expected. (The decrease caused by ethylene alone is presumably

due to increased auxin destruction.) If the data for the derooted plants are now examined, it will be seen that auxin causes a much smaller increase in coleoptile growth than it does in the normal plants.[1] Apparently, then, a substance from the roots is necessary also for longitudinal growth of the coleoptile. It is not possible to say what effect ethylene alone has on this substance, for ethylene decreases coleoptile growth merely by its destructive action on auxin.

Let us now consider the growth in length of the mesocotyl (Fig. 15). The data presented are in agreement with all assumptions made here, as well as with known facts, In normal plants, ethylene alone decreases mesocotyl growth. This may be due to destruction of auxin. It is also probable that swelling itself causes a decrease in longitudinal growth, as it does in peas. When auxin is added in large amounts to the coleoptile tip, it is transported downward, so that it causes increased growth of the mesocotyl as well as the coleoptile. Ethylene and auxin together cause a decrease of growth rate in the mesocotyl, although they cause an increase in the coleoptile. Such an effect is to be expected if the ethylene is causing increased destruction of auxin. In spite of the

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1. It will be noted that ethylene, alone, also causes a smaller decrease in the derooted than in the normal plants. This is only because removing the roots causes a smaller growth rate of the controls, so that less decrease is possible.

destruction, enough auxin is left in the coleoptile to cause growth, but a large part of it has been destroyed by the time it reaches the mesocotyl.

In the derooted plants, auxin does not cause as great an increase in longitudinal growth of the mesocotyls as it does in the normal plants. This was found to be true for coleoptiles also, and the reason is presumably the same in both cases. That is, a substance from the roots is necessary for both coleoptile and mesocotyl growth in length.

Growth of the primary leaf was also measured. It was very greatly decreased both by ethylene and by auxin. In the case of ethylene, the decrease may be due to auxin destruction. Auxin may cause a decrease in primary leaf growth merely by promoting growth of the coleoptile. This has been shown by Went[1935b] to be true in Avena seedlings, where rapid coleoptile growth uses large amounts of food factor, which would otherwise be available for the primary leaf.

Summary of Experiments with Corn:

1. Five different growth processes have been considered, as follows: Mesocotyl growth in diameter, coleoptile growth in diameter, coleoptile growth in length, mesocotyl growth in length, and first leaf growth in length.

2. Removal of the roots causes more or less inhibition of all of these processes. Hence it appears that they depend on substances coming from the roots.

3. Mesocotyl growth in diameter (swelling) is caused by a substance which is in some way activated both by ethylene and by auxin.

4. Coleoptile growth in diameter is caused by a substance which is activated by auxin but not by ethylene. Hence it must be different from the swelling substance.

5. Coleoptile, mesocotyl, and primary leaf growth in length also require something from the roots, but it is impossible to tell from these experiments whether they each require the same substances or different substances, or whether these substances are like either of the two substances described in paragraphs 3 and 4.

Effects of Ethylene upon Bud Growth in Peas

Bud Inhibition: As has been shown by Skoog and Thimann (1934), if the terminal bud of a pea seedling is removed, the highest lateral bud grows out. If, however, heteroauxin is applied to the terminal stump, it inhibits the outgrowth of lateral buds. It is of interest now to consider the effect of ethylene on growth of lateral buds.

To observe this, pea seedlings six centimeters high (with two lateral buds) were divided into four groups and treated as indicated in Table 14. Ethylene concentration was 0.1%, and ethylene treatment lasted for six days. The plants were decapitated.

TABLE 14.

Effect of Ethylene on Bud Growth in Peas

Group	Treatment of Plants	Swellings	Bud Growth
1	No auxin, no ethylene	-	Top bud grew
2	No auxin, ethylene	+	Nearly all buds grew, but abnormally
3	Auxin, no ethylene	+ + +	None
4	Auxin, ethylene	+ + + +	None

It may be seen (group 4) that auxin inhibits bud growth in ethylene as well as in pure air. It is group 2 that is most interesting, however.

The description of these plants at the time of the experiment follows. "When removed from ethylene, buds were distinctly injured. In a few days, both buds grew from most of the plants, but did not grow normally. Most of them grew very slowly, or soon stopped." Only one bud in the whole group grew normally.

Here, again, ethylene and auxin act in different ways. Ethylene evidently caused a failure of the bud-inhibiting mechanism, since both buds grew instead of the top one only. It is possible that the ethylene increased auxin destruction to such an extent that not enough auxin was left to inhibit bud growth.

A similar case was found in one of the experiments on pea swellings (Table 12). Here the plants were not decapitated.

Ethylene caused growth of a few buds, but derooting the plants (without ethylene treatment) caused many more. This seems to indicate that some substance produced in the roots also has a connection with bud inhibition. (See Fig. 10.)

From the data at hand, ~~however,~~ it is impossible to say what controls bud inhibition. The data do indicate, however, that the initiation of the growth of a lateral bud does not depend on the same factors as those responsible for its continued growth after initiation. This follows from the work of Went, (1937, ~~unpublished~~), who has shown that a substance from the roots (probably the previously mentioned "second root substance") is responsible for the continued growth of buds. In this experiment, however, bud growth has been initiated under conditions such that the "second root substance" was lacking.

Conclusions from Part V: Effects of Roots on Growth

It has been shown that ethylene-induced swellings on stems of corn and pea seedlings are like the swellings induced in those plants by auxin in high concentrations. As ethylene increases destruction of auxin, it produces a decrease, not an increase, in the auxin concentration in the plant. There is thus no possibility that ethylene induces the swellings through its action on auxin.

It was previously known that removal of the roots made it impossible for the plant to form swellings in response to auxin.

The same was found to be true for ethylene. This is strong evidence favoring the theory that a substance (called swelling substance) is necessary for swelling formation, and that this substance is formed in the roots. This substance, then, is activated, or made to cause swellings, by ethylene and by auxin. The ethylene cannot act completely independently of auxin, for decapitated (and therefore auxin deficient) pea seedlings will not form swellings in ethylene unless auxin is added. Ethylene will, however, cause swellings in intact and otherwise untreated plants (which do not have the abnormally large amount of auxin necessary for formation of swellings in the absence of ethylene).

The way in which auxin and ethylene activate the swelling substance is unknown. They may affect its transport to the point where the swelling occurs, or they may merely cause it to become active, once it has reached that point. In any case, they are not, alone, responsible for its transport out of the roots; for it comes out of the roots under normal growth conditions which do not promote swelling formation. This is shown by the fact that if the roots are removed and the plant treated with auxin or ethylene immediately, there is still enough swelling substance in the plant to cause swelling formation, but if the plant is not treated until two days after removal of the roots, the swelling substance has disappeared and no swellings will form.

These experiments, therefore, constitute one case in which auxin (in high concentration) and ethylene act in the same way. That is, they both act on the swelling substance, causing it to form swellings. Whether this is a true similarity between ethylene and auxin, or whether it is merely a superficial resemblance, remains to be seen. It is possible that both substances act directly on the swelling substance; but it is also possible that each one sets up an entirely different chain of reactions, both of which happen to affect the swelling substance.

It has also been shown that a substance comes from roots which causes increase in the diameter of the corn coleoptile. This substance is activated by auxin but not by ethylene; hence it must be different from the swelling substance, which is activated by both auxin and ethylene.

Went has shown also that a substance formed in the roots is necessary for elongation of Avena coleoptiles and buds and stems of peas. These experiments have confirmed this (with regard to pea stems), and they indicate that mesocotyl and coleoptile growth in corn is also dependent upon a substance from the roots. The action of ethylene on these substances is unknown; and the study of this point is complicated by the fact that any variations in longitudinal growth resulting from action of ethylene on these substances are superimposed on growth-rate variations resulting from the destruction

of auxin by ethylene. Whether these elongation-promoting substances are all identical or whether they are different, is, of course, unknown.

