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Summary: The paper is a literary review of major works on the subject of geotropism beginning with Dodart in 1703. The review comes up through the years with Astruc, de la Hire, de Monceau, Knight, Johnson, Giltay, Pfeffer, Pinot, Sachs, and Frank to the time of the Cholodny-Went Theory in 1927. Then the work after this time is discussed with stress on important points. A criticism is constructively made of many of the experiments with the criticisms being supported by other opinions of other workers in this branch of plant physiology. The paper ends, but does not climax, as there are still many unanswered questions concerning mechanisms of geotropisms.

ADVISER'S APPROVAL L. Herbert Bruneau

PLANT GEOTROPISM

A Literary Review of Past and Current Concepts

By

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INTRODUCTION

In 1703 Dodart made what is considered to be the first scientific investigation of geotropism when he noted that seedlings laid out to germinate all germinated with their roots extending downward. After this theories of the cause of this geotropic response were developed. They ranged from theories of the root sagging under the weight of its sap to the stem's reaching upward seeking its light sap.

Not much progress was made until the twentieth century. After the development of the Cholodny-Went Theory of Tropisms many scientific investigations into the mechanisms of geotropism were made. These inquiries have become more complex and nore numerous with the application of photographic techniques, rotational apparatus, electrical and magnetic instruments and various other scientific advances. Yet the problems are not all solved by any means. We are just at the dawn of the day when we will find the answers, we hope.

I selected this topic because it seemed to be a typical, but yet unsolved scientific problem. It is a subject that most people have never heard of; yet they see the trees, plants, and bushes everyday and know that the stems and branches grow upward and the roots grow downward. Most people never really understand why these things do as they do, but sometimes some students do wonder why. There are some people who cared enough to investigate the subject and they are the ones who will find the answers. It is to the first group of people that I dedicate my paper and it is about the latter group of people that I have written my paper.

STUDIES IN THE EARLY HISTORY OF GEOTROPISM

The first known treatment of geotropic reactions as a scientific problem is credited to Dodart (1703) who made systematic investigations on the direction of roots and shoots of bean (Phaseolus) seedlings which were laid out to germinate with their micropyle pointing in any direction. He noted that the roots and stems oriented themselves in a vertical direction no matter what way the micropyle lay. Dodart also noted the geotropic curvature of trees and herbs when they were moved from their normal position. From his observations he advanced a hypothesis which said that desiccation of the upper side and moistening of the lower side of the aerial parts of plants might lead to contraction of the fibers of the upper side of the organ and extension of those on the lower side of the organ. For roots he proposed just the opposite. However, Dodart proposed this hypothesis "with tongue in cheek" and admitted his inadequacy to advance a theory of geotropic response.

Astruc in 1709 was the first to suggest that gravity was the reason aerial parts of plants grew upwards. His theory was that the density of the plant juice caused it to be transported on the lower side of the plant parts which lay in a horizontal direction. Thus the lower side grew faster because it received better nourishment than the upper side.

A peculiar theory was proposed by de la Hire (1709) which said that plant sap was light and would ascend in the plant. Thus the growth of the plant part followed the ascending juice. The sap in roots, the theory said, was heavy and caused the downward growth of the roots. This latter idea was adopted later by Bazin in 1741.

Duhamel de Monceau rejected Dodart's theory on the basis of work with seedlings in a chamber of uniform humidity; Astruc's theory because the tip of an inverted main stem will turn upward even though its sap must already be uniformly distributed, and de la Hire's theory because of its entirely speculative nature. Duhamel (1758) himself did not advance a hypothesis, but he did suggest that experimental investigations of the conditions under which the reaction occur be made.

In 1806 Knight made the first real progress into the study of the causes of geotropism. He said:

> The curvatures exhibited by orthotropic plant organs (roots, shoots, coleoptiles) when placed horizontally, is a response to the change with regard to the direction of gravity. This curvature is the result of a difference between the growth rate in the upper and that in the lower side of the organ.

He was also the first to state and prove that the effect of gravity was the same as the effect of centrifugal force. In his paper he describes the construction of a water wheel attached to a second wheel which rotated in a vertical plane. The water imbibed seeds were placed in covered boxes at the edge of the rim of the wheel. The wheel velocity was 150 r.p.m.'s and completely negated the effect of gravity. Knight noted that all seeds when germinated had roots pointing away from the rim of the wheel with the stems growing toward the rim. (The centrifugal force equaled 3.5 g's.)

Knight also performed a similar experiment with the seeds mounted on a horizontal wheel which rotated with a velocity of 250 r.p.m.'s (9.7 g's). He found that these roots also pointed outward in the direction of the radii of the wheel. With an r.p.m. of 80, which produces a centrifugal force of 1g, Knight found that the seedling roots grew obliquely downward and outward with an angle of 45 degrees to the vertical.

The results obtained under these special set of conditions are important because they are quantitative evidence that a plant reacts the same way to gravity and a centrifugal force of 1 g.

Later in 1910 Giltay confirmed by more accurate measurements that the "root orients itself exactly in the direction of the resultant of gravity and the centrifugal force" and there is no other force which any effect upon the roots.

Knight, when trying to explain how the same force (gravity or centrifugal force) might cause roots and stems to grow in opposite directions, followed the line of Bazin and Astruc. In regard to roots, he claimed that the curvature begans at the tip and because the root tip was soft the root tips bent passively under the weight of its own sap. Later it was shown that this was not true.

Johnson in 1829 performed experiments with Phaseolus seedlings to find out it a root tip would exert pressure on a horizontal surface, when the root was surrounded by water. By use of a balance system Johnson found that after two days it required ten grains to restore the former equilibrium. He said that this indicated the root's property to produce an active downward pressure. However, Giltay (1910) pointed out that Johnson's experiment was open to criticism because of his inadequately supported conclusions and his unrefined technique. However, Johnson should receive credit for suggesting a method for the solution of the problem.

Then in 1910 Giltay and Pfeffer in 1904 repeated J ohnson's experiment under more rigidly controlled conditions and found that indeed the root tip during its geotropic curvature did exert a downward pressure which was equal to one hundred and fifty times greater than the weight of the root tip. Such ideas clearly refute the idea of passive sinking.

However, Pinot (1829) performed an experiment which furnished better evidence to the disproof of Knight's idea of passive sinking. Pinot demonstrated the active participation of growing root tissue in geotropic curvature by germinating seeds on a mercury surface. The root tips still grew downward and obviously the growth was not passive. (For references to Pinot's unpublished paper, see Christiansen, 1917.) This key experiment brought about alot of discussion. Dutrochet (1830) immediately denied Pinot's conclusions. Mulder (1829), Payer (1844), van Harreveld (1903), and Durand (1845) confirmed Pinot's results. However, despite this and further evidence, Dutrochet and Hofmeister, both leading nineteenth century plant physiologists, still upheld Knight's hypothesis of passive bending.

The term geotropism was introduced in 1868 by Frank in analogy with the term heliotropism coined by Candolle in 1832. Later when the term heliotropism was replaced by the Greek derivative word, phototropism, barytropism (barytes means weight) should have replaced geotropism but it never did.

However, the main accomplishment by Frank was his realization that the nature of geotropisms was active not passive. Thus, he adopted a concept of "release" or stimulation. This concept stated that the unilateral exposure to gravity releases an active force within the reactive plant parts which causes the geotropic response. To support this hypothesis, he performed an experiment similar to that of Pinot and drew conclusions similar to those of Pinot. Frank did point out, however, that only roots in an active state of growth could perform a geotropic curvature. Although this concept does not seem to be much of step forward, it must be remembered that Frank's hypothesis was the first to move away from hypotheses which were unsupported by evidence or contrary to observation.

Sachs (1875) followed up Frank's views with more research and became convinced of the soundness of his concepts. Sachs and Pfeffer became the strongest supporters of the principle of stimulation which was extended to other physiological processes besides geotropism. The application of this principle suggested by Frank has led to the concept of a reaction chain in which some distinction is made between the various phases. The phases as listed by Larsen (1962) are:

(a) at least one physical phase called susception
(b) several physiological phases. The first physiological phase may be called perception or reception. During this phase a certain "excitation" is created. At a later stage, a phase of transmission of this "excitation" to the site of the reaction can frequently be distinguished. Still later comes the reaction proper, for instance, a geotropic binding and the reaction may be followed by aftereffects. The term stimulation makes up the physical phase and the first (reception) physiological phase.

Several other people made contributions to the study of geotropism. Darwin in 1880 discovered that the perception of gravity is made by the tip of the root. Rother in 1894 concluded that the same was true for coleoptiles. Rutgers (1912) stated that the influence of various temperatures on presentation time is another factor to be studied. Van Ameyden noted that the geotropic curvature was changed in the absence of free oxygen. However, the beggest step forward came after the snythesis of the Cholodny-Went Theory in 1927, which is the next subject to be considered.