It has, therefore, been shown that (in corn, at least) two separate substances promoting growth are produced by the roots. Also a substance (or substances) is produced by the roots and affects elongation. This may be identical to one or the other of the two substances ~~mentioned~~ found in corn.

From the small amount of data available, it appears that loss of roots favors, rather than inhibits, the growth of lateral buds. The buds are unable to elongate, however; as they remain only a few mm. long.

The results of these experiments are not considered as proof of the existence of any of these substances which are said to be formed in the roots; for the substances have not been removed, in active form, from the plant tissue. Nevertheless, the phenomena described are easily explained in this way, and no other simple explanation can be made at the present time.

PART VI.

EFFECTS OF ETHYLENE ON AVENA SATIVA

Superficial Effects of Ethylene upon Avena: Avena plants react differently to ethylene than do peas and corn. As has already been pointed out, their responses to gravity are not affected by ethylene. Also ethylene does not cause enlargement or swelling of the mesocotyl as it does in corn. This may be due to lack of swelling substance. More probably, it is because the seedlings are grown under such conditions that the mesocotyl is extremely small, stops growing at an early age, and probably contains no meristem when the plants are treated with ethylene.

Ethylene does decrease longitudinal growth in Avena seedlings, as it does in peas, corn, and many other plants. This inhibiting effect of ethylene is shown in the following experiment, previously reported by the author (Michener, 1935). Four groups of Avena seedlings were placed for twenty-four hours in, respectively, air, 0.001% ethylene, 02% ethylene, and 2% ethylene. The growth in each of the three groups treated with ethylene was about the same, and was about 30% less than the growth of the controls kept in air. It is also of interest to note here that the ethylene seems to have the same effect in each group, regardless of the 2000-fold range in concentration.

If this reduction in growth is due to action of ethylene on

auxin, the ethylene must act on one or more of the following processes:[1]

1. Auxin production.
2. Auxin transport.
3. Destruction of auxin.
4. Sensitivity of the plant to auxin.

Effect of Ethylene on Production of Auxin: The effect of ethylene on production of auxin was determined by the same method as used for peas. Plants were placed in 0.01% ethylene(with controls in air) for several hours. Then about 1.5 mm. was removed from the tip of each plant. The tips were placed on wet filter paper for forty minutes to two hours (the length of time seemed to make no difference), for the purpose of removing oxidative enzymes liberated at the cut surface, which otherwise might destroy part of the auxin coming out of the tip. After this, the tips were placed on agar blocks (24 tips to each block) and allowed to remain there for two hours. During this time the ethylene-treated tips were in most cases placed again in ethylene. After the removal of the tips, the blocks were tested by the usual test. The results of these experiments are shown in Table 15.

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1. All except number 3 have been suggested by van der Laan.

TABLE 15.

Effect of Ethylene on Auxin Production in Avena

Exp. No.	Time in Ethylene Hrs. As Intact Plants	Time in Ethylene Hrs. While tips on Agar Blocks	Auxin Produced (Curvature in degrees) Ethylene	Auxin Produced (Curvature in degrees) Pure Air
1	$3\frac{1}{2}$	0	4.8 ± 0.6	4.9 ± 0.8
2	13	2	5.4 ± 0.4	5.1 ± 0.5
3	4	0	7.1 ± 0.5	6.2 ± 0.3
4	14	2	4.2 ± 0.3	5.6 ± 0.4
5	16	2	4.5 ± 0.2	4.7 ± 0.3
6	16	2	4.0 ± 0.4	4.4 ± 0.6
	Total		30.0 ± 1.0	30.9 ± 1.3

It appears that there is no difference between auxin production in ethylene-treated and in normal plants. It was somewhat surprising to find these experiments thus in complete disagreement with similar experiments of van der Laan, and no explanation for the discrepancy can be given at present.

Auxin Transport and Auxin Destruction: Van der Laan (1934) has done a number of experiments on auxin transport in Avena seedlings. His data are quite extensive, and show no effect of ethylene on transport. From his data it also appears that ethylene had no effect on destruction.

No extensive check on these experiments has been carried out, although the effect of ethylene on transport was tested in one

experiment. The method used here was the same as that used by van der Laan; i.e., sections were placed in normal and inverse positions, on blocks of pure agar. On top of the sections were placed blocks containing a known amount of auxin. After two hours both top and bottom blocks were removed and tested. The plants used were treated with ethylene in a concentration of 0.01%, for $2\frac{1}{2}$ hours before being cut into sections.

In this case the bottom blocks did not receive enough auxin to be tested, so only the final concentrations in the top blocks are shown in Table 16. The ethylene did not affect significantly the amount of auxin withdrawn from the top blocks; therefore it did not affect transport or destruction.

TABLE 16.

Transport of Auxin in Avena

Treatment of Plants	No. of Plants Used	Auxin remaining in top blocks (Curvature in degrees)	
		Sections in Normal position	Sections in Inverse position
Pure Air	24	7.7	16.4
Ethylene	24	8.3	19.0

A few experiments were also done on destruction of auxin by sections of Avena coleoptile, using the same method as used for pea sections. The data from these experiments are shown in Table 17. The sections were placed on agar blocks (in normal position, not

inverse) and allowed to remain there for two or two and a half hours.

TABLE 17.

Destruction of Auxin by Avena Sections *

Exp. No.	Conc. of Auxin in Blocks (Curvature in degrees) Original Concentration	Final Concentration Ethylene Plants	Controls	No. of Sections on Block	Part of Plant Used	Time in Ethylene Hrs. before Exper.	Ethylene Concentration
1	9.5	12.9 9.7 9.5 10.8	9.0 8.0 9.0 8.0	12	tip base tip base	5-1/4	0.01%
2	24.8	12.1 13.9	16.1 11.6	20	tip base	4-1/2	0.1%
3	8.1	10.3	9.2	24	middle	5-1/4	0.1%
Sum		79.2	70.9				

* (12 plants used for each determination.)

It is clear that in two of these experiments (1 and 3), the sections used contained some auxin, so that the amount of auxin in the blocks was increased instead of decreased. Nevertheless, if ethylene had caused a large increase in rate of destruction, it would have shown in the experiment.

Sensitivity of Avena Seedlings to Ethylene:

(a) Effect on Standard Avena Test: This test was carried out as usual, except that the plants were placed in a large sealed jar

TABLE 18

Effect of Ethylene on the Sensitivity of the Avena Test

Experiment	Ethylene treatment*									Curvature of plants in degrees		Number of rows of plants#	Ethylene concentration
	During growth of plants								During reaction	Ethylene plants	Controls		
	Time in hours before second decapitation												
	7	6	5	4	3	2	1	0					
1										8.5 7.9 8.4	8.4	5	0.01%
2										7.9 7.8	8.7	6	0.01%
3										6.6 6.7	6.2	6	0.01%
4										5.9 5.3	6.8	6	0.2%
5										11.2 9.1 11.7	6.5	5	0.01%
6										9.6 10.6	5.9	6	0.01%
7										10.0	3.6	5	0.01%
8										12.2 10.3 13.6	7.4	9	0.01%
9	← 18 hrs.									13.2 8.2	9.0	6	0.2%
10	(Maximum angle)									30.1 24.1 30.2	23.7	5	0.01%

*Presence of a horizontal line indicates that the corresponding plants were in ethylene; its absence, that they were in pure air.

#The total number or rows for the experiment.