THE CHOLODNY-WENT THEORY

The first paper published by Cholodny dealing with the relationship between tropisms and growth hormones was in 1924. His first investigation showed the lack of geotropic response of a decapitated coleoptile or root stump to gravity, but then he showed that by placing the tip back on the stump cut surface the geotropic response would be restored. From this and other similar work, he arrived at these conclusions:

> (1) Growth hormones play an essential role in the mechanisms of the geotropic reaction.
> (2) In vertically placed stems and roots the growth-regulating substances are equally distributed on all sides.
> (3) As soon as these organs are placed in a horizontal position, the normal diffusion of the growth hormones is disturbed; the upper and lower cortical cells now obtain different amounts of these substances. This unequal distribution is ascribed to a physiological polarity induced by gravity, a polarity which Cholodny postulated in 1918.
> (4) The opposite signs of reactions of roots and shoots fit in with the fact that they react in opposite ways to the growth hormones coming from their tips.
> (5) The problem of geotropism can be traced back to the much simpler problem of the chemical control of growth.

Around the same time Went came to the same conclusions as Cholodny, from his experimentation on the isolation of auxin from the coleoptile tip. He said that the geotropic perception is caused by a polar alteration in the coleoptile cells. Instead of moving rectilinearly the growth regulators are more strongly conveyed towards that side which under geotropic stimulation was turned downwards.

In 1927 Cholodny stated that the apparently contradictory results of various workers on phototropism could be brought into agreement by adapting his hormonal theory of geotropism. He said that the cells of the coleoptile became polarized, then as a result of the unilateral illumination, the continuously produced growth hormones diffuse from the light towards the dark side more rapidly than in any other direction.

Soon after this theory of phototropism was formulated, Went came out with a similar hypothesis, this time with experimental proof. He had found that the growth hormone (auxin) could be determined quantitatively by the growth curvature test and that with no auxin, growth was absent. Thus he concluded that differential growth should be studied from the angle of differential auxin relations. He found that auxin production and distribution on the two sides of the phototropically stimulated coleoptile tips differed and that the auxin was unaffected or undestroyed by light. His experiments showed the principal effect of unilateral illumination is lateral transport of auxin from the light to the dark side.

Other experiments followed, and then a concept was drawn known as the general theory of plant tropisms or the Cholodny-Went Theory. This is presented in the book Phytohormones by Went and Thimann (1927) and they formulate the general theory of plant tropisms in the following way:

> Growth curvatures, whether induced by internal or external factors, are due to an unequal distribution of auxin between the two sides of the curving organ. In the tropisms induced by light and gravity the unequal auxin distribution is brought about by a transverse polarization of the cells which results in the lateral transport of auxin.

Went in 1942 added more evidence to his theory when he showed that the degree of geotropic curvature is directly related to the amount of free diffusible auxin not the total amount of auxin. The remaining auxin which is not free, called bound auxin, may be extracted by the use of chloroform or alcohol and ether. When the tip was removed from the coleoptile, the amount of bound auxin in the stump decreased slowly while the geotropic curvature decreased rapidly. While the tip was being regenerated it was noted that as the amount of diffusible auxin increased so did the ability of the stump to respond geotropically. The conclusion drawn by Went was that geotropic curvature is due to the effect of gravity on the free auxin.

RECENT STUDIES ON MECHANISMS OF GEOTROPISM

After the Cholodny-Went Theory was formulated, many workers in plant physiology sought to furnish further evidence to prove or disprove its application to geotropism. Dolk in 1930 and 1936 found that the amount of growth substance diffusing from the lower half of the horizontal organ is greater than the amount diffusing from the upper half - but the question of how these differences arose was still unknown- of an Avena coleoptile. Navez and Robinson in 1933 on Avena, Dijkman in 1934 on the hypocotyls of Lupinus, Van de Laan in 1934 on the epicotyl of Vicia faba, and Hawker in 1932 on roots all substantiated the Cholodny-Went Theory of plant tropisms.

Dolk, however, was the first to demonstrate that the theory proposed by Went and Cholodny applies completely and in its simplest form to geotropism in Avena. He showed that no growth reaction occurred when coleoptiles were rotated horizontally nor when returned to the vertical. Navez and Robinson confirmed this in 1933. Correspondingly the total amount of auxin diffusing from the coleoptile tips is the same whether they are horizontal or vertical. However, the amount diffusing from the lower half when placed horizontally is 62.5% and 37.5% from the upper half. (This was also confirmed by Navez and Robinson.)

Hence it may be concluded that gravity has no effect on the total auxin production, but that it does affect the relative distribution between the two sides. Unequal distribution and curvatures do not last long after removal of the stimulus (up to 40 minutes); on the contrary, the curvature begins to decrease from the top down, making the zone of maximum curvature near the base. (Dijkman in 1933 and 1934 made similar tests and came out with similar results.)

Dolk also noted that the longer in length the excised tip of the coleoptile, the more definite and greater was the difference in the amount of auxin diffusing from one side of the coleoptile into an agar block than from the other side of the coleoptile into an agar block. He concluded that the ability to transport auxin laterally is not exclusive to the extreme tip of the coleoptile. By further experimentation he proved that the basal parts do indeed have the capacity for lateral transport of auxin. He used coleoptile cylinders from which the tip had been removed. Auxin was added evenly to the apical tip and collected in separate agar blocks from the basal tip. The amount of auxin diffusing out of the lower half exceeded that diffusing out of the upper half by a ratio of 3 to 2. This is the same ratio as was found in the case above using the "natural" auxin.

It should be noted that the experiment lasted only ninety minutes and this was not enough time for the cylinder to regenerate a physiological tip, and therefore, there was no auxin production in the cylinder during the test period. This is very important for proper results and interpretation of the results.

Again Dolk's work was further substantiated by research by other experimenters. Bunning 1939, Brauner and Appel 1960, Gillespie and Briggs 1960 and Went in 1943 offer further prooof for Dolk's work.

Nuernbergk in 1933 curved geotropically a decapitated coleoptile by the use of an auxin derived from urine which was dissolved in an agar block. His work was the first to show that a growth substance or auxin which had been isolated from a nonplant source could cause a geotropic curvature.

Burkholder (1941) first used the synthetic growth hormones (alpha-napthalene-acetic acid, indole-3-propionic acid, indole-3-butyric acid, and indole-3-acetic acid) on Avena seedlings and excised coleoptiles. The hormones were dissolved into water or agar. He concluded that growth substances reduce the curvature of the coleoptiles.

Burkholder's conclusion, although contrary to most other workers' conclusions, remained unchallenged and undisproved until Anker in 1954 showed that Burkholder's concentrations were too high or too low physiologically. (It will become clear why later.)

Anker's work dealt with quantitative studies of the growth substance requirements of excised decapitated coleoptiles during geotropic curvature. He worked with the coleoptiles submerged in solutions made up of the growth substance dissolved in water. He proved that the geotropic curvature was greatest in the solutions whose concentrations were suboptimal for coleoptile growth and that the curvature fails to appear at all in the concentrations which were optimal and supraoptimal for coleoptiles growth. The growth substances which he worked with were indole-3-acetic acid, indole-3-acetonitrile, and alpha naphthalene acetic acid. It should be noted that Anker's experimental success suggests that quantitative experiments on geotropism could be conducted with excised, nondecapitated and decapitated coleoptiles in water and other solutions containing the growth substances.

Brauner (1926, 1927, 1928) showed the establishment of a transverse electrical polarity in a horizontal tissue-living or dead or a model. The upper side of a horizontally placed coleoptile becomes electrically negative with respect to the lower side. This was called the "geo-electric the "geo-electric effect" by Brauner. Amlong 1933, Clark in 1937, and Schrank in 1944 and 1945 carried out further investigations on differences of potential between upper and lower surfaces.

In 1926 and 1927 Cholodny suggested the transverse movement of growth substances resulted from an electromotive force. Then Went in 1932 formulated a theory of polarity in plants which he called "Eine botanische Polaritatstheorie" after studying basic and acidic dye penetration in stem sections of Impatiens. He stated that a cataphoretic transport was the cause of the lateral movement of growth substances. (Cataphoresis is defined as the movement of suspended particles through a fluid under the action of an applied electromotive force.) Went said that the negatively charged anion of the growth substance molecule would be transported to the positively charged lower side of the horizontally placed organ, resulting in an increase in the growth substance concentration in the lower side. However, no evidence yet proves that growth substances are moved by internal electric potentials and no evidence has been brought forward to determine whether or not the potential differences which do exist in a horizontally placed organ are the primary effect, and if they are, whether they are strong enough to move the growth substances, and produce the ensuing geotropic curvature.

An interesting addition to this is a statement made by Schrank in a 1945 paper. He said: "The fact that the transverse electrical polarity is established prior to the unequal distribution (of growth substances) does not necessarily mean that it is the orienting force or polarity which is essential for lateral hormone transport." Then he went on to say that "it means only that the transverse electrical polarity fulfills the prime requirement of being established in the correct sequence."

REVIEW OF NEW IDEAS ON ROOT AND SHOOT GEOTROPISMS

Introduction, Reaction, and Course of the Geotropic Reaction.

It seems necessary that a uniform terminology be adopted for use in this section of the paper dealing with the more recent advances in the study of geotropism. The terminology employed by Larsen concerning the phases of the reaction will be used. These phases are defined on page six of this paper.

Within the past few years many new methods have been developed for studying the geotropic reactions. Instead of relying on the eye to determine the degree of geotropic curvature, photographic techniques have been developed to record the angle of curvature; then the angles are measured microscopically. Obviously these results are more accurate and independent of the investigator's subjective opinion.