TABLE 19

Effect of Ethylene on the Sensitivity of the Avena Test

Summary of Table 18

Ethylene conc.	Ethylene treatment		Auxin conc. mg./cc.	Number rows of plants used	Average Curvature of plants	Increase over controls
	Before reaction (hrs)	During reaction (hrs)				
0.01%	over 2½	1½ or 0	0.001	14	11.1	4.4
	under 2½	1½ or 0	0.001	6	7.6	0.9
	0	1½	0.001	6	7.7	1.0
	0	0	0.001	15	6.7	-
0.01%	over 2½	1½ or 0	0.005	2	30.1	6.4
	0	1½	0.005	1	24.1	0.4
	0	0	0.005	2	23.7	-
0.2%	under 2½	1½	0.001	2	5.9	-0.9
	0	1½	0.001	2	5.3	-1.5
	0	0	0.001	2	6.8	-
0.2%	over 15	0	0.001	4	10.7	1.7
	0	0	0.001	2	9.0	-

containing ethylene in the appropriate concentration. This, of course, meant that the plants grew in a relative humidity of 100% instead of the usual 85%. If treatment was during growth of the plant, this difference was neglected. If it was during the test itself, the controls were put in another closed jar without ethylene.

The data from these experiments are shown in Table 18, where the time of treatment is indicated by the length of the line preceding the figure indicating curvature. These data are also summarized in Table 19.

These experiments indicate clearly that when the plants are treated with 0.01% ethylene for a long period of time before the experiment, their sensitivity to auxin is increased. This is true both for concentrations giving the maximum angle and for lower concentrations. When the plants were treated with ethylene only during the reaction to auxin, or for a short time before that, the ethylene had no appreciable effect.

(b) Effect of Ethylene on the Growth of Coleoptile Sections: The sensitivity of coleoptile sections was tested by the method described by Bonner (1933) in which sections about five mm. long were cut out of the coleoptiles, measured, placed in auxin solution for about fifteen hours, and measured again.

The first test was with sections from plants grown in pure air. After cutting, half of the sections were placed in solutions containing in addition to auxin, 0.01% of saturated ethylene solution, and in an atmosphere containing 0.01% ethylene. The resulting growth is shown in Table 20.

TABLE 20.

Effect of Ethylene on Section Growth *

Auxin Concentration mg. per ~~cc.~~ l.	Air or Ethylene	Growth in 0.1 mm. units	% Decrease Due to Ethylene
0.06	Pure Air	11.8	21%
0.06	Ethylene	9.3	
0.006	Pure Air	8.0	29%
0.006	Ethylene	5.7	

* Original length of sections = 52.7.

At present, no reason can be given for the slight decrease caused by the ethylene in this case. It may be significant, however, that in this test, a much longer time is required for the growth to occur than in the usual Avena curvature test. This means that the ethylene treatment, which lasted during the entire length of the test, was about fifteen hours. In the curvature test, ethylene had no effect when applied only during the time of the bending, but this time was only ninety minutes.

In further experiments the plants were ethylene-treated before being cut into sections, instead of during the test. Here the effect of ethylene was apparently reversed. Sections from plants treated with 0.1% ethylene grew much more than sections from untreated plants. (See Experiment 1, Table 21.) This experiment was repeated (2, Table 21) with the difference that the plants were repeatedly decapitated before being cut into sections, thus creating a deficiency of auxin. There the ethylene caused a much smaller increase in growth over the controls.

These experiments were repeated, using 0.2% ethylene (Table 22). Here the intact and decapitated plants were grown at the same time, so that the two groups are comparable. Also sections were taken not only from the top of the coleoptiles but from the middle and base as well. In this case the growth was small, and was not significantly affected by the ethylene.

Discussion and Summary of Experiments on Avena: When ethylene-treated plants were used in Avena tests, they gave greater curvatures than normal plants. Sections from ethylene-treated plants grew as much as, or more than, sections from normal plants. Van der Laan (1933) placed blocks containing auxin on tops of decapitated coleoptiles, and measured their growth in ethylene and in air. The ones in ethylene grew faster for the first four hours; thereafter they grew somewhat more slowly. Therefore ethylene certainly does not

TABLE 21.

Growth of Sections from Plants Treated with 0.01% Ethylene

Experiment	Auxin Concentration mg./l.	Air or Ethylene	Growth (in 0.1 mm. units)	Increase due to Ethylene	
I	0	air	1.0	380%	Intact plants,
	0	ethylene	3.8	"	treated 6 hours
	0.03	air	5.6	116%	with ethylene.
	0.03	ethylene	12.1	"	
II	0	air	2.4	75%	Decapitated* plants
	0	ethylene	4.2	"	treated 8 hours
	0.03	air	7.9	14%	with ethylene.
	0.03	ethylene	9.0	"	

*Decapitated 7, $4\frac{1}{2}$, and $2\frac{1}{2}$ hours before cutting sections.

TABLE 22.

Growth of Sections from Plants Treated with 0.2% Ethylene

Ethylene treatment - 6 hours before experiment.

Decapitation - 6, $3\frac{3}{4}$, and 2 hours before cutting of sections.

Auxin Concentration mg./~~c.c.~~ l.	Air or ethylene	Intact Plants Growth (0.1 mm. units)	Intact Plants Increase due to the ethylene	Decapitated Plants Growth (0.1 mm. units)	Decapitated Plants Increase due to the ethylene
0	air	1.9	+0.8	1.9	-0.2
0	ethylene	2.7		1.7	
0.03	air	5.9	0	6.2	-1.1
0.03	ethylene	5.9		5.1	

Sections from tip of coleoptile

decrease sensitivity of coleoptiles to auxin. (The increase will be mentioned later.) Furthermore, it seems reasonable to conclude that nothing except auxin is lacking in the ethylene plants, since artificial addition of auxin brings growth at least as rapid as in the controls.

According to other experiments, ethylene does not affect production, transport, or destruction of auxin. This apparently leads to a paradox, for ethylene has been shown to have no effect on the factors which are known to influence auxin concentration in the plant, yet the growth rate of ethylene-treated plants is abnormally slow unless auxin is added.

The reason for these anomalous results is at present unknown. One point which makes the data presented appear doubtful is the disagreement between this work and that of van der Laan on the effect of ethylene on auxin production in Avena coleoptiles. It might be said of this and other experiments, both of this author and of van der Laan, that they were done with different exposures to ethylene, with plants that were not always the same age, etc.; and that therefore they are difficult to compare. Ethylene inhibits growth of intact Avena seedlings, however, under all conditions in which it has been tried.[1]

---

1. Repeated attempts were made to extract auxin from whole ethylene-treated and normal coleoptiles by means of the chloroform method described by Thimann (1934). These were unfortunately unsuccessful.

The fact that ethylene increases sensitivity to auxin favors the belief that there is, for some reason, a lack of auxin in ethylene-treated plants. As already mentioned, not only auxin, but food factor and probably one other substance are necessary for growth. If auxin is lacking, growth will not occur, and other growth factors will accumulate. The plant is then more sensitive to auxin becuase of the accumulation of the other growth factors. (Schneider, 1936, unpublished). If ethylene causes an auxin deficiency, we may have just this situation in the Avena coleoptile. Such a view is supported also by the fact that ethylene had a much smaller effect on the sensitivity of coleoptiles which had been decapitated several times, since they had an accumulation of food factor (caused by lack of auxin) even in the controls.

There is still the possibility that lack of some other growth factor could be limiting growth, if the maximum growth rate in these experiments on sensitivity is less than that of normal intact coleoptiles. In this case, ethylene could cause a deficiency of one of the other growth factors, which would limit growth in the intact coleoptile but not in experimental plants with a slower growth rate. This possibility is ruled out, however, for the experiments of Du Buy (1933) showed that the normal growth rate of coleoptiles three centimeters high is ~~0.035~~ 0.35 cm. per hour. Van der Laan, in his experiments on sensitivity, used a concentration which

gave a growth of 0.125 cm. per hour in plants not treated with ethylene. Also, in one experiment described here, ethylene increased the maximum angle produced by blocks containing a concentration of about 40 units per cc.

These experiments have shown, then, that auxin is capable of increasing the growth-rate of ethylene-treated plants to, and beyond, that of normal plants. They do not, however, show how the ethylene causes a decrease in growth rate.

PART VII.

STIMULATION OF ROOTS BY ETHYLENE

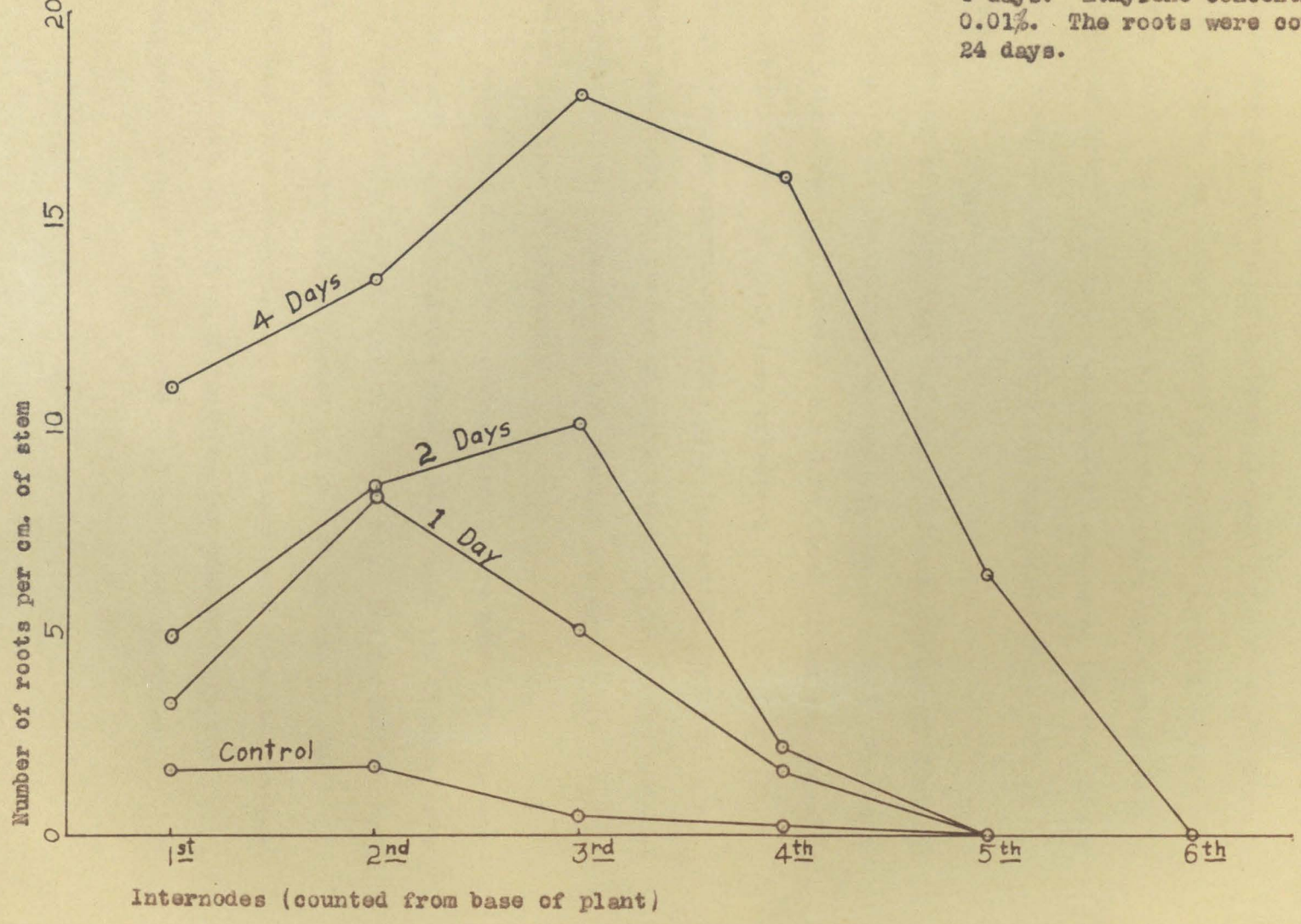
Effects of Ethylene on Root Growth in Marigolds

Preliminary Experiments and Experimental Method: As has been told previously, ethylene stimulates growth of adventitious roots on the stems of many plants. In a few preliminary experiments, ethylene did not stimulate root growth in potted plants of Helianthus, Pisum, or Avena. In Lycopersicon and Tagetes, it was found, in agreement with Zimmerman and Hitchcock (1933) that root production is greatly increased by treatment with ethylene (See Fig. 16.) African marigolds were selected for further experiments along this line, as they form roots which can be easily counted, they have no lateral branches when young, and their foliage was not damaged by ethylene in low concentrations.

In these experiments, the plants were grown in the green house to a height of about twenty centimeters, at which time the fourth and fifth internodes were generally in the stage of rapid elongation. At this time, they were treated ~~in~~ with ethylene in concentrations between 0.02% and 0.5%, for periods of 1, 2, and 4 days. After this the plants were placed under a glass box, which kept the humidity near the saturation point. The roots were then counted from time to time.

Fig. 16. Roots on the stem of an ethylene-treated marigold plant.

Fig. 17. Root growth in marigold plants treated with ethylene for 1, 2, and 4 days. Ethylene concentration was 0.01%. The roots were counted after 24 days.



Description of Experimental Results: Although the results obtained were rather irregular, and the number of plants rather small, it was obvious that the plants treated for four days produced more roots than those treated for shorter periods of time. (See Fig. 17.) The number of roots also appears to increase with the length of treatment, but - apparently because of the individual variations among the plants - there are many cases where this is not true.

Concentration of ethylene had little effect on root production, within the range of concentrations used, (0.02% to 0.5%.) The most effective concentration, however, was 0.2%, both higher and lower concentrations being slightly less effective.

Some of these experiments also give indication that ethylene treatment increases the number of roots appearing on internodes that were not formed (or at least had not reached a stage of rapid growth) at the time of treatment.

In a later experiment much older plants were used. In these plants, in which rapid vegetative growth had ceased and flowers were forming, very little root formation took place. The number of roots varied greatly, but rarely exceeded one or two per cm. of stem, after 56 days from the beginning of the experiment. It will be seen from Fig. 16 that the number of roots in the younger, rapidly growing plants is very much greater - 10 or 20 roots per cm..

Furthermore, in these older plants, ethylene had no observable effect on root formation.

As previously mentioned, when the terminal bud stops rapid growth and forms an inflorescence, there is a period when vegetative growth is very slow. After this, however, several rapidly growing laterals develop. It has been observed that these, when placed in a moist atmosphere, form roots as do the rapidly growing young plants. The most interesting point, however, is that root production may, in these cases, also be found on the main stem, extending downward through one or two internodes from the point of attachment of a lateral.

In another experiment with old plants, auxin paste (about 0.1%) was applied to the stems above the lateral branches. A small number of roots were formed in close proximity to the point of application of the auxin.

Discussion: These experiments were not continued, as these marigold plants were unsatisfactory as experimental material. This was because of their great individual variability, and the difficulty of growing them in large numbers.

The stimulation of adventitious root growth by ethylene is clearly shown in these experiments. The experiments with old plants with active lateral branches also indicate that some substance which travels downward in the stems causes increased root

production. This is evidently not an effect of auxin alone, however, for auxin applied to the main stem above the lateral branches did not cause extensive root formation.

Effect of Ethylene on Root Formation in Pea Cuttings

Experimental Method: The object of the following experiments was to observe the effects of ethylene on root formation in pea cuttings. The cuttings were prepared according to the method of Went (1934), of which a brief description follows. Seedlings of Alaska peas, grown to a height of about 10 cm., were cut below the first leaf (third node) and above the first node, thus giving sections of stem comprising the second and third internodes and the included node with its scale. These were placed successively in 0.05% $KMnO_4$ solution for four hours, and in water for four hours, inverted in auxin solution for fifteen hours (the upper end was split lengthwise for a distance of 1 cm. to facilitate entry of auxin). Finally they were placed, normally again, in 2% sucrose solution. Roots were counted after a week, and again after two weeks. The two weeks' count is the only one considered in the data here presented.