Larsen in 1953 and 1956 published papers describing the use of agar plates to grow the roots between. This prevents any irregular distribution of water found when the experiment is performed in damp air. Audus and Brownbridge, as well as Rufelt, have worked with roots in an aqueous solution of growth substances as described earlier in the work of Anker. It has proven to be a very satisfactory method. Other workers have used immersion techniques and added to it the use of a klinostat to rotate the roots. Larsen has criticized the interpretations of Audus and Brownbridge concerning his experiments involving the use of the klinostat on the basis that the rotation velocity decreases the growth rate as well as differentially affecting curvature development.

Different velocities affect the curvature in different ways and, therefore, Larsen state that the velocity of rotation is necessary for proper interpretation of the results; yet it is seldom reported.

It was stated earlier that the time lapse for using decapitated coleoptiles should not exceed ninety minutes from the time of their decapitation because after that time the regeneration of the physiological tip will mask the results of the experiment. Obviously, this limits the length and degree of geotropic curvature, but Anker claims that an accuracy may be achieved by use of photographic methods and a protractor. Rufelt asked for a statistical analysis on Anker's claim of accuracy, but as yet one has not been published.

The presentation time may be defined as the duration of a stimulation that is required to produce a reaction. The time is usually determined by finding the stimulation time required to produce visible curvatures in one half of the plants used in the experiment. Larsen has criticized this method on the grounds that unstimulated plants often develop a slight curvature. He has suggested that a mean curvature produced by several stimulations be determined for a set of different times of stimulation; then the true minimum time can be obtained by extrapolation. This method is best for an accurate determination of a true minimum reaction. However, Rufelt has defended the classical method as accurate in all cases except those where the true minimum reaction is to be determined. In all other cases both methods give the necessary linear connection between intensity of stimulation and magnitude of curvature. Thus the classical method would be of use where measurements of changes in the presentation time are all that is needed.

There seem to be no methods which can really determine quantatitively the stages in the reaction chain before the reaction itself. However, certain workers have attempted to determine the perception time by following up the theory that the geoelectric effect causes a unilateral distribution of auxin. Brauner, Hertz, and Lundegardh have developed methods which attempt to measure the change in the potential difference in the organ, but so far the methods and results are not without severe criticism.

The course of the geotropic reaction is the next subject under discussion. Larsen has written a paper which contains many interesting points. He stated that during the first hour to two hours following the horizontal placement of the roots, the roots curve at a relatively constant rate. Then after the two hours, the rate of curvature decreases due to the decrease in the stimulation since the angle serving as stimulation is decreasing. When the roots are rotated on a horizontal klinostat as has been done by Audus and Brownbridge,the curvatures after stimulation are the same as those continually stimulated. However, the roots which were rotated on the klinostat straightened out after two hours and Audus and Brownbridge interpreted this as autotrophic straightening. Larsen has said that their interpretation of the results is incorrect. He claims that the straightening, or negative curvature, resulted from the omnilateral stimulation incurred on the klinostat after the original curvature was complete and no longer served as a stimulus.

Rufelt worked with wheat roots which were immersed in a solution, but which were not rotated. He described the development of the curvature as it began. A positive curvature begins at the extreme tip of the root then "moves" further down the root with the tip becoming straight behind the shifting bend due to the negative geotropism mentioned earlier. The first stages of positive and negative tropisms are reversible, while the later stages of positive tropism are not. This later stage appears when the bending reaches the area of the meristem. Rufelt also found that the root curvatures were influenced in rate by the pH, temperature and oxygen supply. At a pH value of 7 to 7.5 the roots curved faster than at low pH values of 5 to 6. The roots also curved faster as low temperatures and also at high oxygen supply. This last point concerning the effect of oxygen is interesting because some workers such as Audus have aerated their growth solutions, but have not allowed for this effect.

Other workers when working with different species such as corn, pea and Avena have substantiated the conclusions made by Larsen concerning the depression of the positive curvature rate several hours after stimulation with the only difference between the results being the absolute time when this occurs. In the case of corn roots, the time at which the curvature rate decreased was found to be four to five hours.

Bennet-Clark, Younis and Esnault in 1959 worked with roots with their tips extended upward in a moist environment of air. Their results confirmed those of Nemec (1901) and Zimmermann (1927) and stated that the roots are able to completely curve 180 degrees to a position with the tip extending downwards. However, the rate of curvature was more rapid at the beginning than later, and it was more rapid than the curvature of a root from a 90 degree angle at the beginning of the time. This is in keeping with the results stated earlier by Larsen who noted that the initial rate of curvature was faster than the final due to the decrease in stimulation due to the decrease in angle. However, when the plants were inverted in soil or sand, their curvature was not always completed. This was explained by one author as the interference of the haptotropic stimulation on the geotropic one. This in itself is another complete subject and one upon which we shall spend no more time here.