Except for the time during which treatment with ethylene occurred, these plants were kept in a dark room at a relative humidity of about 70% and a temperature of 25° C. For treatment with ethylene, they were placed in a dessicator over moist sodium

nitrate, which is in equilibrium with a relative humidity of 66%. (Loomis and Shull, 1937.) Controls were placed in a similarly equipped dessicator without ethylene.

Effect of Ethylene on Uptake of Auxin: The test for root formation was carried out exactly as described by Went, except for the fifteen hours during which the cuttings were inverted in auxin solution. During this time they were placed in ethylene, to test the effect of ethylene on uptake of auxin. The results are shown in Table 23.

TABLE 23.

Effect of Ethylene on Uptake of Auxin by Pea Cuttings

Experiment	Auxin Concentration	Roots per 10 cuttings: Uptake of auxin in ethylene	Roots per 10 cuttings: Uptake of auxin in pure air	Ethylene Conc.
1 (80 plants)	0.5 mg. per liter	24	26	0.005%
	pure water	6	2	
2 (100 plants)	0.7 mg. per liter	36	40	0.001%
	pure water	14	10	
3 (300 plants)	34 mg. per liter	41	42	0.001%
	6.8 mg. per liter	37	37	
	1.71 " " "	30	33	
	0.34 " " "	28	28	
	0.068 mg. " "	31	28	
	pure water	17	21	

It may be seen from these data that ethylene has no effect upon uptake of auxin from the solution. Since uptake is dependent on

transport of auxin, this is in agreement with the previously described experiments on transport. There is, however, one point of apparent disagreement with previously described results. Since ethylene increases auxin destruction, why does this added destruction not cause a decrease in the number of roots formed? It is possible that the ethylene concentration used here does not affect destruction, or that the auxin enters and acts on the cutting during the time before the ethylene causes an increase in destruction (which was previously shown to be several hours).

Effect of Ethylene Applied During the Growth of the Roots: Here the experiment was carried out according to the method of Went except that the plants were placed in ethylene for varying lengths of time after uptake of auxin; (i.e., while standing in sucrose solution). The results of such experiments are shown in Table 24.

TABLE 24.

Effect of Ethylene on Root Growth in Pea Seedlings

Experiment	Treatment of Cuttings	Eth. Conc.	Roots per 10 cuttings With Auxin	Roots per 10 cuttings Controls
1	Ethylene 7 days	0.01%	0	0
	Pure air		61	32
2	Ethylene 7 days	0.005%	0	0
	Ethylene 2 days	0.005%	26	14
	Pure air		26	2
3	Ethylene 7 days	0.001%	0	0
	Ethylene 2 days	0.001%	32	½
	Pure air		40	10
4	Ethylene 5 days	0.001%	3	½
	Ethylene 2nd-6th day	0.001%	3	1
	Ethylene 1 day	0.001%	38	4
	Pure air		40	6
5 (relative humidity 93%)	Ethylene 6 days	0.001%	0	0
	Ethylene 4 days	0.001%	4	0
	Ethylene 2 days	0.001%	10	0
	Pure air		33	0

Examination of this data shows only one case (exp. 3) where the number of roots appears to have been increased by the ethylene. This difference is probably only accidental, as it does not appear in the other experiments.

In addition to this, it may be seen that 5 to 7 days' ethylene treatment almost completely inhibited root formation. All cuttings thus treated showed wilting and other signs of damage due to the toxic action of ethylene.[1]

Ethylene treatments of one or two days apparently also cause a reduction in the number of roots in some cases. Probably this is also an effect of the toxic action of ethylene.

The position of the roots formed on the ethylene-treated cuttings is the same as on the controls, - that is, very close to the base of the cutting.

These experiments have shown, therefore, that ethylene has no effect upon production of roots in these cuttings (except when it causes injury to the roots). In this respect, pea cuttings differ from woody cuttings of Salix, which will be described in the next section.

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1. One of the signs of ethylene injury, observed here as well as in intact plants of several species, is drying of the tissues to an extraordinary degree. For this reason it was thought that injury might be decreased if the experiment were done in a higher humidity. Consequently experiment 5 (Table 24) was done at a humidity of about 93%, but there was apparently no decrease in the degree of injury.

This type of injury was seen in these pea cuttings and in potted pea plants, where the leaves were completely killed. Yet the author has never seen it in the young seedlings described in preceding parts of this paper.

Effects of Ethylene on Root Formation In Hardwood Cuttings

Preliminary Experiments: Cuttings of peach, apple, and apricot were treated with ethylene and then kept in the dark at a relative humidity of 90% and a temperature of 25° C. No roots were formed, either in controls or treated cuttings, or in cuttings treated with auxin paste. An attempt was made to cause root formation in apple twigs by leaving the twig on the tree, enclosing part of it in a tube containing a small amount of ethylene, and applying auxin paste above this tube. This also gave no results.

Cuttings of Cottonwood (Populus trichocarpa) produced many roots, and the number was slightly higher in ethylene-treated plants than in the controls. The difference was so slight, however, that these were considered unsatisfactory as experimental material.

Experimental Procedure: The most satisfactory material found for these experiments was cuttings of Salix. These cuttings were of year-old wood, about 18 cm. long and 0.6 to 1.2 cm. in diameter. They were made as uniform as possible, and none with lateral branches were used. During the experiment they were placed in one-quart Mason jars, with water about two cm. deep in the bottom. In some cases they were placed directly in the water, and in some they were hung over the water. In the latter case a strip of filter paper was tied to the base of the cutting and allowed to hang in

the water. It seemed to make little difference whether they were placed directly in the water or over it.

Part of the plants were treated with auxin by applying auxin paste (one part auxin to 2000 of lanoline). This was applied to a small area near the top of the cutting, from which the outer layer of bark had been removed. After this the cuttings to be treated with ethylene were placed in a gas-tight, light proof incubator, kept at 25°, where they remained for two weeks. The incubator was opened, ventilated, and re-charged with ethylene every two or three days. The controls were kept in the dark room, also at 25°. After two weeks, the plants were removed and the roots counted.

Using the methods described here, several experiments were performed, in each of which groups of ten to fifteen cuttings were treated in different ways, as follows: (1) controls, (2) 0.1% ethylene for two weeks, (3) heteroauxin applied to the top of the cutting in the form of lanoline paste, (4) treated both with heteroauxin and 0.1% ethylene. After such treatment, it is very noticeable that auxin increases root formation and decreases bud growth. Ethylene has these effects also, but in addition it changes the distribution of the roots, so that they are found along the entire length of the cutting instead of being concentrated at its base. (See Figs. 18 and 19.) Counts were made of the number of roots in various sections of the cuttings (See Table 25). This will be discussed later.

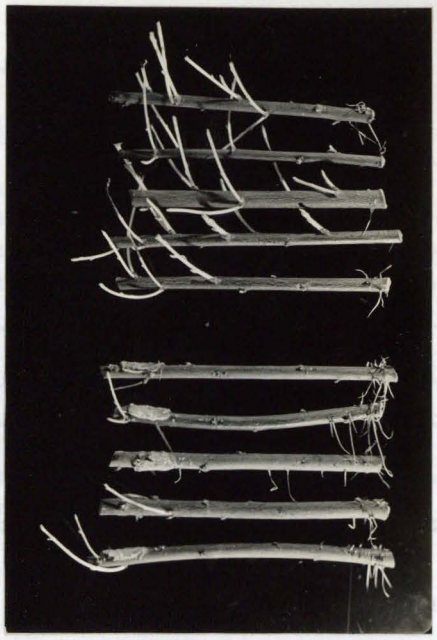

Fig. 18.

Fig. 19.

Effects of auxin and ethylene on Salix cuttings.