In both shoots and roots illumination produces an increase in the positive geotropism and causes the negative (straightening) geotropism which normally follows to be retarded and often it causes it to fail to appear at all. Another example of a case where the negative response fails to follow is found in the work of Dolk and de Wit who both used Avena coleoptiles. Coleoptiles decapitated immediately after geotropic stimulation still curve in the direction in which they were stimulated to curve, but no negative geotropic response follows unless the physiological tip is replaced. No artificial supply of auxin will cause this negative bending. Thus Rawitscher concludes from this evidence that curvature and straightening are two entirely different processes.

Effects of Growth Regulators on the Geotropic Reaction.

The first experiments to review which will help to give an understanding of the different effects obtained from the growth regulatory substances are three by Anker. In the first experiment in 1954 he ran tests using three different auxins in order to determine the concentrations of each auxin which was optimal for growth and that which was optimal for the geotropic reaction. He found that the geotropic reponse is limited to a narrow range of growth substance concentrations, and that the auxins themselves vary in the amount of this concentration. However, the optimum concentration for the geotropic response was quite low in comparison to the optimum concentration for growth. This latter fact makes up what is called the auxin concentration rule.

In 1956 Anker published a paper which was centered around the auxin concentration rule. In this experiment he sought to determine what relationships existed between the auxin concentration and the geotropic response and the growth response. He found that the magnitude of the geotropic curvature is dependent upon the growth rate for the upper and lower sides of the coleoptiles. The curvature develops when geotropic stimulation causes part of the auxin to diffuse to one side giving an increased growth rate on that one side. However, when the concentration is high, both sides have the optimum amount of auxin, and then any difference in concentration resulting from geotropic stimulation is without influence.

Anker used the auxin concentration rule in 1958 to interpret the influence of pH effects on the geotropic reaction. He postulated that if the pH of the media did promote or inhibit growth when the auxin was supplied externally, then these effects might be the result of a difference in the degree of auxin dissociation. However, another possibility existed: that the change in pH might also change the acidity of the cytoplasm and thus change the activity of cell enzymes. This last possibility has been the subject of a controversy of whether the internal pH is independent of the external pH. (Albaum et al 1937, Rietsema 1950, Van Santen 1940, Simon and Beevers 1952 and Blackman and Robertson-Cunninghame 1953.) The whole thing is centered around the fact that no one can tell the exact difference between a cell micro-climate pH and the cytoplasmic pH. The first is important for auxin action and the second for auxin transport or geotropic curvature. The geotropic curvature would be facilitated by the presence of the auxin in an ion form.

By using a dissociable indole acetic acid (IAA) and an undissociable indole acetic nitrile, Anker tried to discriminate between the effect on pH on IAA dissociation and its possible effect on the cytoplasm and cell elongation. Anker found, as Audus in 1949 had found, that root growth inhibition is independent of pH. The only effect of the acid pH was a quicker penetration of the cytoplasm by the auxin within a narrow range of concentrations.

Rufelt in 1957 worked with wheat roots and flax roots to determine why the different auxins varied in their concentrations which were optimal for geotropic response. He found that the different effects of the auxins were due to their influence upon separate physiological processes. Root geotropism could be affected by growth substances in either of the following ways: (1) by a direct effect on the elongation in progress or (2) by an effect on very young cells whose elongation are later inhibited or stimulated. Coumarin is an example of the second type.

A point which I found important to keep in mind while reading an experiment concerning the effect of hormones on growth and geotropism is the difference in the length of time for which the experiments are run. The experiments dealing with growth effects are run for at least twenty-four hours, whereas the experiments dealing with the geotropic response are run for not more than ninety minutes immediately following decapitation. Thus the growth experiments do not show the effects of adaptation phenomena which often peak and dissipate within six to ten hours. The geotropic reaction does show the influence of the adaptation phenomena. The conclusion made by Ruflet is that the results of a growth substance on a geotropic curvature can be compared with the results of a growth substance on growth only if discretion is used. Thus a direct comparison of these two processes is not as valid as some workers would lead you to believe. Audus, for one example, is capable of using this faulty comparison with too much emphasis and too little caution.