Fig. 18. Top. Controls
Bottom. Cuttings treated with auxin.

Fig. 19. Top. Cuttings treated with ethylene.
Bottom. Cuttings treated with ethylene and auxin.

These cuttings were placed in a horizontal position during the experiment. Other experiments showed that the position of the cutting with respect to gravity had little, if any, effect on root formation and bud growth.

TABLE 25.

Effects of Ethylene and Auxin on Root and Bud Growth

In Salix Cuttings

Portion of Cutting	Controls	Ethylene alone	Auxin alone	Ethylene and Auxin
Roots				
Basal cm.	7.7 ± 0.4	6.0 ± 0.5	8.0 ± 0.5	7.0 ± 0.3
Second cm.	2.6 ± 0.3	3.8 ± 0.4	4.5 ± 0.3	5.3 ± 0.5
Remainder	1.7 ± 0.3	6.4 ± 0.9	10.6 ± 1.4	28.2 ± 2.2
Total roots	11.9 ± 0.6	16.2 ± 1.2	23.1 ± 1.5	40.5 ± 2.3
Number of buds growing out	7.4 ± 0.4	6.1 ± 0.4	2.5 ± 0.3	0.8 ± 0.2

Six experiments of this kind were done, all giving essentially the same results. The age of the wood made very little difference, as the results were the same in an experiment done in June with wood only two or three months old instead of a year old. When second-year wood was used, the number of roots decreased somewhat toward the end of the winter, but this effect was small. When the cuttings were placed horizontally instead of vertically, there was slightly less concentration of roots in the basal centimeter of the cutting, but otherwise the results were the same. The results of representative experiments are presented in Table 25 and, graphically, in Fig. 20. Results of the remaining four experiments are presented as an appendix.

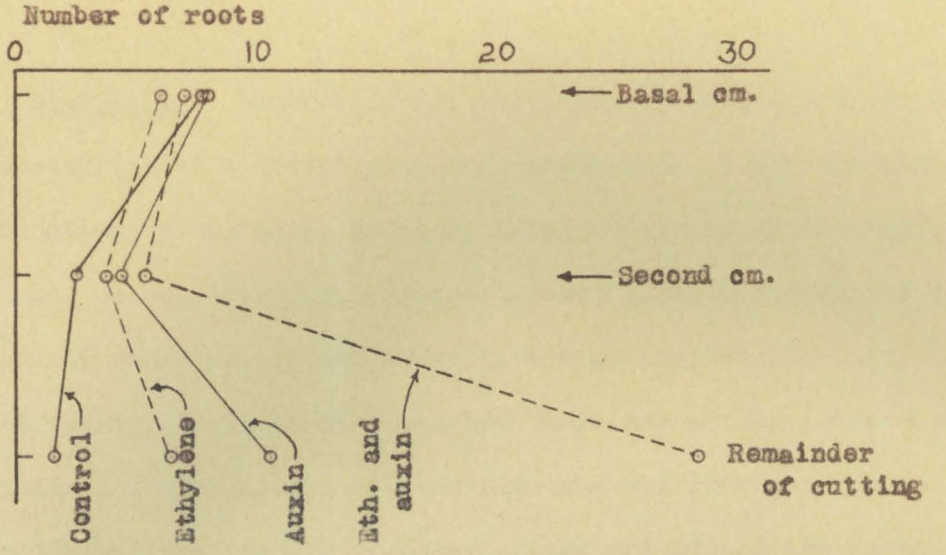

Fig. 20a. Distribution of roots in ethylene- and auxin-treated Salix cuttings.

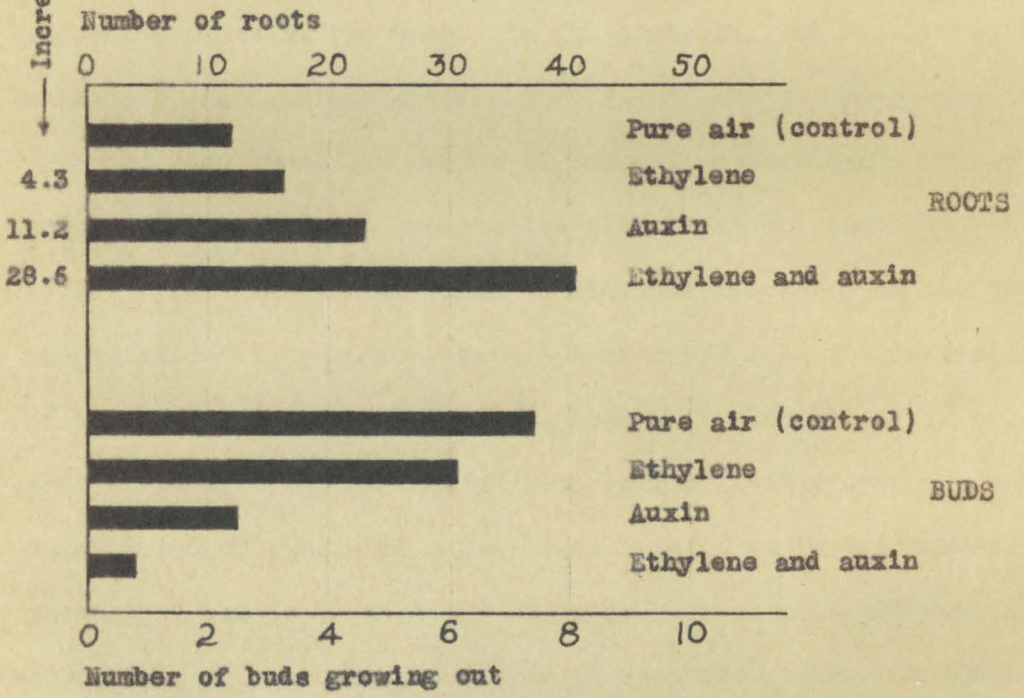

Fig. 20b. Total number of roots, and the number of buds growing out, in ethylene- and auxin-treated Salix cuttings.

Discussion: Superficially, these experiments appear to indicate that ethylene acts directly on root formation. A more careful examination of the data, however, shows that this is incorrect.

Let us consider the increase in root formation over the controls caused by ethylene, by heteroauxin, and by the two together, (as shown in Fig. 20.) If ethylene and auxin are acting independently to cause root formation, it is clear that the increase due to ethylene and heteroauxin together should not exceed the increase due to ethylene plus the increase due to auxin. Yet it does very definitely in this, as well as in several other experiments.

If we compare the distribution of roots in controls and cuttings treated with auxin, we see that they both produce about the same number of roots at the base. In the controls, the number rapidly falls off with increasing distance from the base. This is to be expected, for a certain amount of auxin is undoubtedly present in the cutting, and it is transported polarly toward the base, where it accumulates. When heteroauxin is added artificially at the top of the cutting, it is also transported downward. As its diffusion out of lanoline paste into the living tissue is slow, there is a continuous downward passage of heteroauxin lasting over a considerable period of time, and a resultant rise in auxin concentration throughout the whole cutting. In the upper part of the cutting there is a corresponding increase in root formation. At the base

this increase is very small, for, even in the controls, there is enough auxin to produce nearly the maximum number of roots which can be produced by auxin. Hence, at the base, the increase caused by adding heteroauxin is only slight.

Now let us consider the cuttings treated with ethylene. If ethylene is acting directly, should it not increase the root formation even at the base of the cutting? Actually it has no effect or, more often, causes a slight decrease.

It may be argued, of course, that a maximum number of roots is reached at the base, which cannot be exceeded by any treatment. If this were true, it can easily be seen that ethylene would cause no increase. It is nevertheless impossible to explain (assuming a direct action of ethylene) why the addition of heteroauxin, either to ethylene plants or controls, always causes some increase in root formation, at the base as well as elsewhere; while an addition of ethylene, either to controls or to plants with heteroauxin, almost always causes a slight decrease in root formation at the base, though it causes an increase in the remainder of the cutting.