There is still another comparison which is frequently made without consideration of the pitfalls involved. It is often forgotten that the growth does not occur primarily at the tip, but in the area of elongation located back of the tip. Thus because of these differences in function and physiological activity, it would not be safe to compare the two regions without exercising caution. In a work by Audus found in the Journal of Experimental Botany, Volume 8, he bases his whole experiment and conclusions on the first assumption that these two areas are comparable. He does not impose any restrictions or suggest that the two areas are not the same, but goes on to draw his conclusion regardless of the original questionable assumption. This line of criticism is taken by Larsen, 1962.

Another consideration to keep in mind is a point brought up by

Overbeck (1926) who showed that the geotropic reaction can be straightened partly by means of plasmolysis. This means that the curvature is reversible to a certain extent under a process which is not a growth process. This complication is not usually encountered, but sometimes it is present.

Various other substances have an influence upon the geotropic reaction. Indole 3 butyric acid (IIBA) can invert the positive geotropic reaction. Evidence seems to indicate that it serves as an antagonist to the indole acetic acid which produced the positive curvature; however, the proof is not yet complete.

Audus and Brownbridge have stated that growth inhibition indole acetic acid concentrations (IAA) or 2, 4, D concentrations increased the reaction time of the roots and reduced the rates of the curvature. Growth rates of the upper and lower side were inhibited which caused the reduced curvature. Then Audus and Browhbridge go on to suggest that "the same relationship exists between the growth rate and endogenous auxin concentration in the entire root as is found between the growth rate and applied auxin for the root segments excised from the extending zone." From what has been mentioned before in this discussion we know that this comparison does not appear to be valid without more evidence to support it. However, Audus and Brownbridge go on to explain what they claim occurs by a rather complicated exposition which we shall not go into. (Larsen and Rufelt).

The influence of auxins on the geotropism of shoots is harder to study than that of roots because the shoots cannot be submerged in a growth solution. (The shoot will not take up the auxin like the root does from a solution.) However, investigators have supplied auxins to the cut surface of decapitated organs. Positive geotropic responses were invoked by Roberts with IAA, 2 naphthyloxyacetic acid, naphthalene, acetic acid and 2,4,5, T MIBC ester and negative responses from 1 naphthoxy acetic acid. This last substance was found to inhibit root growth to some extent besides causing the negative response. Schrank reported the differential effects of 2,3,6, trichlorobenzoic acid on growth and geotropic curvature. Since it is known already that the mechanisms invoking the growth and the geotropic response are complex, it is not surprising to find that some growth regulators have different effects on growth and geotropic reaction. In this particular case, the curvature is inhibited, but the growth reaction is not increased. (With IAA, when the curvature is inhibited the growth rate is increased overall.)

Stimulation.

The classic statolith theory is presented in a paper by Audus and Brownbridge. The starch grains are assumed to be the acceptors of the force of gravitation. The stimulation perceived by the starch grains would cause the release of an enzyme which would concentrate in the lower part of the root, according to the theory. This enzyme is supposed to serve as an inhibitor for the side where it is localized. Since the other side is not inhibited, a curvature resulting from differential growth occurs. Audus and Brownbridge have tried to demonstrate the ability of the starch grains to serve as the link between the gravitational force and geotropic stimulation by attempting displacement of the diamagnetic starch grains in a strong magnetic field. The authors however, state that this experiment did not supply evidence to support the theory.

Hertz and Graham have suggested that such bodies as mitochondria within the cell may produce the geoelectric potential difference found in cells after geotropic stimulation. Supporting evidence for this is found in the work of Ziegler who has shown the displacement of some cell organelles in a gravitational field.

The statolith theory in a modified form has been advanced by Larsen. His idea is that the statoliths are not free to move but pivot as a pendulum pivots. The unbalanced pendulum upsets some kind of electrical balance which sets the reaction in motion. However, many criticisms of this theory have been put forth and as of yet no substantial support of the theory has been made.

Although the mechanism of displacement is uncertain, several experimenters have confirmed the fact that the auxin is unilaterally distributed following a geotropic stimulation. It is possible that the auxin was displaced unilaterally or that it was produced unilaterally. Much work has been done to support both views. So far work with radioactive IAA has failed to support the view that the auxin is unilaterally transported. However, the work by Anker with Avena coleoptiles which have been decapitated tends to support the idea of unilateral transport. He found that decapitated coleoptiles do not respond geotropically unless auxin is supplied from the environment. Thus he concluded that the auxin is transferred unilaterally to produce the geotropic response since an external source of auxin can be utilized to produce a reaction. If the auxin had to be produced on the side where it was to be used, a uniform external source would not initiate the geotropic reaction.

Brauner and Hager have suggested another idea. They theorized that not the auxin but a cofactor is translocated across the tissue. In several experiments they tried to separate the geotropic stimulation from the reaction. In one experiment they placed the seedlings in four degree Centigrade temperature where they placed the seedlings horizontally to stimulate them. No reaction takes place at this temperature, but when the seedlings are returned to the vertical in a twenty degree temperature, they curve geotropically in proportion to the stimuli they received. In another set of decapitated coleoptiles which were place in a darkroom for four days no geotropic reaction took place, despite the fact that they were stimulated in the twenty degree temperature. Thus, the authors concluded that the plants could not produce a curvature due to auxin depletion, and the fact that a geotropic stimulation can take occur without a reaction causes them to suggest that stimulation takes place in the following steps:

> Under the influence of the gravitational force, the easily diffusible cofactor is translocated toward the lower side of the organ. After that, a period of metabolic binding of this substance sets in at the places where it is accumulated. Oxygen is consumed in the binding process, and no geotropic reaction was obtained after stimulation in a nitrogen atmosphere. The cofactor then combines with auxin to form a complex active in stimulating growth. Thus the cofactor is translocated during the stimulation of auxin-free hypocotyls, but a growth reaction takes place only after the addition of IAA.

In opposition to Brauner and in support of the lateral transport concept is the work of de Wit, who has said that "without growth substance there is no geotropism". In an experiment where decapitated coleoptiles were kept horizontal in water then vertically in growth solution, no geotropic curvature resulted. Thus he concludes from the evidence that in the absence of IAA no preparatory processes are induced under the influence of gravity. As a check he placed these coleoptiles in a horizontal position in growth substance and found that they did not curve any quicker than those which had not been subjected to this "preparatory process". The evidence of de Wit outweighs the evidence of Brauner and Hager at this time according to Rufelt. Further work on this subject is still needed though before any definite conclusions can be made.

However, some of the apparent conflicts in the results may be discussed in relation to the work of Guttenberg and Buchsel (1944). In an experiment performed by these workers IAA and a diffused substance obtained from the tips of coleoptiles were found to have identical effects when applied to intact coleoptiles. When these two substances were next applied to auxin-free cylinders, the diffusate showed an immediate effect whereas the IAA showed no effect until the third hour. The curvature of the coleoptile receiving diffusate was not as great as the curvature of the coleoptile receiving IAA. The authors concluded that the diffusate, or auxin, is not the same as IAA: Indole acetic acid serves as an activator for the auxin precursor. Thus from this and further experiments from de Wit, it is safe to conclude that there is a basic difference in the mode of reaction of whole and decapitated coleoptiles, and that IAA and auxin might not be the same substance.

Yet it is still hard to explain why radioactive IAA did not give the expected results concerning lateral transport, since we have seen that most evidence tends to support lateral transport. Rufelt states that if Brauner and Appel are correct in their asumption that a cofactor is displaced, then the results of the radioactive IAA tests are more understandable. Another possibility is that radioactivity could accelerate the regeneration of the physiological tip. This is denied by de Wit, but his evidence for denial is no stronger than the evidence for the idea that radioactivity does accelerate the regeneration of the tip. It would seem as if someone would perform an experiment instead of hypothesizing.

In 1960 Brauner and Appel presented a paper in which they discussed their experiment which they claim demonstrates lateral transport of a growth substance or growth regulator of some type. By separating the coleoptile tip with a mica plate it was found that the growth was inhibited and the curvature was greater than in the control.

Rufelt in 1957 suggested that a geotropic stimulus causes an increase in auxin production on the lower side of the root which causes the positive geotropic response. He suggested that the negative response

response is produced by the increase of a different growth substance on the upper side. The cause of this increase is theorized as a geoelectric effect.

Anker however, still maintains that one auxin is translocated laterally to cuase differential growth. He does not postulate directly the cause for the following negative curvature, but he does say that an increase in auxin content to a level supraoptimal (and thus inhibitory) for curvature and maximum for growth could be similar to the mechanism for development of negative geotropisms.

CONCLUSION

In conclusion I would like to say that I feel like a person would who had stepped in during the middle of an Alfred Hitchcock movie and was unable to stay until the end. The suspense is thrilling, but the question of "How will it end?" is gnawing. I can estimate and examine evidence to find what has gone before, but I will not be able to determine the end until it happens. Then I will be able to look back and say "I knew it all the time."

Many different and carefully worked out theories exist dealing with various factors relating to geotropism, however, little concrete may be stated about the HOW and the WHY. We can only say absolutely that geotropism does occur, that is is caused by gravity, that roots and stems react in opposite ways to the gravity stimulus, that some sort of auxin is responsible for the tropism, that the auxin is either produced on the stimulated side or somehow transported there, and that differential growth seems to be the cause of the curvature. That is not a lot to know after two hundred and sixty years of investigation. Yet we are finding out more every day which is adding more evidence to the solution of the mystery of geotropism.

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