An attempt was made to exhaust the supply of substances necessary for root formation in cuttings. This was done by placing cuttings in jars, without other treatment, and allowing them to grow roots for about six weeks, - the theory being that in doing so

they would use up their supply of "root-forming substance", or whatever is necessary for root formation. At the end of this six weeks period, all the roots and growing buds, and the basal two cm. of the cutting, were removed; and the cuttings were treated with ethylene and auxin as in the previously described experiment.

The possibility was considered that these cuttings would form more roots - and therefore more rapidly exhaust their supply of "root-forming substance" - if the roots were removed every few days instead of only at the end of a considerable period. This was found to be true, though the constant removal of roots had no great effect. (See Table 26.) This method was used for further experiments.

TABLE 26.

Effect of Constant Removal of Roots on Root Formation in Salix

Treatment of Cuttings during period of three weeks.	Total number of Roots formed during three weeks period (by 24 cuttings)
All roots removed five times	757
No roots removed until end of period	575

In cuttings treated in this manner, addition of auxin had a greater effect than in the previously described experiment on the number of roots formed in the basal portion of the cutting. In the upper part of the cutting, the effect of auxin was the same as before.

The effect of ethylene is somewhat doubtful, though in one experiment (See appendix, Tables 29 ~~32 and 33~~), it had no more effect than in the previously described experiment. This is further evidence that ethylene acts indirectly to cause root formation.

The possibility has been considered that the roots produced in these cuttings grew from pre-existing root primordia. The cuttings contain some such root primordia, which can be very easily seen if the bark is stripped away from the wood. The number of these primordia is small, however, compared to the number of roots which the cuttings formed in these experiments.

It is also of interest to note the effect of ethylene and auxin on bud growth in these cuttings, and to compare it with the effect of these substances on root formation. (See Fig. 20.) It may be seen that auxin (either with or without ethylene) caused a great reduction in the number of buds which grew out. This is in accord with the work of Skoog and Thimann (1934), which showed the inhibiting effect of auxin on bud growth in peas.

Ethylene also causes a considerable decrease in the number of buds growing out, though its effect is not as great as that of auxin.

The experiments on Salix cuttings have shown, therefore, that ethylene does not act directly to cause root formation; since its action depends partly on the amount of auxin in the cutting, and

since it often causes a slight decrease in root formation at the base of the cutting. It has also been shown that ethylene, like auxin, increases root formation but decreases bud formation. At the time these experiments were done, these facts seemed to indicate that ethylene was in some way affecting auxin. Consequently experiments were carried out to test this hypothesis.

Effect of Ethylene on Auxin Transport in Salix: First to be considered was the possibility that ethylene could inhibit the downward transport of auxin, thus causing more of it to remain in higher parts of the stem. To test this, transport experiments were done, using the method described earlier. For these experiments, internodes of willow twigs about a mm. in diameter were cut into 5 mm. sections. Twelve sections were then placed on a standard agar block with another agar block above them. The top block contained heteroauxin in a concentration of 250 units per cc.[1] After 2 to 2½ hours, the top block and sections were removed, and the bottom block was cut into 12 parts and tested for auxin content by the Avena test. The results of these experiments are shown in the Tables 27 and 28.

From these experiments two conclusions may be drawn. First, auxin is transported polarly ~~and~~ downward in woody Salix stems just as it is in the herbaceous plants which have been investigated.

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1. That is, a concentration which would give 250° curvature in the Avena test; or twenty-five times the concentration necessary to give 10° curvature.

TABLE 27.

Effect of Ethylene on Transport of Auxin in Salix
(Ethylene Treatment During the Experiment)

Ethylene concentration %	Total no. of plants	Auxin content of bottom block, expressed as degrees of curvature in Avena test			
		Sections normal		Sections inverse	
		Ethylene	Pure air	Ethylene	Pure air
0.01	48	13.6	13.7	-1.8	-1.7
0.05	48	3.7	5.1	-1.1	-0.5
0.1	48	9.3	10.2	-2.4	-1.5

TABLE 28.

Effect of Ethylene on Transport of Auxin in Salix
(Ethylene Treatment Before the Experiment)

Treatment of plants	Auxin content of bottom block
No ethylene treatment	6.8
Ethylene only before transport test	5.5
Ethylene only during transport test	5.7
Ethylene before and during transport test	5.3

Ethylene treatment: 0.1% ethylene for four hours.

All sections normally oriented with respect to gravity.

Second, neither polarity nor rate of transport were affected by the treatment with ethylene. This is ~~the same as~~ in agreement with other experiments having to do with the effect of ethylene on transport of auxin.

Other Possible Modes of Action of Ethylene on Salix: Since the observed phenomena are not a result of an effect of ethylene on auxin transport, they must be due to an increase in the amount or activity of the auxin, or the sensitivity of the plant to auxin. If not, then the action of ethylene on root production must have no direct connection with auxin.

The only way in which the amount of auxin can be increased is by an increase in production. This is exceedingly unlikely, since production of auxin is not known to occur in material of this kind, and furthermore there is no evidence whatever that ethylene can cause an increase in auxin production.

These experiments do not absolutely preclude the possibility that ethylene causes an increase in the activity of auxin, or the sensitivity of the plant to auxin. It is very unlikely, however, in view of the fact that ethylene, in several cases, caused a decrease in the number of roots in the basal cm. of the cutting.

This brings us then, to the last of the alternatives stated above, - that the action of ethylene on root production must have no direct connection with auxin, or with possible effects of ethylene on auxin.

Discussion of Effects of Ethylene on Root Formation

It has previously been known, and it is shown again by these experiments, that auxin stimulates root formation. It is also evident that ethylene stimulates root formation. Formerly it seemed possible that ethylene in some way increased the action of auxin in the plant, thereby causing more roots to be formed; but this hypothesis has now been shown to be incorrect, since ethylene does not increase concentration or activity of auxin in Salix cuttings.

Since these experiments were done, Went (1936; 1937, in press) (Cooper, 1936) has done experiments with pea seedlings which indicate that some substance other than auxin is necessary for root formation. The transport of this substance is brought about by auxin, but auxin does not directly stimulate root formation. Since ethylene does not act directly, and does not increase the root-stimulating power of auxin, it is reasonable to suppose that it in some way activates this "root-forming substance", thereby causing it to form more roots than it otherwise would. It will probably be possible to test this hypothesis, using pea seedlings, as experimental material; for, by the use of different experimental methods, pea seedlings can now be ethylene-treated without the toxic effects which caused difficulty in the experiments on root formation described here.

It appears then, that the effect of ethylene on root formation resembles its effect on swellings in peas and corn. In both cases, ethylene and auxin each act (directly or indirectly) on another substance. It must not be concluded from this, however, that ethylene and auxin are alike in their action, though such is not impossible.

Summary of Experiments on Root Formation:

1. These experiments have shown that ethylene stimulates formation of roots on marigold plants.

2. In non-toxic concentrations, ethylene failed to affect root formation in pea cuttings tested according to the method of Went. It is probable, however, that this toxic action of ethylene can now be avoided by using different experimental methods.

3. Both ethylene and auxin stimulate root formation in Salix cuttings, but ~~that~~ the two substances are not necessarily the same in their action.

4. Ethylene does not have any effect on the auxin in the cuttings which could cause the auxin to give increased root formation.

5. These facts, together with experiments of Went, make it probable that neither ethylene nor auxin act directly, but that both of them act on another hormone which causes root formation.

PART VIII.

DISCUSSION

A number of effects of ethylene on plant growth have been described in this paper. They may, for convenience, be divided into three groups: (1) effect of ethylene on geotropism; (2) effect of ethylene on longitudinal growth as a result of auxin deficiency, and (3) effects of ethylene on various growth phenomena resulting from its action on hormones other than auxin.

It has been shown by van der Laan (1933) that ethylene affects the lateral transport of auxin which is associated with geotropic response. This is evidently true also in the case of phototropism, since ethylene-treated pea seedlings are not phototropically active. Longitudinal transport of auxin has been tested in pea, Avena, and Salix, and in no case was it affected by ethylene. It follows from this that, in the pea, at least, lateral transport and longitudinal transport of auxin must be different processes, since one is affected by ethylene, while the other is not. This conclusion is complicated, however, by the fact that ethylene does not affect lateral transport in Avena.

Van der Laan has shown that ethylene did not merely inhibit, but reversed lateral transport in Vicia seedlings. The present author confirmed this by obtaining negatively geotropic curvatures in ethylene-treated pea seedlings. Is it possible that this reversed lateral transport is responsible for ethylene-induced epinastic

curvature of leaves? This is a point which should be investigated.

Such a hypothesis would not explain the effect of auxin on leaf epinasty, but it is not necessary that it should. According to Crocker, Zimmerman, and Hitchcock (1935), ethylene and heteroauxin are identical in their power to produce leaf epinasty. This, however, is apparently based only on the fact that ethylene and heteroauxin both produce epinasty. In the absence of further facts on the subject, there is no reason to suppose that they produce epinasty by the same process.

According to van der Laan, ethylene causes a decrease in auxin production. This results in decreased auxin content in the plant, to which he attributes all effects of ethylene not concerned with lateral transport (i.e., all effects other than geotropism and phototropism). The present author, however, failed to find any effect of ethylene on auxin production in Avena. In pea seedlings, ethylene caused a large decrease in the auxin extractable (by diffusion) from tips, but this may be a result only of the increased destruction caused by ethylene - a factor which van der Laan did not consider. Proof is lacking, therefore, that ethylene causes a decrease in auxin production.

The author agrees with van der Laan in stating that ethylene does not affect auxin transport, and that, in Avena, it causes an increase in sensitivity to auxin. Also, auxin destruction does not appear to be affected by ethylene in Avena. Thus ethylene has no

action on any of the factors which are known to affect the amount of auxin in the plant (production, transport, and destruction of auxin). Nevertheless, ethylene inhibits growth in such seedlings; but the addition of auxin brings the growth back to normal. These contradictory results have not yet been explained. It is probable that some important factor has been left out of consideration.

As has been stated, ethylene causes a great increase in destruction of auxin in peas. This is sufficient to account for the reduction in growth (with other factors to be discussed later). It may be, of course, that whatever reduces growth in Avena is also active in peas, but its effect is superimposed on that of auxin destruction.

The difference in destruction between pea and Avena is not (at present, at least) necessarily to be regarded as qualitative. It is known that peas have a very much higher rate of destruction than Avena. A given increase in auxin destruction in pea seedlings may be very large, while a proportional increase in Avena may be so small as not to be easily detectable.

Van Overbeek (1935) has shown that increase in destruction in nana corn over normal corn is correlated with an increase in activity of oxidative enzymes. Although no experiments have yet been done, it seems probable that the ethylene-induced increase in auxin destruction may be correlated with a similar increase in

enzyme activity. If so, this will be in agreement with various workers (see Part I.) who have shown that ethylene increases enzyme activity in fruits and other plant materials. It may be, then, that some (or all) of the effects of ethylene on growth are to be attributed to effects on enzyme activity.

So far only the effects of ethylene on longitudinal growth and on geotropic reactions have been mentioned. These are the only ones which may be attributed to effects of ethylene upon auxin. The other effects of ethylene upon growth result from action of ethylene upon other substances. Thus, ethylene-induced swelling of pea stems and corn mesocotyls is not influenced by auxin (except that a small amount of auxin must be present); but they cannot form in the absence of a substance which comes from the roots.

Since auxin-induced swellings are also unable to form without a substance from the roots, (Went, 1937) it is concluded that ethylene and auxin both act on a substance which is necessary for swelling formation. Through its action on this swelling substance, ethylene and auxin both have an indirect effect on stem elongation; for, as previously explained, swelling formation inhibits stem elongation.

A similar situation is found in the case of root formation. In Salix cuttings, ethylene ~~auxin~~ does nd act directly to cause root formation, nor does it have any effect on auxin which could cause the auxin to give increased root formation. It must, therefore, act on the root-forming substance suggested by Went (1936, 1937). In this respect

it is like auxin, which also does not act directly to cause root formation, but causes activity of the root-forming substance.

There are, then, at least two cases in which ethylene appears to act in the same way as auxin - by affecting growth hormones other than auxin. However, as was remarked in the discussion of leaf epinasty, this merely means that ethylene and auxin have the same effect, not that they act by means of the same process.

SUMMARY

1. It has been shown that ethylene can produce negative geotropism, at least under certain circumstances, in pea seedlings.

2. Ethylene does not affect transport of auxin in any of the plants tested. There is no evidence that it directly affects sensitivity of plant tissue to auxin. Proof is lacking that it decreases production of auxin.

3. In Avena, ethylene has no observable effect on auxin destruction. Hence it is also impossible to account for a decrease in auxin content of the seedling, yet addition of auxin gives normal growth.

4. In pea seedlings, ethylene causes an increase in auxin destruction; thereby giving decreased longitudinal growth.

5. In pea and corn seedlings, stem swellings are caused by the action of ethylene on a hormone which comes from the roots.

6. In corn seedlings, it has been shown that at least two hormones come from the roots. Both are acted upon by auxin, but ethylene only affects one of them.

7. Ethylene affects root formation by means of its action on the root-forming hormone of Went (1936), or some other substance different from auxin.

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APPENDIX

TABLE 29

Further Experiments on Root-Formation in Salix

		Number of Roots Growing Out*					
		Basal cm.	Second cm.	Lower half (ex. 1st 2 cm)	Upper half	Total	Buds growing out
I.	Controls	12.1	2.7	0.9		15.6	
	Ethylene	11.1	3.9	10.5		25.4	
	Auxin	21.6	5.3	6.1		33.2	
	Eth. & auxin	14.5	5.3	25.8		46.4	
II.	Controls	7.1	0.5	0		7.6	2.7
	Ethylene	7.3	1.8	2.5		11.5	2.9
	Auxin	13.6	4.8	2.5		20.9	0
	Eth. & auxin	10.4	5.6	24.0		40.0	0
III.	Controls	4.3	2.1	0.7	0.2	7.2	8.1
	Ethylene	6.4	4.1	9.6	4.6	24.6	11.2
	Auxin	7.8	5.7	4.7	4.2	22.4	1.6
	Eth. & auxin	6.8	5.8	16.2	15.9	44.7	0.8
IV.	Controls	6.3		0.7	0	7.0	5.8
	Ethylene	14.1		9.0	3.4	26.6	6.1
	Auxin	18.0		7.4	5.1	31.2	3.5
	Eth. & auxin	22.0		20.0	20.3	62.2	2.8
V.	Controls	3.8		0.6	0	4.5	2.4
	Ethylene	2.8		4.5	3.1	10.6	1.3
	Auxin	8.7		4.6	8.4	21.6	0.4
	Eth. & auxin	6.1		6.7	12.2	25.0	0

*The average number of roots per cutting, for 12 to 20 cuttings.

These experiments were done in the same way as the one for which the results are given in Table 25.

Further data: Experiment I. Ethylene concentration is 0.1%. Roots were not counted separately on the top half and the lower half of the cutting.

Experiment II. This is the only experiment in which cuttings were made

from wood only two or three months old. In other experiments the wood was a year old. In this experiment the ethylene concentration was 0.2%.

Experiment III. Here the cuttings were placed horizontally. In all other experiments they were in a vertical position. Ethylene concentration is 0.2%.

Experiments IV and V. Cuttings used in these experiments were "starved" for root-forming substance, by being allowed to grow many roots, which were removed before the beginning of the experiment. The roots were not counted separately in the first and second cm. The ethylene concentration is 0.1%.